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Proceedings of the 43rd Annual Meeting, Southern Soybean Disease Workers (March 9-10, 2016, Pensacola Beach, Florida)

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2016

Southern Soybean Disease Workers

43rd Annual Meeting

March 9-10, 2016

Pensacola Beach, Florida

PROCEEDINGS

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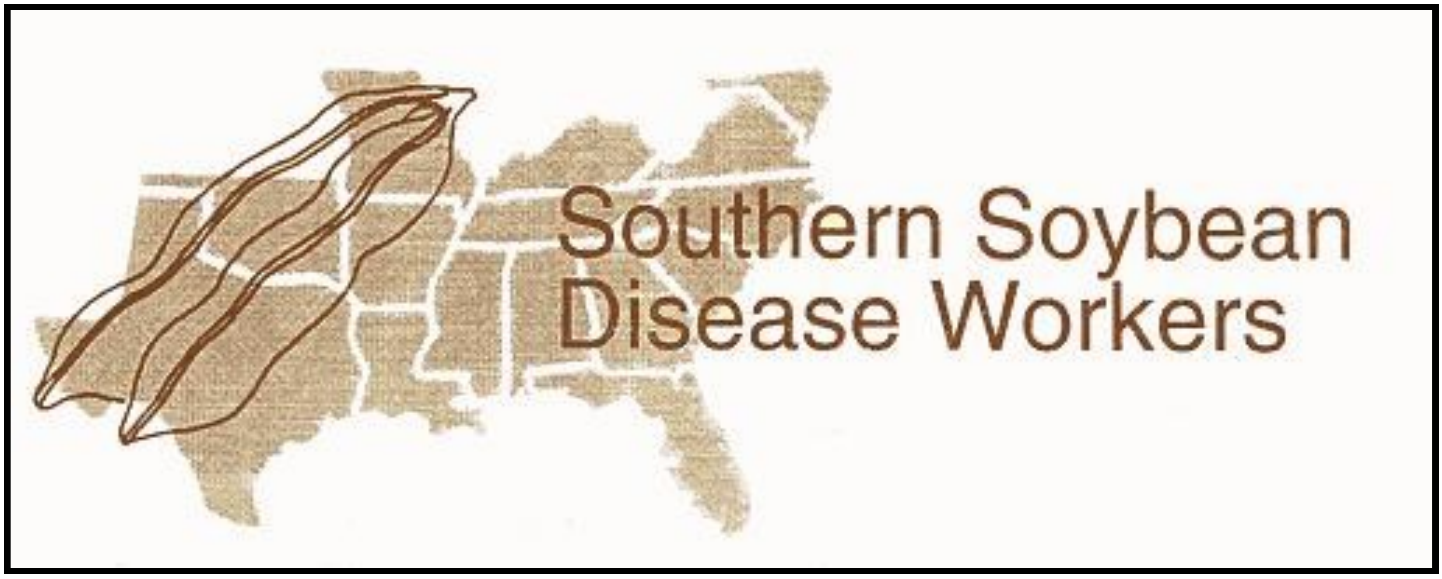

PROCEEDINGS OF THE
SOUTHERN SOYBEAN DISEASE WORKERS
43rd ANNUAL MEETING

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43rd Annual Meeting of the Southern Soybean Disease Workers

March 9-10, 2016

Pensacola Beach, Florida

Wednesday, March 9th, 2016

11:30-1:00	**Registration (White Sands)**
1:00-1:15	**Introductions** Trey Price, SSDW President
1:15-2:45	**Soilborne Disease Symposium** Tom Allen, Moderator
1:15-1:30	**Integrated management of sudden death syndrome** D. Mueller, L. Leandro, Y. Kandel, C. Bradley, M. Chilvers, A. Tenuta, and K. Wise
1:30-1:45	**Field screening for stem canker: an outdated exercise or a necessary service for soybean growers?** K. Rowe and T. Kirkpatrick
1:45-2:00	**Nematode associated diseases in soybean** C. Overstreet, Edward C. McGawley, D. Xavier-Mis, and M. Kularathna
2:00-2:15	**Characterization of taproot decline in southern soybean** M. Tomaso-Peterson, T. Allen, P. Price, R. Singh, and T. Spurlock
2:15-2:30	**Frontline tactics to manage Phytophthora stem and root rot** A. Robertson
2:30-2:45	**Open discussion on symposium topics**

Trey Price

2:45-3:00 **Break (White Sands)**

3:00-4:15 **Graduate Student Research Competition**

Travis Faske, Moderator

3:00-3:15 **Causative agents for the green stem disorder of soybeans in Louisiana**

B. Ward, C. Robertson, and R. Schneider

3:15-3:30 **Solubilization of cercosporin and its use for reproducing symptoms of Cercospora leaf blight of soybean**

E. Silva, M. Liu, J. Zhang, C. Robertson, Z. Liu, and R. Schneider

3:30-3:45 **Effect of droplet size on foliar fungicide application in soybean**

S. Butler, H. Kelly, T. Mueller, and G. Kruger

3:45-4:00 **Virulence assessment of strobilurin-sensitive and -resistant *Cercospora sojina*, the causal agent of frogeye leaf spot in soybean**

N. Brochard, M. Tomaso-Peterson, T. Allen, and R. Melanson

4:00-4:15 **Application thresholds in controlling *Cercospora sojina*, the causal agent of frogeye leaf spot**

J. Jordan and H. Kelly

6:00-8:00 **Social and Banquet (Poolside)**

Thursday, March 10th, 2015

7:00-8:15 **Breakfast (Coral Reef)**

7:30-8:30 **Registration**

8:30-9:45	**Graduate Student Research Competition (continued)** Terry Spurlock, Moderator
8:30-8:45	**Assessment of ILeVO for management of root-knot nematodes in soybean** C. Jackson, T. Faske, M. Emerson, and K. Hurd
8:45-9:00	**Evaluating the physiological impacts of fungicide phytotoxicity in Mississippi soybean** J. Mansour, M. Tomaso-Peterson, A. Henn., J. Bond, T. Irby, and T. Allen
9:00-9:15	**Benefit of secondary nutrition in reducing *Macrophomina phaseolina* colonization in Mississippi soybean** T. Wilkerson, M. Tomaso-Peterson, B. Golden, S. Lu, A. Johnson, and T. Allen
9:15-9:30	**Detection of a mycovirus from soybean rust and mycoviruses from other biotrophic fungi using a practical method for the extraction of viral dsRNA** R. Herschlag, S. Khankhum, and R. Valverde
9:30-9:45	**Effect of *Macrophomina phaseolina* inoculation, irrigation and cultivar on soybean yield** M. Zaccaron and J. Rupe
9:45-10:00	**Break (White Sands)**
10:00-12:00	**Contributed Papers** Eduardo Silva, Moderator
10:00-10:15	**Phenotypic characterization of *Cercospora sojina* isolates collected from wide geographical areas** A. Mengistu, J. Ray, J. Smith, and H. Kelly

10:00-12:00	**Contributed Papers** Eduardo Silva, Moderator
10:00-10:15	**Phenotypic characterization of *Cercospora sojina* isolates collected from wide geographical areas** A. Mengistu, J. Ray, J. Smith, and H. Kelly
10:15-10:30	**Effect of flower and pod removal on soybean senescence and comparison to green bean syndrome** J. Rupe, B. Holland, and A. Steger
10:30-10:45	**Competition studies of QoI resistant and sensitive *Cercospora sojina* isolates, the causal agent of frogeye leaf spot** B. Lin, H. Kelly, H. Yu, and A. Mengistu
10:45-11:00	**Frequency and distribution of QoI resistant *Cercospora sojina* in Virginia** H. Mehl and T. Zhou
11:00-11:15	**A survey of Arkansas soybean nematodes, 2014-2015** K. Sullivan, J. Robinson, and T. Kirkpatrick
11:15-11:30	**Nuclear proteins controlling soybean rust resistance** B. Cooper
11:30-11:45	**Nuts, bolts, frogeye leaf spot, and the UUOT** T. Allen, T. Faske, C. Hollier, P. Price, T. Spurlock, and H. Young
11:45-1:00	**Lunch (on your own)**
1:00-2:30	**Business Meeting**

Southern United States Soybean Disease Loss Estimates for 2015

Allen, T.W.[1], Bradley, C.A. [2], Damicone, J.P.[3], Dufault, N.S.[4], Faske, T.R.[5], Hollier, C.A.[6], Isakeit, T.[7], Kemerait, R.C.[8], Kleczewski, N.M.[9], Koenning, S.R.[10], Mehl, H.L.[11], Mueller, J.D.[12], Overstreet, C.[6], Price, P.P.[13], Sikora, E.J.[14], Spurlock, T.N.[15], and Young, H.[16]

[1]Mississippi State University, Stoneville, MS; [2]University of Kentucky, Princeton, KY; [3]Oklahoma State University, Stillwater, OK; [4]University of Florida, Gainesville, FL; [5]University of Arkansas, Lonoke, AR; [6]Louisiana State University, Baton Rouge, LA; [7]Texas A&M University, College Station, TX; [8]University of Georgia, Tifton, GA; [9]University of Delaware, Newark, DE; [10]North Carolina State University, Raleigh, NC; [11]Virginia Tech, Suffolk, VA; [12]Clemson University, Blackville, SC; [13]Louisiana State University, Winnsboro, LA; [14]Auburn University, Auburn, AL; [15]University of Arkansas, Monticello, AR [16]University of Tennessee, Jackson, TN

