2020 MSPB Annual Report

Project # 14-2020

Title: **Determination of organisms affecting soybean seed quality and fungicide efficacy in reducing associated losses.**

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Background and Objectives

Reductions in soybean grain quality can occur as a result of warm wet weather that precedes physiological maturity (R8), delays harvest and seed diseases. Several different fungi can cause soybean grain and seed quality reductions; however, one of the most damaging pathogenic fungi is *Phomopsis longicolla*. Grain elevators in Mississippi report an average of 7% damage (or greater), depending on the year. Many variables contribute to post-harvest grain quality reductions including pathogen and insect damage, grain color, soybean kernel size, splits, and reduced test weight. In locations where the soybean end use is seed, decreased vigor, and germination are also a concern for seed companies as a result of this disease complex. In some years, such as 2018, Phomopsis seed decay caused economic losses in both the southern and northern U.S. soybean production systems (average loss = 1.68%, or 87 million bushels or $8.56/ac). In 2017, as reported by the Southern Soybean Disease Workers, an estimated 52,500 metric tons of grain production were lost to seed-associated diseases across 16 southern states accounting for an estimated 0.2% of all economic losses. Symptoms of Phomopsis seed decay include shriveled, elongated seed which appear chalky. Infection can be more severe when harvest is delayed and environmental conditions continue to be warm and humid during the growth stages that precede harvest (R7 and R8). As a means of reducing Phomopsis seed decay in subsequent seasons the current management strategies include crop rotation with non-hosts, tillage, fungicide applications during pod-fill, and resistant cultivars. However, information regarding efficacy of fungicide applications and cultivar resistance within the current commercial offerings is limited. The objectives of this research will improve soybean resistance to reduced grain quality by screening germplasm and developing new and improved breeding lines, and develop best management practices to address the soybean production issues associated with reduced grain quality.

Objective 1: Determine the causal agent of reduced soybean seed quality in harvested soybean seed.
Lab assays to determine the organisms present on 2019 harvested seed are on-going and almost complete.

Objective 2: Determine efficacy of fungicide on reducing growth of pathogen causing seed rot in vitro.
Nothing to report-lab experiments to begin 2021.

Objective 3: Evaluation of mechanisms for pathogen entry into seed.

Objective 4: Evaluate response of new soybean germplasm exposed to environmental conditions which promote reduced seed quality.

Report of Progress/Activity

Objective 1: Determine the causal agent of reduced soybean seed quality in harvested soybean seed.

Methods

Thirty randomly selected soybean seed from each plot harvested during 2018, 2019, and 2020 from the rainout shelters 1-5 will be used to explore the diverse community of organisms that are present on or in

harvested soybean grain. All grain will be surface-disinfected by soaking in a 10% solution of bleach for one minute and rinsed in sterile distilled water three times. The seed will be plated on sterile Petri dishes containing Acid potato-dextrose agar (85% lactic acid) and regular PDA and incubated at 25±1°C for seven days. These freshly prepared subculture plates will be used for DNA extraction.
DNA of each isolate will be extracted using the Norgen Biotek Yeast/Fungi DNA Isolation Kits (Norgen Biotek Corp, ON, Canada). The extracted DNA will be used as template DNA for PCR reaction. Each isolated DNA will be amplified using three different primers to amplify regions of ribosomal DNA using polymerase chain reaction (PCR) for proper identification. Internal transcribed spacer (ITS 4 and 5) regions of ribosomal DNA, Large subunit (LSU), and Translation Elongation Factor 1-α (TEF1-α) primers. After amplification electrophoresis will be carried out to confirm the various band sizes for each set of primers. Samples of each isolate will be sent for sequencing with Eurofins Genomics. From these results, each fungus isolated will be identified to the genus and species level.

Results
Lab assays to determine the organisms present on 2020 harvested seed are on-going and almost complete. All seed samples from 2018 and 2019 shelters have been processed. Results of identified organisms are present below (bolded names represent the fungi recovered with the greatest frequency during 2018-2020 [2020 results are from shelters 1 and 2, results from the adjacent plots are still pending])

- ***Fusarium armeniacum (fulciforme, graminearum, incanitum, incarnatum/equiseti complex)***
- ***Phomopsis longicolla***
- ***Cercospora complex***
- *Epicoccum sorghinum*
- *Alternaria alternate*
- *Macrophomina phaseolina*
- *Colletotrichum truncatum*
- *Cladosporium tenuissimum (cladosporiodes)*
- *Diaporthe longicolla*
- ***Corynespora cassiicola***

Objective 2: Determine efficacy of fungicide on reducing growth of pathogen causing seed rot in vitro. Nothing to report-lab experiments to begin summer 2021.