Since 1974, soybean disease loss estimates for the southern United States have been published in the annual proceedings of the Southern Soybean Disease Workers (SSDW). Summaries of the results from between 1977 and 2010 have been published in numerous refereed scientific journals (6,8-11,13-20). The most recent disease loss estimates from 2010 to 2014 have been published annually in the SSDW proceedings (1-5,7). In addition, a website through the University of Illinois Extension Service is available and summarizes the yield loss estimates from both the northern and southern U.S. from 1996 through 2014. The website can be accessed at:

http://extension.cropsci.illinois.edu/fieldcrops/diseases/yield_reductions.php

Various methods were used to obtain the disease losses, and most individuals relied on more than one. The methods employed included: field surveys, plant disease diagnostic clinic samples, variety trials, and questionnaires to Cooperative Extension staff, research plots, grower demonstrations, private crop consultant reports, foliar fungicide trials, sentinel plot data, variety trial ratings, and "pure guess". In the case where individuals have retired, another individual was contacted to aid in continuing the disease loss estimates project. The production figures for each state were collected from the USDA/NASS website in mid-January 2016. Production losses were based on estimates of yield in the absence of disease. The formula used to derive production losses was: potential production without disease loss = actual production ¸ (1-percent loss) (decimal fraction). Rounding errors may occur in the tables provided below, specifically Table 2 and 3, due to the presence of "trace" estimates of disease which were estimated to be approximately 1×10^{-9}. Total losses in the form of percent disease loss by state and total losses in millions of bushels were determined by averaging the loss by state with the inclusion of the trace estimates.

Soybean acreage in the sixteen southern states covered in this report in 2015 decreased compared to that reported in 2014 by 4.4% (1). Eleven states reported a yield reduction between the 2015 and 2014 season. The 2015 average per acre soybean yield was 38.9 bushels per acre, an 11% decrease in average yield compared to the 2014 average yield (43.7 bu/A). In 2015, more than 832 million bushels were harvested from 19.6 million acres from 16 southern states accounting for a 13.6% reduction in the total harvest. The 2015 total acres harvested, average yield in bushels per acre, and total production in each state are presented in Table 1. Percentage loss estimates from each state are specific as to causal organism or the common name of the disease (Table 2). The total average percent disease loss for 2015 was 7.7% which accounted for a 5.6% decrease in percent disease loss compared to that reported for 2014 (8.16%). In terms of the disease losses in millions of bushels, the 2015 disease losses accounted for 88.98 million bushels in lost potential production, or a 21.4% decrease over the losses incurred during the 2014 production season (113.2 million bushels).

Table 1. Soybean production in 16 southern states in 2015.

State	Acres	Bu/Acre	Yield in Bu (1,000's)
Alabama	490	41	20,090
Arkansas	3,170	49	155,330
Delaware	173	40	6,920
Florida	31	38	1,178
Georgia	315	43	13,545
Kentucky	1,810	49	88,690
Louisiana	1,395	41	57,195
Maryland	515	40	20,600
Mississippi	2,270	46	104,420
Missouri	4,480	40.5	181,440
North Carolina	1,790	32	57,280
Oklahoma	375	31	11,625
South Carolina	405	26	10,530
Tennessee	1,720	46	79,120
Texas	115	26	2,990
Virginia	620	34.5	21,390
TOTAL	19,674		832,343
		Avg. 38.9	

Acknowledgments

Funding was obtained from the United Soybean Board to aid in compiling the southern disease loss estimates for the SSDW Disease Loss Estimate Committee.

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Table 2. Estimated percentage loss of soybean yield due to diseases from 16 southern states during 2015.

	% yield suppression by state																
Disease	**AL[a]**	**AR**	**DE**	**FL**	**GA**	**KY**	**LA**	**MD**	**MS**	**MO**	**NC**	**OK**	**SC**	**TN**	**TX**	**VA**	**AVG**
Anthracnose	0.01	0.10	0.00	0.10	0.25	0.05	0.25	0.00	0.02	0.00	0.90	0.10	0.01	0.50	0.00	0.50	**0.19**
Bacterial diseases	0.00	0.01	0.00	0.10	0.00	0.00	0.10	0.00	0.01	0.00	0.20	0.10	Tr	0.00	0.00	0.01	**0.04**
Brown stem rot	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	**0.00**
Cercospora leaf blight	0.10	0.10	0.50	Tr	Tr	0.10	2.00	0.50	1.60	0.25	0.20	0.05	0.75	0.05	0.00	0.50	**0.41**
Charcoal rot	2.50	2.20	0.01	Tr	Tr	0.80	0.50	0.01	2.00	0.00	0.00	2.00	0.05	1.00	0.10	0.10	**0.67**
Diaporthe/Phomopsis complex (seed rot)	0.10	0.05	0.01	0.00	0.00	0.05	0.00	0.01	0.01	0.00	1.50	0.20	5.00	1.00	0.00	1.00	**0.56**
Downy mildew	0.00	0.00	0.00	0.10	0.00	0.01	0.00	0.00	0.00	0.00	Tr	0.00	0.00	0.00	0.00	0.01	**0.01**
Frogeye leaf spot	1.00	1.50	0.01	Tr	Tr	1.00	2.50	0.01	1.75	0.50	0.40	0.05	0.01	2.60	0.00	1.00	**0.71**
Fusarium wilt and root rot	Tr	0.03	0.10	0.00	Tr	0.00	0.00	0.10	0.00	0.05	0.00	0.00	Tr	0.01	0.00	0.10	**0.02**
Other diseases[c]	0.00	0.00	0.00	0.00	0.00	0.00	1.50	0.00	2.10	0.00	0.03	0.00	0.02	0.00	0.10	0.00	**0.23**
Phytophthora root and stem rot	0.00	0.01	0.00	0.10	0.00	0.40	0.00	0.00	0.00	2.00	0.60	0.10	0.01	0.00	0.00	0.10	**0.21**
Pod and stem blight	Tr	0.08	0.00	0.00	1.00	0.10	0.50	0.00	0.01	0.50	1.00	0.15	1.00	0.00	0.00	0.50	**0.30**
Purple seed stain	0.10	0.01	0.00	0.00	Tr	0.01	0.10	0.00	0.01	0.00	0.10	0.05	0.50	0.05	0.00	0.10	**0.06**
Reniform nematode	0.50	0.00	0.00	0.00	0.25	0.00	2.00	0.00	1.75	0.00	0.00	0.00	0.50	0.01	0.00	0.00	**0.31**
Root-knot nematode	0.50	3.60	1.00	0.10	3.00	0.00	2.00	1.00	2.15	0.10	0.70	0.25	1.00	0.00	0.00	1.00	**0.98**
Soybean cyst nematode	0.25	0.80	2.00	0.00	0.10	2.50	0.00	1.50	0.70	2.00	2.00	2.00	1.00	2.50	0.00	3.00	**1.27**
Other nematodes[d]	0.00	0.00	0.00	0.00	0.25	0.00	0.10	0.00	0.00	0.00	0.25	0.00	1.00	0.00	0.00	0.50	**0.13**
Rhizoctonia aerial blight	0.00	0.01	0.00	0.25	0.00	0.00	0.50	0.00	0.75	0.00	0.01	0.00	Tr	0.00	0.00	0.00	**0.10**
Sclerotinia stem rot (white mold – *Sclerotinia sclerotiorum*)	Tr	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
Seedling diseases	1.00	0.20	0.03	0.10	0.10	1.00	0.25	0.03	0.50	3.00	0.10	0.20	0.03	1.50	0.10	0.50	**0.53**
Septoria brown spot	0.00	0.10	0.02	0.50	0.00	0.50	0.25	0.02	1.25	0.00	0.05	0.25	0.15	1.25	0.00	0.10	**0.27**
Southern blight	Tr	0.05	0.01	0.00	0.25	0.00	0.10	0.01	0.01	0.00	0.30	0.05	0.04	0.00	0.00	0.00	**0.05**
Soybean rust	0.10	0.00	0.00	0.75	Tr	0.00	0.10	0.00	0.05	0.00	0.00	0.00	0.02	0.00	0.10	0.00	**0.07**
Stem canker	1.00	0.10	0.00	0.00	0.00	0.10	0.10	0.00	0.35	0.00	0.07	0.05	Tr	0.25	0.00	1.00	**0.18**
Sudden death syndrome	Tr	0.10	0.00	0.00	0.00	0.30	0.10	0.00	0.01	0.50	0.07	0.10	0.01	0.50	0.00	0.01	**0.24**
Virus diseases[e]	0.50	0.05	0.50	0.00	0.00	0.20	0.00	0.50	0.05	0.00	0.20	0.10	0.02	0.00	0.00	0.10	**0.14**
Total % disease	**7.56**	**9.10**	**4.20**	**2.10**	**5.20**	**7.12**	**12.95**	**3.70**	**15.04**	**8.90**	**8.68**	**5.80**	**11.12**	**11.17**	**0.40**	**10.14**	**7.70**

[a]Rounding errors may exist
[b]Tr = trace (0.000000001)
[c]Other diseases listed included: Phymatotrichopsis root rot (TX), Pythium root rot (DE, MD), red crown rot (LA, MS), taproot decline (AR, LA, MS), target spot (AR, MS, NC, SC).
[d]Other nematodes listed included: Columbia lance nematode (NC, SC), sting nematode (GA, NC, VA), stubby root nematode (VA).
[e]Virus diseases listed included: *Bean pod mottle virus* (AL, AR, KY, MS, NC), *Soybean mosaic virus* (AL, AR, MS, NC), *Soybean vein necrosis virus* (AL, AR, DE, KY, MD, MS, VA), *Tobacco ringspot virus* (KY, NC).

Table 3. Estimated suppression of soybean yield (Millions of Bushels) as a result of disease during 2015.

	yield suppression by state (millions of bushels)																
Disease	**AL[a]**	**AR**	**DE**	**FL**	**GA**	**KY**	**LA**	**MD**	**MS**	**MO**	**NC**	**OK**	**SC**	**TN**	**TX**	**VA**	**TOTAL**
Anthracnose	0.00	0.17	0.00	0.00	0.04	0.05	0.16	0.00	0.00	0.00	0.15	0.01	0.00	0.42	0.00	0.06	**1.07**
Bacterial diseases	0.00	0.02	0.00	0.00	0.00	0.00	0.07	0.00	0.01	0.00	0.15	0.01	0.00	0.00	0.00	0.00	**0.26**
Brown stem rot	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	**0.03**
Cercospora leaf blight	0.02	0.17	0.04	0.00	0.00	0.10	1.31	0.11	1.92	0.45	0.15	0.01	0.08	0.04	0.00	0.28	**4.69**
Charcoal rot	0.54	3.76	0.00	0.00	0.00	0.76	0.33	0.00	2.40	0.00	0.08	0.27	0.04	0.85	0.00	0.00	**9.04**
Diaporthe/Phomopsis complex (seed rot)	0.02	0.09	0.00	0.00	0.00	0.05	0.00	0.00	0.01	0.00	0.38	0.01	0.00	0.85	0.00	0.03	**1.44**
Downy mildew	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
Frogeye leaf spot	0.22	2.56	0.00	0.00	0.00	0.95	1.64	0.00	1.98	0.91	0.57	0.01	0.00	2.46	0.00	0.57	**11.89**
Fusarium wilt and root rot	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.09	0.00	0.00	0.00	0.01	0.00	0.00	**0.18**
Other diseases	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	2.52	0.00	0.92	0.00	0.01	0.00	0.00	0.00	**4.44**
Phytophthora root and stem rot	0.00	0.02	0.00	0.00	0.00	0.38	0.00	0.00	0.00	3.63	0.46	0.01	0.00	0.00	0.00	0.00	**4.50**
Pod and stem blight	0.00	0.14	0.00	0.00	0.14	0.10	0.33	0.00	0.00	0.91	0.77	0.01	0.00	0.00	0.00	0.09	**2.47**
Purple seed stain	0.02	0.02	0.00	0.00	0.00	0.01	0.07	0.00	0.00	0.00	0.08	0.01	0.00	0.04	0.01	0.03	**0.28**
Reniform nematode	0.11	0.00	0.00	0.00	0.04	0.00	1.31	0.00	2.10	0.00	0.08	0.00	0.17	0.01	0.00	0.00	**3.81**
Root-knot nematode	0.11	6.15	0.07	0.00	0.43	0.00	1.31	0.21	1.98	0.18	0.61	0.03	0.33	0.00	0.00	0.28	**11.71**
Soybean cyst nematode	0.05	1.37	0.14	0.00	0.01	2.39	0.00	0.32	0.60	3.63	1.53	0.22	0.17	2.12	0.00	0.85	**13.41**
Other nematodes	0.00	0.00	0.00	0.00	0.04	0.00	0.07	0.00	0.00	0.00	0.38	0.01	0.42	0.00	0.00	0.00	**0.91**
Rhizoctonia aerial blight	0.00	0.02	0.00	0.00	0.00	0.00	0.33	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.65**
Sclerotinia stem rot (white mold – *Sclerotinia sclerotiorum*)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
Seedling diseases	0.22	0.34	0.00	0.00	0.01	0.95	0.16	0.01	0.60	5.44	0.05	0.02	0.02	1.27	0.01	0.03	**9.14**
Septoria brown spot	0.00	0.17	0.00	0.01	0.00	0.48	0.16	0.00	0.72	0.00	0.11	0.02	0.01	1.06	0.00	0.03	**2.78**
Southern blight	0.00	0.09	0.00	0.00	0.04	0.00	0.07	0.00	0.01	0.00	0.31	0.01	0.00	0.00	0.01	0.00	**0.52**
Soybean rust	0.02	0.00	0.00	0.01	0.00	0.00	0.07	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.16**
Stem canker	0.22	0.17	0.00	0.00	0.00	0.10	0.07	0.00	0.42	0.00	0.00	0.00	0.00	0.08	0.00	0.18	**1.20**
Sudden death syndrome	0.00	0.17	0.00	0.00	0.00	0.29	0.07	0.00	0.01	0.91	0.38	0.02	0.00	1.48	0.00	0.28	**3.62**
Virus diseases	0.11	0.09	0.00	0.00	0.00	0.19	0.00	0.11	0.06	0.00	0.15	0.01	0.00	0.00	0.00	0.00	**0.76**
Total disease loss	**1.67**	**15.55**	**0.30**	**0.03**	**0.74**	**6.80**	**8.51**	**0.79**	**15.73**	**16.15**	**7.31**	**0.69**	**1.27**	**10.70**	**0.03**	**2.71**	**88.98**

[a]Rounding errors may exist
[b]Tr = trace (0.000000001)
[c]Other diseases listed included: Phymatotrichopsis root rot (TX), Pythium root rot (DE, MD), red crown rot (LA, MS), taproot decline (AR, LA, MS), target spot (AR, MS, NC, SC).
[d]Other nematodes listed included: Columbia lance nematode (NC, SC), sting nematode (GA, NC, VA), stubby root nematode (VA).
[e]Virus diseases listed included: *Bean pod mottle virus* (AL, AR, KY, MS, NC), *Soybean mosaic virus* (AL, AR, MS, NC), *Soybean vein necrosis virus* (AL, AR, DE, KY, MD, MS, VA), *Tobacco ringspot virus* (KY, NC).

Integrated management of sudden death syndrome

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Sudden death syndrome (SDS) is one of the most recognizable diseases in most of the soybean-producing areas. SDS is a soilborne fungal disease with two phases — a root rot phase and a leaf scorch phase. The foundation of SDS management is to plant SDS-resistant soybean cultivars. However, in years when environmental conditions are very conducive for SDS development, host resistance alone may not provide adequate control. We investigated management options that could help resistant cultivars be as effective as possible, even in years favorable for SDS. Management strategies studied were crop rotation, tillage, planting date, and seed treatments. Of these, crop rotation away from corn and soybean and effective seed treatments were the most promising management strategies to help resistant cultivars for SDS management.

Field Screening for Stem Canker: An Outdated Exercise or a Necessary Service for Soybean Growers

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Southern stem canker (then *Diaporthe phaseolorum* var *caulivora*) was first reported in Arkansas in 1984. Between 1985 and 1989 the disease became increasingly widespread statewide causing significant soybean crop losses in many areas of the state. In late 1989, following particularly devastating and widespread soybean losses due to this disease, the Arkansas Soybean Promotion Board requested that the University of Arkansas, Division of Arkansas develop a soybean cultivar screening program to identify commercial cultivars and advanced breeding lines with resistance to the disease. The Arkansas Stem Canker Cultivar Screen was initiated in 1990, and has been conducted continuously on an annual basis since that time. Although over the years this program has expanded to include various other pathogens under the title "Arkansas Soybean Cultivar Disease Screening Program, stem canker has continued to be a part. Techniques and procedures as well as personnel and philosophies have changed during the 26 year history of this program. Today, an array of cultivars with high levels of resistance to southern stem canker (now *Diaporthe phaseolorum* var *meridionalis*) are available to growers in Arkansas and the other southern states. Screening data from about 260 cultivars and lines each year since 2010 indicate that about 90% of the cultivars that are enrolled in the Arkansas Official Variety Trial are highly resistant to stem canker. The history of the Arkansas stem canker screen and the outlook for future screening efforts will be discussed.