Objective 3: Evaluation of mechanisms for pathogen entry into seed.

Methods
Field trials were established in 2019 and 2020 to evaluate red banded stink bug effect on seed quality. Trials were arranged as a randomized complete block with 21 soybean lines as treatments consisting of: three commercial lines as checks, one *Phomopsis*-resistant line (SS93-6181), and 17 advanced breeding lines. The trials contained single row plots of each entry, replicated three times. All plots were inoculated with a *P. longicolla* spore suspension consisting of beta conidia at the R5.5 growth stage. Plots were covered with metal frame mesh cages and infested with 2 times the normal threshold rate of red banded stink bug (Fig 2.). Cages were removed 12 days post infestation and plants were allowed to mature. Yield, visual damage, and feeding injury levels were evaluated.

Results
Numerical differences in visual damage were observed during 2019 with up to a 36% decrease when compared to the commercial checks. During 2020 significant differences were observed between treatments with up to 64% reduction in visual damage when compared to the commercial checks (Table 5).

Objective 4: Evaluate response of new soybean germplasm exposed to environmental conditions which promote reduced seed quality.

Methods
During 2019-2020, field trials were established under rainout shelters (n=2) consisting of greenhouse cold frames covered with translucent plastic. Rainout shelters were used to more effectively control the moisture input and mimic an environment conducive for the development of grain decay. Two additional identical trials were established adjacent to the rainout shelters to evaluate treatments in a natural occurring environment. Trials were arranged as a randomized complete block with 21 soybean lines as treatments consisting of: Three commercial lines as checks, one *Phomopsis*-resistant line (SS93-6181), and 17 advanced breeding lines. Seed were planted on 17 May, 2 July, and 17 June respectively, to 76.2 cm centers with nine seed per 30.5 cm. The trials contained single row plots of each entry, replicated three times. All plots were inoculated with a *P. longicolla* spore suspension consisting of beta conidia at the R5.5 growth stage. Shelter 1 remained fungicide non-treated while Shelter 2 received a 293 ml/ha fungicide application consisting of trifloxystrobin + prothioconazole (as Stratego YLD) at R7. Shelters were simultaneously overhead irrigated for approximately 200 h post-inoculation over a period of 61 days. Plots were hand-harvested 2 weeks after R8 to determine plot weight (g) and grain damage (%). Observations of visual grain damage from each plot included purple-stained seed, and shriveled, moldy kernels considered to be the result of Phomopsis seed decay as evaluated on a scale of 0 to 10 with 0=no damage and 10=no healthy kernels (Fig.1). In addition, grain samples were evaluated by Mid-South Grain to assess damaged kernel total (DKT; %).

Results
Significant differences in damage, up to 74% between entries across years, were observed in the non-fungicide shelter (Table 1). DB06X06-093 provided equal to or less overall visual damage when compared to the commercial checks in each year. Progeny 4211 provided up to 75% greater plot weight compared to other entries. Numerical differences in damage and plot weight were observed across years between entries in the fungicide-treated shelter (Table 2). On average, DB06X06-093 reduced damage by 27% across years when compared to the commercial checks. On average, DB06X06-093 reduced damage by 51% when compared to the commercial checks. Numerical differences in damage, up to 75% between entries were observed in the non-fungicide plots (Table 3). Significant differences in damage and plot weight were observed between entries in the fungicide-treated plots (Table 4). On average, entries reduced visual damage by up to 64% when compared to the commercial checks. Regardless of year, differences occurred between entries; however, additional research is needed to determine the potential of germplasm in reducing seed quality losses.

Discussion
Although some level of damage occurred regardless of treatment, differences in resistance to damage of harvested grain were observed between lines suggesting that some level of tolerance may be inherently available within the currently available germplasm. Some new germplasm lines may have the potential to reduce mature seed damage and will be breeding reservoirs for future seed quality research. In some years visual damage was significantly decreased in plots receiving a late season application of fungicide;

however, results were not consistent across years. Insect feeding did increase damage in 2019 and 2020; however, red banded populations per year will determine the impact on damage. Additional research is needed to determine the potential of germplasm in reducing seed quality losses.

Impacts and Benefits to Mississippi Soybean Producers

Outreach

Project components have been discussed at board meetings including the Mississippi Soybean Promotion Board summer tour and at the Mid-South Soybean Promotion Board summer and winter meetings.

End Products–Completed or Forthcoming

A poster entitled "**Evaluation of new germplasm associated with reducing losses associated with poor quality grain and organisms present post-harvest"** summarizing 2018 field data and a preliminary set of molecular identification of organisms present was presented at the American Phytopathology Society national meeting (Plant Health 2109) August 2-7, 2019 in Cleveland Ohio.