Nematode Associated Diseases in Soybean

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The soybean cyst (SCN), root-knot, and reniform nematodes are serious pests of soybean in the southern U.S. Losses from these nematodes were estimated at 32.3 million bushels in 2014. Both soybean cyst and reniform nematodes have rapidly spread during the past 50 years. Although soybean cyst nematode has been found in all the southern states, major losses currently occur only in those further north including Kentucky, Missouri, North Carolina, Delaware, Oklahoma, and Virginia. SCN was once widely distributed and caused substantial losses in some states such as Louisiana. Incidence and damage from this nematode in Louisiana has drastically declined over time relegating it to an insignificant status. Symptoms associated with nematodes have changed during the past 40 years. Soybean cyst and reniform nematode caused serious stunting of soybean as well as associated chlorosis in the past but plants now are often asymptomatic, yet still sustaining significant losses in yield. All three of these major nematodes of soybean are known to have different races or virulent phenotypes that complicate the development of management plans. The use of resistant cultivars has resulted in quick changes in pathogenicity for SCN, allowing the development of new races to develop. However, almost all of the current cultivars of soybean have resistance coming from a single source, PI 88788, which limits the effectiveness of resistance in some locations. Reniform nematode shows considerable variability in pathogenicity and reproduction on cotton and soybean but has not had a defined classification system developed yet for the U.S. that can be used with soybean. Changes have occurred over time with management including higher yielding and more tolerant/resistant cultivars, better use of incorporating resistance in rotations, and changes in nematicides and methods of application.

Characterization of taproot decline in southern soybean

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A malady of soybean has been observed in the ArkLaMiss soybean production system for the last decade. Foliar chlorosis and necrotic stippling, and stunting of soybean may appear as early as vegetative growth stages (V6-V8). However, chlorosis is evident at full bloom (R2), but is most evident during pod fill. A distinct symptom of this disease is mummified taproots which cause the soybean plant to break at the soil line. Black stroma and white mycelium can be observed at the base of the plant, tap, and lateral roots. Fruiting structures termed 'dead man's fingers' are common at the base of dead plants as well as emerging from crop residue. Inside the dried taproot, white mycelia colonize the pith and serve as a source for fungal isolation.

Spatial analysis of infection counts conducted in Arkansas soybean fields concluded chlorotic plant symptoms associated with the disease were spatially correlated to dead plants. Clustered symptomatic plants in the field coupled with the monocyclic disease pattern corresponds to the behavior of a soilborne pathogen. The recurrence of the disease in continuous soybean is most likely due to inoculum produced in the previous crop. Infested residue from soybean stubble and buried roots likely serve as primary inoculum for the subsequent season. On-farm Louisiana studies in 2014 determined disease incidence was significantly greater in continuous soybean compared to rotation with corn. Also in 2014, differences in susceptibility to the disease were observed in soybean variety trials. Disease incidence was prevalent in 2014 throughout the Tri-state area due most likely to cooler temperatures and above normal precipitation. A yield loss trial, conducted in one MS soybean field, determined that in severe situations yield losses on the order of 18% could be observed.

A fungus is consistently isolated from infected soybean in ArkLaMiss. The colonies are initially white, circular and flat, with undulate margins. Mature colonies develop alternating black to grayish-black rings. Stroma and fruiting structures may develop in mature cultures. Plant symptoms have been replicated in greenhouse and growth chamber inoculation studies; however, re-isolation of the fungus has not been achieved. Work continues at each university to attain successful completion of Koch's postulates. Due to the unique mummified rot of affected soybeans, we propose the name taproot decline of soybean.

Frontline tactics to manage Phytophthora Stem and Root Rot

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Phytophthora stem and root rot of soybean is caused by the oomycete *Phytophthora sojae.* Symptoms of the disease include a characteristic brown lesion that extends up the soybean stem. Soybean is susceptible to infection by *P. sojae* throughout the growing season and infection occurs when soils are saturated. In the past decade, estimated losses in the U.S. due to this disease amount to over 330 million bushels.

Soybean and *P. sojae* have a gene-for gene relationship. Resistance (*Rps*) genes in the host recognize specific genes (*Avr*) in the pathogen. Isolates of *P. sojae* are classified into pathotypes (formally known as races) based on their ability to cause disease on soybean lines that each contain specific *Rps* gene. Studies that have evaluated the diversity of *P. sojae* over the past half-century have shown that the pathogen is becoming increasingly complex. It is not uncommon now to recover isolates of the pathogen that are virulent on multiple *Rps* genes.

There are various tactics that farmers can use to reduce PSRR and protect yield but there is no silver bullet. An integrated approach of resistant varieties, seed treatments and cultural practices is recommended.

Both single gene (*Rps*) and multigenic resistances to *P. sojae* occur in soybean and both should also be considered as tactics to manage PSRR. A soybean variety with a single *Rps* gene may be completely resistant to infection by the pathogen, or may be susceptible and die when infected – it depends on the pathotype of the isolate infecting the plant. Because the population of *P. sojae* in a field may contain numerous pathotypes, planting a soybean variety with a specific *Rps* gene is not always the answer to reducing yield loss due to PSRR. Partial resistance or field tolerance is multigenic, and while this type of resistance does not prevent infection, reduced colonization does enable the plant to survive and yield.

Seed treatments may reduce the risk of early season stand loss from PSRR, but farmers should be aware their protection is short-lived. Moreover, higher rates of fungicide are often required to protect germinating seedlings.

Cultural practices that improve soil drainage can also reduce the occurrence of PSRR and associated yield losses.

Causative Agents for the Green Stem Disorder of Soybeans in Louisiana

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Soybean (*Glycine max*) is a major crop in the U.S., Brazil and Argentina. An emerging problem in soybean production is the green stem disorder (GS), which is prevalent especially in Brazil and throughout the U.S. The disorder causes stems to remain green and supple after pods and seeds have matured, dried, and are ready for harvest. GS results in a loss of grain quality, higher input costs, and more frequent problems in harvesting. Since its description, numerous factors have been implicated as causes of GS, both abiotic and biotic. This study aimed to determine which of these factors result in higher GS incidence in Louisiana. Field experiments were conducted in 2014 and 2015 in which glyphosate, foliar applied nitrogen, water availability, two fungicides, and insecticides and their interactions were evaluated. Percentages of GS plants in each treatment were recorded when control plots were at harvest maturity (R8). Excess water and fungicides caused an increase in GS incidence during both years. Glyphosate and foliar nitrogen application were linked to increases in GS incidence in 2014 and 2015, respectively. Interactions among these factors also will be presented.

Solubilization of Cercosporin and its Use for Reproducing Symptoms of Cercospora Leaf Blight of Soybean

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Considered one of the most devastating soybean diseases in the Mid-South of the United States, Cercospora leaf blight (CLB) was recently found to be caused by *Cercospora* cf. *flagellaris* in the United States, Brazil and Argentina. Resistant varieties are not available in Louisiana, and fungicide resistance is widespread. The pathogen produces a toxin, cercosporin (CCP), that induces the production of reactive oxygen species leading to peroxidation of membrane lipids, nutrient leakage, and consequently death of cells. Typical symptoms of CLB include purple discoloration of leaves exposed to sunlight and leaf blight in later stages of the disease, which can lead to yield losses of 20% or more. Recent findings showed that *C. flagellaris* biomass is similar in symptomless and purple leaves, but blighted leaves had higher fungal biomass. In addition, CCP was not detected in symptomless leaves, but was detected in low (5µg/g fresh weight) and high (17µg/g fresh weight) concentrations in purple and blighted leaves, respectively. These findings suggested that CLB symptoms are more related to CCP concentrations than fungal biomass. In several attempts to reproduce CLB symptoms using pathogen mycelium under greenhouse settings, only blight symptom (but never purple) were obtained. Because the development of CLB symptoms was not closely related to pathogen biomass, the goal of this work was to attempt to reproduce blight and purple symptoms in leaves treated with CCP. In order to be taken up by plants and translocated to leaves, CCP, which is not water soluble, was solubilized by a natural solubilizing agent (SG) from stevia (*Stevia rebaudiana*; Asteraceae) leaves. By processing SG and CCP together using a solvent evaporation method, a water-soluble nanomicelle-CCP (NM-CCP) sample was obtained. Petioles with leaves (leaf cultures) from five-week-old soybean cultivar Pioneer 95Y61 were treated with solutions of 0, 1, 5, 15 and 40µg CCP per ml deionized water at pH 7.3 in NM-CCP. A vehicle treatment (SG) also was included. Each treatment had three replications. Leaf cultures were placed in a light chamber with 400-watt metal halide bulbs with a light intensity of 9,000 lux. Leaf symptoms were monitored at 6, 20, and 48 hours after inoculation (HAI). At 6 HAI, leaf cultures treated with 40µg NM-CCP showed reddish veins but no necrotic lesions. The control, vehicle, 1 µg, and 5µg NM-CCP treatments did not show any symptoms, whereas the 15µg treatment showed a light red discoloration in the veins. At 20 HAI, symptoms in the 40µg treatment progressed to vein necrosis; leaves in the 5 and 15 µg treatments showed red veins; and leaves in the remainder of the treatments were unchanged. At 48 HAI, the 5µg treatment caused dark red to purple pinpoints in the leaf blade; the 15µg treatment produced a dark discoloration of the veins; and the 40µg treatment resulted in a severe necrotic area adjacent to veins. In summary, we reproduced typical CLB symptoms, including purple discoloration and blight, following treatments with solubilized CCP.