A poster entitled "**Evaluating the response of new soybean germplasm to environmental conditions that promote reduced grain quality."** summarizing 2018-2019 field data was presented at the Southern Division American Phytopathology Society meeting February 9-12, 2020 in Charleston, South Carolina.

A poster entitled "**Evaluation of stinkbug damage, Phomopsis infection, and grain quality new soybean germplasm.** summarizing 2019 stinkbug trial data was presented at the American Phytopathological Society National Meeting (Virtual), August 9-12, 2020.

Mississippi Soybean Promotion Board Research Roundup (December 9, 2020) "Evaluation o Soybean Lines for Managing Mature Seed Quality Issues"

A poster entitled "**Reducing losses associated with poor grain quality using advanced soybean germplasm in Mississippi"** summarizing 2018-2020 field data was presented at the Southern Division American Phytopathology Society meeting (virtual), February 9-11, 2020.

Fig. 1. Mature soybean pods and seed from field plots located at the Delta research and Extension Center in Stoneville, MS during 2020.

Fig 2. Insect cages surrounding plots infested with red banded stink bug.

Table 1. Harvest data (g/plot) and visual damage from non-fungicide treated soybean plots conducted under a rainout shelter in Stoneville, MS.

	Plot weight (g)			Visual damage (0-10 scale)		
Breeding line [a]	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**
Asgrow 4232RR2Y	**814.8 abc**	**567.6**	**821.0 b**	**1.3 d**	**7.7 abc**	**4.7 a-g**
Credenz CZ3841LL	**887.1 ab**	**608.9**	**402.9 bcd**	**3.0 bcd**	**5.7 c-g**	**5.3 a-e**
Progeny 4211	**1,042.8 a**	**565.9**	**1,395.5 a**	**1.3 d**	**8.7 ab**	**2.7 d-g**
SS93-6181[a]	553.5 b-e	580.5	612.7 bcd	1.7 d	3.7 fg	4.0 b-g
10061-236-11	390.7 d-h	527.6	714.7 bcd	2.7 cd	4.7 efg	4.0 b-g
10076-121-11	688.8 a-d	463.6	560.9 bcd	2.0 cd	6.3 b-e	5.7 a-d
11030-541-210	252.6 e-h	416.6	469.7 bcd	6.7 a	5.3 c-g	4.0 b-g
11030-541-24	394.8 d-h	361.7	390.2 bcd	3.7a-d	6.0 c-f	3.7 b-g
11030-541-26	501.5 c-f	361.8	350.6 cd	3.5 a-d	7.0 a-e	5.0 a-f
11030-541-28	42.3 h	322.5	547.1 bcd	2.0 cd	6.7 a-e	2.3 efg
11030-541-29	411.4 d-g	410.5	631.5 bcd	4.3 a-d	6.3 b-e	4.0 b-g
11043-224-81	387.1 d-h	470.1	328.5 d	6.7 a	7.3 a-d	5.0 a-f
11043-224-91	409.0 d-g	484.1	625.3 bcd	3.0 bcd	5.7 c-g	6.3 abc
11043-225-72	587.9 b-e	558.9	773.5 bc	3.3 a-d	6.0 c-f	3.3 c-g
65-414-132-1	447.6 def	464.7	566.7 bcd	1.3 d	5.0 d-g	2.7 d-g
DB06X06-093	439.3 def	407.3	824.6 b	1.3 d	3.3 g	2.0 fg
LD06-7620	382.2 d-h	263.2	359.6 cd	6.3 ab	9.0 a	7.7 a
LG03-4561-14	547.2 b-e	423.6	418.5 bcd	3.0 bcd	5.3 c-g	6.7 ab
Pella 86	401.8 d-h	334.3	568.5 bcd	4.7 a-d	7.0 a-e	4.7 a-g
PI 587982A	73.2 gh	300.9	403.2 bcd	5.3 abc	3.3 g	4.0 b-g
PI 594619	173.8 fgh	262	305.1 d	2.0 cd	6.0 c-f	1.7 g
Mean	481.7	436.0	572.40	3.34	6.0	4.28
LSD (α=0.05)	363.6	238.9	440.19	3.65	2.5	3.33
p-value	0.0002	0.0921	0.0049	0.0213	0.0013	0.0494

[a] Breeding line presented in bold indicates a commercial line serving as a check. The single line denoted with shaded cells indicates the line was previously determined to be resistant to Phomopsis seed decay.

Table 2. Harvest data (g/plot) and visual damage from plots receiving an application of trifloxystrobin + prothioconazole conducted under a rainout shelter in Stoneville, MS.