Effect of droplet size on foliar fungicide application in soybean

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Introduction of new herbicide tolerant soybean require growers to use drift-reducing nozzle technology that produces very-coarse to ultra-coarse droplets. Due to the frequency of tank-mixed pesticide applications, cost associated with utilizing drift-reduction technology, and the reduction in coverage from applying solutions with coarser droplets, growers making applications with these type nozzles could affect other foliar pesticides. Soybean infected with *Cercospora sojina*, the causal agent of frogeye leaf spot (FLS), could be negatively impacted by changes in application techniques. Field experiments were conducted in Jackson and Milan, Tennessee in 2014-2015 to evaluate the influence of droplet size on foliar fungicide efficacy, residual, and coverage in soybean infected with FLS. A premix of azoxystrobin and difenoconazole was applied using a 141 L ha^{-1} carrier volume through 3 spray nozzles with varying droplet spectra. Spray nozzles included: Teejet XR11002 (VMD = 247 µm) considered an industry standard, Teejet TTI11002 (839 VMD = µm) labeled for use in future herbicide-tolerant soybean, and Teejet AIXR11002 (VMD = 505 µm) representing the finest droplet spectra that could potentially be labeled for use. Atomization analysis were conducted using field application parameters and fungicide solutions via laser diffraction to determine VMD. Visual control ratings were taken 21 days after application (DAA). Leaf tissue samples were collected 0, 2, 7 and 14 DAA for azoxystrobin quantification analyzed using high-powered liquid chromatography/mass spectrometry. Soybean plots were harvested for yield comparison. FLS isolates were collected from each location and characterized as resistant or sensitive via germination assay and real-time qPCR. Response curves of FLS isolates to azoxystrobin were developed to analyze levels of control at each leaf sampling date in comparison to sample quantification. Coverage analysis was also conducted by adding PTSA tracer dye to fungicide solutions, arranging jars in the upper and lower canopy, and analyzing solutions captured using a fluorometer. No significant differences were found amongst treatments in regards to visual control ratings, soybean yield, and azoxystrobin concentration at 0, 2, and 7 DAA, however, the TTI11002 was the only treatment significantly greater than the untreated 14 DAA. Results suggest that the reduction in coverage from drift-reduction nozzle technology may not negatively affect azoxystrobin/difenoconazole efficacy on FLS in soybean. Utilizing ultra coarse droplets may also increase the residual of azoxystrobin in soybean.

Virulence Assessment of QoI-Sensitive and -Resistant Isolates of *Cercospora sojina*, the Causal Agent of Frogeye Leaf Spot in Soybean

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Frogeye leaf spot (FLS), a foliar disease of soybean, is caused by the fungus *Cercospora sojina* Hara. Quinone outside inhibitor (QoI; strobilurin) fungicides, such as azoxystrobin and pyraclostrobin, have been the industry standard for effective FLS management. However recently, reduced fungicide efficacy has been observed by growers following stand-alone QoI applications in the presence of FLS in FLS-susceptible cultivars in the Mississippi soybean production system. The threat of fungicide resistance following the detection of QoI-resistant *C. sojina* isolates in other Mid-south states resulted in a survey of soybean producing counties in MS during 2013 and 2014. This research demonstrated that the majority (>90%) of *C. sojina* isolates collected from 76 MS counties, were resistant to the QoI fungicides following the detection of the G143A substitution using PCR-RFLP-based analysis. Due to the shift from QoI-sensitive to -resistant isolates causing FLS in MS, the fitness of resistant isolates warrants investigation. Loss of virulence may be expressed as a fitness cost due to the development of fungicide resistance. Therefore a virulence assessment of QoI-resistant isolates collected in MS was conducted and compared to QoI-sensitive isolates and their ability to cause FLS.

Twenty-four *C. sojina* isolates, 11 QoI-sensitive and 13 QoI-resistant, were randomly selected for the virulence assessment from the isolate repository collected during 2013 and 2014. Cultivar Dyna-Gro 37RY47 soybean seedlings were inoculated with each QoI-sensitive or -resistant isolate at growth stage V1-V2. Conidial suspensions were adjusted to 1×10^5 conidia/ml for a final volume of 30 ml and sprayed on the seedlings with a CO_2-pressurized sprayer using a single cone nozzle. Inoculated seedlings were incubated in a humidity chamber for four days before being transferred to an open bench top with supplemental humidity. Disease severity was assessed 21 days post-inoculation using a 0 to 10 rating scale where 0 indicates no disease and 10 is complete blighting. The experiment was conducted in the greenhouse and was arranged in a completely randomized design with three replicates for each isolate and a water control. Data were subjected to analysis of variance and means were separated with Fisher's protected LSD ($\alpha = 0.05$).

QoI-sensitive and -resistant *C. sojina* isolates were pathogenic to soybean seedlings. Disease severity was significantly different among *C. sojina* isolates within sensitivity groups. Despite disease severity differences within sensitivity groups, no differences in observable severity were noted between QoI-sensitive and -resistant isolates. The QoI-resistant isolates of *C. sojina* appear to be equally as virulent as the sensitive isolates and appear stable in their ability to cause FLS. Alternative field management measures may need to be explored for increased fungicide efficacy when FLS-susceptible cultivars are planted in the Mississippi soybean production system.

Application thresholds in controlling *Cercospora sojina*, the causal agent of frogeye leaf spot

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Soybean production has dramatically increased over the last decade throughout much of the Southeastern and Midwestern United States. Numerous diseases have impacted production, and many strategies have been implemented for their control. Foliar diseases have primarily been controlled via variety selection and foliar fungicide applications. One such disease is frogeye leaf spot (FLS), caused by the fungus *Cercospora sojina.* Damage such as reduced photosynthetic area, loss of leaves, and pod necrosis has caused yield losses that have approached as much as 60%. Sequential fungicide applications of an azoxystrobin/difenoconazole premix were used to determine how best to control the FLS while and determine if yields would be directly impacted by disease control. The experiment was conducted during the field seasons of 2014-2015 at a total of 5 locations. Two varieties were used, one susceptible to FLS and one resistant to FLS. A randomized complete block design was used with 10ft by 30ft 4 row plots and 4 replications. Applications were initiated at the beginning of reproduction and sequential applications were applied at 2-3 week intervals. Disease ratings were taken during the growing season using a percent area effected rating system. Yield was collected on the center two rows of each plot. Analyses indicates that variety selection and disease pressure dramatically influences the benefits of sequential applications in relationship to yield benefits and disease control. Future management recommendations should consider variety response and on site disease pressure. While sequential applications may not be warranted in all situations they may be important in protecting and maintaining crop production goals.

Assessment of ILeVO for management of root-knot nematodes in soybean

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Fluopyram (ILeVO) was registered as a soybean seed treatment in 2014 to manage soilborne fungal pathogens and plant-parasitic nematodes. Few studies have investigated the extent of soybean root protection by ILeVO against the root-knot nematode (RKN), *Meloidogyne* spp. The objectives of this study were to evaluate the efficacy of ILeVO for suppression of RKN in the field and developing root systems at high, moderate, and low RKN populations in the greenhouse. Field trials were conducted using susceptible and moderately resistant soybean cultivars in 2014 and 2015 with Armor 53-R16, Delta Grow DG 4940, and Armor 55-R22, Delta Grow DG 4970, respectively. Cultivar selection changed between years to align with maturity group selection in the production field. Treatments consisted of ILeVO, fluopyram applied as an in-furrow (IF) spray, Avicta, Poncho/VOTiVO, ILeVO + Poncho/VOTiVO, and a non-treated control (NTC). Phytotoxicity was observed along the edge of cotyledons from ILeVO treated seed; however, this had no impact on soybean stand or vigor. There was a significant treatment x cultivar interaction in 2014 due to low gall counts among several treatments that were unrelated to a specific cultivar. In 2015, fewer (P = 0.10) galls were observed roots treated with Avicta and fluopyram IF than the NTC on DG 4940. On DG 4970, a lower (P = 0.10) percentage of galling was observed on soybean roots treated with ILeVO, Avicta, and Poncho/VOTiVO than the NTC. Nematode reproduction differed among treatments and soybean cultivars. No effect of seed treatment on nematode reproduction was observed on DG 4940 and 55-R22; however, on DG 4970 and 55-R16, fewer (P = 0.10) eggs were collected on roots treated with ILeVO + Poncho/VOTiVO, Avicta, and Poncho/VOTiVO than the NTC or fluopyram IF. Though yield was higher on both cultivars in 2014 than 2015, a greater (P = 0.10) yield was observed in both years with ILeVO than the NTC. Greenhouse trials were conducted to evaluate these treatments on DG 4970 at a high (1,000 egg/cc soil), moderate (100 eggs/cc soil) and low (10 eggs/cc soil) RKN damage levels. Treatments responded similarly regardless of damage levels. Inoculum level increased percent root galling on greenhouse root systems as eggs/cc soil increased from low to high. A lower (P = 0.10) root galling was observed on roots treated with Avicta, ILeVO, ILeVO + Poncho/VOTiVO, and fluopyram IF than the NTC or Poncho/VOTiVO. Nematode reproduction was reduced (P = 0.10) across damage levels by ILeVO, ILeVO + Poncho/VOTiVO, and fluopyram IF compared to the NTC and Poncho/VOTiVO. In these studies ILeVO provided early-season root protection against RKN that was similar in magnitude to that of other seed treatment nematicides such as Avicta and Poncho/VOTiVO.

Evaluating the physiological impacts of fungicide phytotoxicity in Mississippi soybean

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Frogeye leaf spot (FLS), caused by *Cercospora sojina* Hara, recently re-emerged as an important foliar disease not only within the Mississippi soybean production system, but throughout the southern U.S. The renewed interest in FLS is the result of the G143A substitution detected within the fungal population, which has resulted in the development of widespread resistance to the quinone outside inhibitor (QoI) fungicides in Mississippi. In turn, this has caused growers to utilize alternative management methods that include the planting of FLS-resistant soybean cultivars and application of alternative fungicide modes of action (MOA). One major drawback to applying these alternative MOAs can be the development of phytotoxicity on the soybean leaves. The phytotoxicity is oftentimes the result of systemic activity caused by a "curative" fungicide, such as following the application of a demethylation-inhibitor class fungicide (FRAC 3). However, phytotoxicity as a result of foliar fungicide application is not unique to the DMI fungicides and can also result following the application of some unknown MOA products (such as dodine, FRAC U 12) as well as methyl benzimidazole carbamates (such as Topsin FRAC 1). Field trials were conducted in 2015 to assess fungicide efficacy on QoI-resistant FLS and the resulting phytotoxicity on a FLS-susceptible cultivar. The candidate fungicides chosen have previously been observed to produce moderate to severe phytotoxicity regardless of growth stage at application timing. The fungicides included dodine (1.5 pint/A of Elast), prothioconazole (3 fl oz/A of Proline), tebuconazole (4 fl oz/A of Monsoon), and trifloxystrobin + prothioconazole (4 fl oz/A of Stratego YLD). Three foliar nutrient products were selected to be tank-mixed with the various fungicide MOAs in an attempt to reduce observable phytotoxicity. The treatments included applications of each product were made as a separate application and tank-mixed with the foliar nutrient. Applications were made at the R3 growth stage in 15gallons/A of water with an adjuvant (0.25% NIS v/v as Induce). Visual assessments of FLS and phytotoxicity were made pre- and several times post-application. A 2 ft. section of plants from one of the middle two rows was hand-harvested at physiological maturity (R8). Plant heights, number of pods, and number of nodes were recorded to determine the physiological effects of phytotoxicity. Analyzed data revealed that certain MOAs significantly reduced FLS severity compared to the non-treated. In addition, prothioconazole (as Proline) applied at 21.92 ml/ha resulted in significantly greater phytotoxicity than the non-treated, a difference of 29.4%. Although the analysis revealed a significant increase in phytotoxicity, the resulting yield proved to be greater than the non-treated. None of the foliar nutrient additives provided a significant reduction in observable phytotoxicity compared to the non-treated. In addition, plant heights and number of nodes per plant were not significantly different from the non-treated.

Benefit of secondary nutrition in reducing *Macrophomina phaseolina* colonization in Mississippi soybean

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Macrophomina phaseolina (Tassi) Goid (*Mp*) is a ubiquitous soilborne fungal pathogen that causes charcoal rot (CR) of soybean. Charcoal rot limits profitability on an annual basis comparable to other notable soilborne diseases such as sudden death syndrome and seedling disease. Throughout the southern United States, an estimated 400 million bushels have been lost to CR over the past 20 years. Typically, CR occurs in years with low moisture, and high temperatures although CR has been observed in fields regardless of environment and likely as a result of other stress-related situations. Current management options include selection of resistant varieties, crop rotation, and cultural practices that reduce stress on plants. *Macrophomina phaseolina* infects over 500 hosts including rotational hosts such as corn, cotton and grain sorghum, therefore, in fields with historically high populations of *Mp*, crop rotation is not always a management option. Although the presence of CR is dictated by a conducive environment, tissue colonization occurs regardless and additional management options are needed to reduce the effects of *Mp*. The objective of this research was to reduce *M. phaseolina* colonization of field grown soybean by supplementing with secondary nutrients, specifically calcium (Ca) and magnesium (Mg) at three different application timings (at plant, R1, at plant fb R1). In 2014 and 2015 non-irrigated, *Mp*-inoculated field trials were conducted in Stoneville, MS with a CR-susceptible and a CR-moderately-resistant cultivar. Treatment applications consisted of 1,000 lb/acre rates of Ca and Mg alone and in combination. Non-inoculated, non-treated plots served as the controls. Root samples were taken at the R3, R5, R7, and R8 growth stages by removing the entire root system from within each plot. Colony forming units (cfu) were used to quantify *Mp* colonization from ground root tissue and enumerating cfus in a *Mp* semi-selective medium. A steady increase in cfus occurred throughout the season in both cultivars regardless of treatment. In all cases, the greatest number of cfus were recovered from plants sampled at R8 rather than at prior growth stages. In some cases, applications of calcium and magnesium reduced colonization when compared to the control.

Detection of a mycoviruses from soybean rust and other biotrophic fungi using a practical method for the extraction of viral dsRNA

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In plants and fungi infected with RNA viruses, large (~1.0-20 kbp) double-stranded RNAs (dsRNAs) are found in the form of genomic segments of dsRNA viruses and replicative forms of single-stranded RNA viruses. These dsRNAs have been extracted from virus-infected plants and fungi infected with mycoviruses, and have been used for viral disease diagnosis, virus identification, and to clone and sequence plant and fungal RNA viruses. There is a need for dsRNA extraction methods from plants and fungi that are simple, fast, and economical. Over the past 50 years, many methods for large dsRNA extraction from virus-infected plant, animal, fungal, and bacterial tissues have been reported in the literature. The majority of them require a relatively large amount of tissue which can present a problem when only limited amounts are available. We have developed a practical dsRNA extraction method from plants and fungi that is fast, economic, versatile, and requires small amounts of tissue. This method was successfully used to obtain dsRNAs from plants infected with RNA viruses and fungi infected with mycoviruses. When the method was used to extract dsRNA from plant tissues infected with two types of biotrophic fungi (rusts and powdery mildews), dsRNAs that resemble those of mycoviruses were obtained. Of 15 plant species infected with rusts, 12 contained putative mycoviruses. Of 14 plant species infected with powdery mildews, nine contained putative mycoviruses. Although mycoviruses with dsRNA genome have been reported in rusts and powdery mildews, the relatively high number of them that yielded putative mycoviruses was surprising.

During the spring of 2015, leaves of kudzu (*Pueraria lobata*) infected with soybean rust (caused by *Phakopsora pachyrhizi*) were tested using the practical dsRNA extraction method. Similarly, during the fall of 2015, soybean plants in the field infected with soybean rust were tested. Results indicated that a putative mycovirus was infecting the fungus. After electrophoretic analyses, of dsRNA extracts from kudzu and soybean infected with rust, six distinct dsRNAs were obtained. These dsRNAs were similar to those reported for mycoviruses. The role of the putative mycovirus of *P. pachyrhizi* is not known. However, it is possible that it could affect the physiology of the fungus and therefore the disease it causes Current research in our laboratory aims at conducting a molecular characterization of the virus and to determine the occurrence in *P. pachyrhizi* from different geographical locations.

The practical method we developed is similar to other previously published dsRNA extraction methods; however it contains several improvements that increase the overall extraction efficiency and also the practicality of using dsRNA as reagent for plant and fungal virus detection, identification, and nucleic acid sequencing. Moreover, this method could be very helpful to researchers interested in virome analyses of phytobiomes.

Effect of *Macrophomina phaseolina* inoculation, irrigation and cultivar on soybean yield

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Soybean charcoal rot, caused by *M. phaseolina*, is often reported to be more severe under drought stress. To improve disease management it is necessary to understand the effect of irrigation regimes on soybean yield loss caused by charcoal rot. In the years of 2011 and 2013 four soybean cultivars, Osage, Ozark, Hutcheson, and R0158F, were grown in field plots with and without inoculum and either irrigated throughout the season (FSI), only irrigated until R5 (UR5I), or not irrigated (NI). In 2011, there were no significant differences in yield between the cultivars, but yields were significantly greater in non-inoculated than inoculated plots, 1848 and 1723kg ha^{-1}, respectively, and greater in FS than IUR5 or NI, 2770, 1487, and 1116 kg ha^{-1}, respectively. In 2013, a three-way interaction occurred between cultivar, irrigation and inoculation. In the non-inoculated plots, there were no differences between cultivars but yields in FS were much greater than UR5I and NI, 2498, 1648, and 1624 kg ha^{-1}, respectively. In the inoculated plots, Osage had the highest yield and Hutcheson had the lowest yield in the FS treatment, in UR5I, Ozark had the highest yield and Hutcheson had the lowest yield, but in NI, there were no differences between cultivars. Adding inoculum of *M. phaseolina* at planting had a significant effect on the plant resulting in lower yields each year. In 2013, this effect was greatest in FS.

Phenotypic Characterization of *C. sojina* Isolates Collected from Wide Geographical Areas

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Frogeye Leaf Spot (FLS), caused by *Cercospora sojina*, is common in the southern and southeastern soybean production region of USA. Recently, FLS has spread further northward into Midwestern soybean growing states, including Ohio, Indiana, Wisconsin, Michigan, Illinois, Iowa, and Missouri and southern states including Alabama, Mississippi, Louisiana, Arkansas, Tennessee, Kentucky, North Carolina, and Virginia. Unfortunately, strobilurin resistant isolates of *C. sojina* have been detected in Tennessee, Illinois, Kentucky, Alabama, Arkansas, Mississippi, Missouri, Indiana, North Carolina, Virginia and Louisiana since 2010 reducing FLS control options. Few soybean genes conferring resistance to FLS have been identified, among which the *Rcs3* gene from cultivar Davis has provided the most durable resistance against all known isolates of FLS in USA. However, this single gene resistance presents a risk as it is only a matter of time before it is defeated by *C. sojina.* Pathogen grouping of *C. sojina* isolates is needed to differentiate and identify germplasm with new genes. Frogeye isolates were collected from a wide geographic area around the United States. Over 83 isolates of *C. sojina* were screened on a set of 12 soybean differential cultivars. Host reactions were assessed at 14, 21 and 28 days after inoculation. A rating score from 0 to 9 was used for disease assessment. Cluster analysis was able to separate isolate x differential responses into multiple groups. Additionally, the number of lesions, lesion size, how far the lesion moved from the infection site and the total number of nodes the infection has moved on the plant were recorded. This approach allowed classification of the most virulent pathotype, that may infect all genotypes; pathotypes with moderate infection; the least virulent pathotype infecting only some but not all; and pathotypes with no infection on all genotypes. The degree of infection on the 12 differentials provided data that showed various levels of aggressiveness of isolates within pathotypes. This will be used as a tool for breeders and others to screen useful resistant genes against the various *C. sojina* pathotypes.

Effect of Flower and Pod Removal on Soybean Senescence and Comparison to Green Bean Syndrome

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In 2009, green bean syndrome (GBS) was observed in a number of soybean fields in northern Prairie County, AR. Affected areas ranged from small spots to entire fields. Almost all of the plants in the affected areas failed to mature. These plants had few if any mature pods, but had a proliferation of small pods at the nodes. There were suggestions that stink bugs may have been involved, but the symptoms also implied a virus or phytoplasma. Dr. Ioannis Tzanetakis at the University of Arkansas extensively sampled affected plants, but did not find any pathogen consistently associated with these symptoms. GBS did not appear in the same location each year, and there were no consistent cultivar associations nor associations with soil types, soil nutrition, drainage or chemical applications. It has been known for some time that soybean senescence can be delayed or prevented by reducing the number of pods, but it was not known if this type of injury would also result in the bud proliferation observed with GBS. To determine if GBS symptoms could be induced by removing flowers or pods, a test was conducted at the Arkansas Agricultural Research and Extension Center, Fayetteville, AR, in 2011 and 2012. Two late maturity group (MG) four cultivars (Asgrow 4907 and UA 4910(2011 only) or R05-4114 (2012 only) and two MG five cultivars (Asgrow 5605 and Osage) were planted in single row plots, 3 m long with rows 91 cm apart. There were four replications arranged in a randomized complete block design. The tests were planted on 13 June 2011 and 1 June 2012. At flowering, each row in each plot was thinned to 20 plants. At the R2, R4, R5 or R6 growth stage, all of the flowers or pods were removed from ten plants in one half of the row. The other half of the row served as a control. Beginning 12 September 2011 and 27 August 2012, the growths stages of treated and control plants were determined on a weekly basis. At the end of the season, the percent of green, yellow and defoliated plants and pod distribution were determined. Since resulting growth patterns within a treatment were similar each year, only the 2012 results will be presented. In addition, the responses of all cultivars to flower and pod removal were comparable, so results were compiled across cultivars. Growth stage and pod distribution data were determined as the number of replications that were at a specific category. Ratings began on 27 August when the control plants were at R4 and R5. Removal of flowers or pods delayed maturity in all treated plants. At the end of the season when the control plants reached R8, those with the flowers removed at R2 were at R7 and R8, those with the pods removed at R4, R5, or R6 were at growth stages R6 to R7, R5 to R6, and R3 to R5, respectively. Similarly, the percentage of green, yellow, and defoliate plants reflected the growth stages with all of the R2 treated plants being defoliated at the end of the season and most of the R5 and R6 treated plants being green. Pod distribution was similar to the control with the R2 treated plants, but pod density became less with each subsequent treatment. The R6 treated plants had only a few scattered pods on most plants. Bud proliferation was observed in 10-20% of the plants in 9 of 16 plots of the R4 treatment. Our results show that removal of flowers and pods at different growth stages not only delayed or prevented senescence, but reduced the total number of pods. Since these treatments resulted in limited bud proliferation, it is unlikely that GBS is caused simply by a one-time loss of flowers or pods.

Competition studies of QoI resistant and sensitive *Cercospora sojina* isolates, the causal agent of frogeye leaf spot

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Frogeye leaf spot (FLS), caused by *Cercospora sojina*, is a yearly foliar disease in Tennessee and causes substantial economic losses if not properly managed. Quinone outside inhibitor (QoI) fungicides are often used to manage FLS, but *C. sojina* isolates have developed resistance to this class of fungicides. Since the majority of soybean fields contain both QoI resistant and sensitive isolates, a better understanding of similarities and differences between such isolates in their biology and aggressiveness in causing FLS symptoms is needed. A greenhouse study was conducted at six different spore ratios of QoI resistant and sensitive isolates: 1:0, 9:1, 1:1, 1:9, 1:99, and 0:1. When the plants reached V4 growth stage, three treatments—no fungicide (water control), Quadris (azoxystrobin), and Quadris-Top (azoxystrobin plus difenoconazole) were applied to three replications where each replication consisted of four soybean plants (Blackhawk cultivar). Results of the greenhouse studies showed that: 1) Disease severity increased as the proportion of QoI-resistance in the inoculum increased, indicating that QoI-resistant isolates are more aggressive; 2) Quadris fungicide only inhibited FLS caused by QoI sensitive isolates and when the proportion of QoI resistance was less than or equal to 10%; 3) Quadris-Top fungicide provided more effective control of FLS, not only by reducing symptoms but also by delaying disease development. This work will contribute to understanding differences in QoI resistant and sensitive *C. sojina* isolates and identifying any differences in effective control measures for FLS.

Frequency and distribution of QoI resistant *Cercospora sojina* in Virginia

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Frogeye leaf spot (FLS), causal agent *Cercospora sojina*, is an important foliar disease of soybean. FLS can be managed using crop rotation, planting disease resistant soybean varieties, and applying fungicides during reproductive growth stages of the crop. Growers have become increasingly reliant on fungicides for management of foliar diseases in soybean, especially the quinone outside inhibitor (QoI) class of fungicides due to their broad-spectrum activity against many fungal pathogens. Recently, high frequencies of QoI-resistant *C. sojina* have been reported from the Mid-South region of the U.S., and in 2013 a QoI-resistant isolate was confirmed from North Carolina. In 2015, a survey was initiated to determine the frequency and distribution of QoI-resistant *C. sojina* in Virginia. FLS infected soybean leaves were collected from throughout the state, and QoI resistance was determined using a previously developed PCR assay that targets the mutation conferring resistance in a majority of FLS isolates (G143A mutation). Thus far, 113 isolates have been analyzed for samples collected from 26 fields in 7 counties where outbreaks of FLS occurred in 2015. The G143A mutation conferring QoI resistance was confirmed from isolates originating from 5 of the 7 counties and 10 of the 26 fields. In fields were QoI-resistant *C. sojina* was detected, frequencies of the mutation ranged from 20 to 100%. Currently, DNA is being isolated directly from an additional 48 leaf samples and the frequencies of the G143A mutation will be quantified using real-time PCR. Additional samples will be collected in 2016 to monitor for shifts in frequencies of QoI-resistant FLS associated with the Virginia soybean crop.

A Survey of Arkansas Soybean Nematodes

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Arkansas has produced roughly 1.3 million hectares of soybeans annually for the past five years. Many formerly monocultured cotton fields are now regularly rotated into soybean and corn. Historical data from surveys of Arkansas soybean fields conducted during the period of 1980-1986 indicate that the soybean cyst nematode was detected in an average of 61% of the fields surveyed while root-knot nematodes (*Meloidogyne* spp.) were detected in only 3% of the fields. The lesion nematode (*Pratylenchus* spp.), also reported to be an economic pest of soybean was present in 40% of the fields. Recently (2014 and 2015) a survey of soybean fields, sponsored by the Arkansas Soybean Promotion Board was completed. Soil samples, representing no more than 40 acres, were collected by farmers and crop consultants from soybean fields according to guidelines available in an on-line course that was developed specifically for this survey. Satisfactory completion of the course was mandatory in order to participate in the survey. Fields were sampled during the late summer and fall each year and submitted to the Arkansas Nematode Diagnostic Laboratory for assay.

A total of 1,635 fields were sampled during the two-year survey. The soybean cyst nematode was present in about 20% of the fields while root-knot and lesion nematodes were found in 22% and 20% of the fields, respectively. The reniform nematode, *Rotylenchulus reniformis*, which was not detected in the 1980-1986 survey was found in 8% of the fields studied. In addition to a general assay of nematodes, soybean cyst nematode populations were isolated from 12 arbitrarily-selected fields and characterized by race and HG-type in the greenhouse. Races 2 and 5 were the predominant races detected. When these populations were characterized by HG-type, types 1.2.5.7, 2.5.7, and 5.7 were most frequently found. Our results indicate that soybean cyst nematode incidence may be declining in Arkansas soybean fields, while root-knot nematode incidence is increasing. Lesion nematode incidence was similar to that found in the historical survey. The reniform nematode is now present in some soybean fields in Arkansas. Soybean cyst nematode biotype identities as characterized by either race or HG-type were relatively consistent across the populations tested.

Nuclear proteins controlling soybean rust resistance

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The soybean immune system is not well-characterized, and a better understanding of it is needed to develop resistant plants to the soybean rust fungus *Phakopsora pachyrhizi*. To find soybean proteins that contribute to resistance, the susceptible Williams 82 cultivar was compared to a resistant Williams 82 inbred isoline harboring the *Rpp1* rust-resistance gene. The goal was to examine nuclei where transcription factors and other proteins accumulate to govern transcriptional and other biochemical changes controlled by *Rpp1*. The abundances of approximately 2,300 proteins observed in both susceptible and resistant plants were measured by mass spectrometry, and clustering was performed to reveal sets of differentially accumulating proteins linked to *Rpp1*-mediated resistance. Among the proteins found were transcription factors, chromatin-associated proteins, DNA polymerases, DNA repair enzymes, nucleolar proteins, spliceosome components, nuclear pore proteins, and other proteins with likely activity in the nucleus. Genes for candidate proteins linked to disease resistance were cloned and expressed by a plant virus to test gene functionality by virus-induced gene silencing. After silencing, the normally-resistant *Rpp1* plants developed rust symptoms and accumulated rust fungal RNA and protein. Silenced plants also had reduced amounts of RNA for the soybean Myb84 transcription factor and soybean isoflavone O-methyltransferase, both of which are important to phenylpropanoid biosynthesis and lignin formation. These data reveal that rust infection leads to the accumulation of transcription factors and other proteins in the soybean nucleus and that these proteins support the immune system through *Rpp1*.

Nuts, Bolts, Frogeye Leaf Spot, and the UUOT

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Managing frogeye leaf spot (FLS) continues to be an important issue since the first observations of quinone outside inhibitor (QoI)-resistant *Cercospora sojina* in 2010. Since that time, countless efficacy trials have been conducted throughout the southern United States to determine the effect of application timing as well as product chemistry on QoI-resistant *C. sojina* populations. Even though numerous fungicide products are labeled for managing FLS (n > 55), few products observationally reduce disease severity even though they may reduce yield losses as a result of the disease. Since 2014, numerous anecdotal statements have been made by agricultural professionals regarding the presence of fungicide resistance and how using some agricultural products could potentially reduce the yield losses attributed to FLS fungicide resistant fungal populations. Fungicide efficacy trials were conducted with eight candidates to determine the effect (FLS severity and yield) on a FLS-susceptible variety at six locations (either Armor DK 4744 (n=5 locations) or Dyna-Gro 37RY47 (n=1 location)). The fungicide products applied alone included copper hydroxide (as CuproFix; 2 lb/A), mancozeb (as Koverall; 2 lb/A), tetraconazole (as Domark; 4 fl oz/A), potassium phosphite + tebuconazole (as Viathon; 2 pt/A), and azoxystrobin + propiconazole + thiophanate-methyl (as Quadris + Tilt + Topsin; 4 fl oz/A + 4 fl oz/A + 10 fl oz/A). In addition, tank mixes included several different product combinations: a fungicide and a nutritional product applied as azoxystrobin + 5-0-0 (N-B-Mo) (as Quadris, 4 fl oz/A + Manniplex B Moly, 2 qt/A), and two tank mix combinations with a fungicide and an insecticide including azoxystrobin + diflubenzuron (as Quadris + Dimilin, 4 fl oz/A + 2 fl oz/A) or trifloxystrobin + prothioconazole + flubendiamide (as Stratego YLD + Belt, 4 fl oz/A + 2 fl oz/A). Fungicide efficacy was judged at each location by rating FLS severity from 0 to 9 or estimating as a percentage. Plots were harvested at all locations and yield was analyzed in SAS following tests of normality. Averaged across all locations, the tetraconazole application resulted in a 4.3 bu/A increase over the non-treated followed closely by the three-way MOA (azoxystrobin + propiconazole + thiophanate-methyl). Phytotoxicity following the application of some of the fungicides was observed in Arkansas, at one location (albeit on a minimal basis), and in Mississippi and Louisiana.

Minutes of the 42nd Annual Meeting of the Southern Soybean Disease Workers

11-12th March 2015

Pensacola Beach, FL

SSDW General Session:

Sixty-two participants representing university, industry, government and commodity groups convened at the Hilton Pensacola Beach Gulf Front Hotel in Pensacola Beach, Florida, for a lively day and a half session addressing soybean disease concerns.

President Craig Rothrock greeted all participants and transferred moderation to Vice President Trey Price. The opening presentations were comprised of the first round of the graduate student paper competition, followed by the contributed paper session.

A banquet was held during the evening and awards were presented to Loren Giesler (The Boyd Padgett Beat the Bushes Award for Excellent Fundraising, presented by Trey Price), Heather Kelly (Future Leader, Friends of Southern IPM, presented by Henry Fadamiro), and Jim Marois (Distinguished Service, SSDW, presented by Ray Schneider).

During the second day, seven additional students participated in the graduate paper competition bringing the total to fifteen students representing Louisiana State University (LSU), Mississippi State University (MSU), University of Tennessee, and Southern Illinois University (SIU). These were followed by general session presentations.

SSDW Business Meeting:

President Rothrock called the meeting to order at 11:56 on the 12th of March. The first order of business was recognizing the student awards.

Jeff Standish (MSU, co-advised by Maria Tomaso-Peterson and Tom Allen) won first place for his presentation "Investigating Fungicide Sensitivities beyond the QoIs in *Cercospora sojina* from Mississippi." Eduardo Chagas da Silva (LSU AgCenter, advisor Ray Schneider) won second place for his presentation "A New Perspective on Cercospora Leaf Blight Symptoms on Soybean." Nick Frederking (SIU, advisor Jason Bond) won third place for his presentation "Efficacy of Seed Treatments for Management of *Fusarium virguliforme* and *Heterodera glycines*." Students received monetary awards and plaques for their accomplishments.

Trey Price was recognized for his role as the local arrangements chair and the success of the venue. The following sponsors: Arysta LifeScience, BASF, Bayer CropScience, Cheminova, DuPont, FMC, Pioneer, and Valent, were recognized for their financial support in assisting defrayment of meeting costs. The gavel was passed from outgoing President Rothrock to in-coming President Price.

Minutes of the 42nd Annual Meeting of the Southern Soybean Disease Workers

(continued)

Minutes provided by Patricia Bollich were presented and approved by the membership. The membership approved Treasurer's report showed a balance of $3011 as of 12/31/14. With the efforts of Loren Giesler, it was projected to be closer to $6000 at the end of first quarter. Unfortunately, Myra Purvis will no longer be able to remain as Treasurer. Membership voted and accepted Patricia Bollich as her replacement.

Tom Allen, Chair – Disease Loss Estimate Committee for the Southern United States, provided highlights from the 2014 sixteen state soybean season. An addendum was provided to the proceeding distributed.

Membership was unaware of any retirements or deaths since the 41st meeting. New faculty included Vinson Doyle, Assistant Professor of Mycology at the LSU AgCenter.

New business topics:

Ray Schneider – would like to establish a web site/server to store SSDW meeting abstracts, proceedings, etc. (http://ssdw.net). Tom Allen noted he has scanned copies of all past meetings (42 years). R. Schneider will see what it will entail to make them searchable. Anticipated costs for the use of the server will be $300/year. Melvin Newman proposed and membership accepted the formation of a committee to oversee the web page. Members include: Ray Schneider, Tom Allen, Doug Jardine, Craig Rothrock and Danise Beadle.
Proposal for the 2016 meeting dates and location was met with overwhelming approval to retain the same venue and time. (March 9-10, 2016 Pensacola Beach, FL) Invitations will be extended to NCERA groups that may wish to precede our meeting.

The business meeting concluded at 12:25 with the election of Terry Spurlock as in-coming vice president.

The officers for SSDW for 2015–2016 are Trey Price, LSU, AgCenter—president; Terry Spurlock, University of Arkansas—vice president; Danise Beadle, Eurofins Agroscience Services, Inc.—secretary; Patricia Bollich, LSU, AgCenter—treasurer; Tom Allen, MSU—chair, Disease Loss Estimate Committee.

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