	Plot weight (g)			Visual damage (0-10)		
Breeding line [a]	**2018**	**2019**	**2020**	**2018**	**2019**	**2020**
Asgrow 4232RR2Y	**495.6**	**540.2**	**647.5 cde**	**2.7**	**8.0 abc**	**3.0**
Credenz CZ3841LL	**707.7**	**716.6**	**732.2 cde**	**1.7**	**6.7 b-e**	**3.3**
Progeny 4211	**722.9**	**535.7**	**882.8 abc**	**3.0**	**8.3 abc**	**3.0**
SS93-6181[a]	827.0	453.6	122.3 f	1.3	7.7 abc	4.3
10061-236-11	530.3	436.4	390.1 def	1.7	6.3 b-e	3.0
10076-121-11	675.9	502.1	748.7 b-e	2.0	7.0 b-e	2.5
11030-541-210	303.5	389.1	438.4 def	4.0	8.3 abc	4.3
11030-541-24	237.7	402.3	1,121.5 ab	3.0	7.0 b-e	4.3
11030-541-26	237.8	429.7	1,197.8 a	4.7	7.3 a-d	5.3
11030-541-28	451.8	461.7	751.1 b-e	3.7	7.3 a-d	3.3
11030-541-29	476.6	413.9	609.0 cde	3.3	6.7 b-e	4.7
11043-224-81	367.0	464.5	493.7 def	3.3	5.0 de	3.3
11043-224-91	659.9	356.6	607.2 cde	2.3	7.7 abc	2.7
11043-225-72	430.8	298.1	688.8 cde	1.7	6.7 b-e	3.0
65-414-132-1	529.6	471.2	579.1 cde	3.0	5.0 de	2.7
DB06X06-093	548.0	400.6	557.5 cde	2.3	5.0 de	2.3
LD06-7620	435.3	281.9	489.9 def	6.0	9.7 a	3.5
LG03-4561-14	388.8	366.0	576.1 cde	3.3	4.7 e	3.0
Pella 86	386.8	377.2	757.8 bcd	2.3	7.0 b-e	2.7
PI 587982A	260.0	336.3	370.1 ef	2.0	5.7 cde	3.0
PI 594619	346.4	236.9	913.1 abc	4.7	6.3 b-e	1.7
Mean	480.7	422.5	664.45	2.9	6.8	3.29
LSD (α=0.05)	524.6	231.8	384.87	3.8	2.5	2.31
p-value	0.6373	0.0919	0.0012	0.6996	0.0227	0.3139

[a] Breeding line presented in bold indicates a commercial line serving as a check. The single line denoted with shaded cells indicates the line was previously determined to be resistant to Phomopsis seed decay.

Table 3. Harvest data (g/plot) and grain quality evaluations as visual damage (%) from non-fungicide treated soybean plots conducted in Stoneville, MS.

	Plot weight (g)		Visual damage (0-10 scale)	
Breeding line [a]	**2019**	**2020**	**2019**	**2020**
Asgrow 4232RR2Y	**263.11**	**722.9 ab**	**8.67 a**	**2.7**
Credenz CZ3841LL	**318.78**	**835.8 a**	**5.34 a-d**	**4.7**
Progeny 4211	**262.6**	**839.6 a**	**7.67 ab**	**4.5**
SS93-6181	149.09	707.2 ab	7.67 ab	3.3
10061-236-11	152.47	700.1 ab	4 e	1.7
10076-121-11	159.44	482.7 a-d	5 bcd	2.7
11030-541-210	172.12	703.1 ab	8 ab	3.0
11030-541-24	197.5	387.5 bcd	5 bcd	3.3
11030-541-26	211.77	656.5 abc	6.34 a-d	3.3
11030-541-28	213.62	719.7 ab	7 abc	4.0
11030-541-29	218.12	467.0 bcd	4 cde	4.3
11043-224-81	239.39	548.9 abc	5 bcd	3.7
11043-224-91	244.47	593.8 abc	8.34 ab	4.7
11043-225-72	249.17	676.1 ab	4.5 de	3.0
65-414-132-1	271.79	466.2 bcd	7.34 abc	1.0
DB06X06-093	285.7	652.2 abc	7.34 abc	5.3
LD06-7620	293.76	147.9 d	7.67 ab	3.7
LG03-4561-14	301.28	703.5 ab	6.34 a-d	4.0
Pella 86	322.35	303.1 cd	7.34 abc	2.7
PI 587982A	384.81	619.9 abc	6.34 a-d	2.7
PI 594619	412.4	415.1 bcd	8 ab	1.3
Mean	254.01	583.99	5.59	3.29
LSD (α=0.05)	309.45	365.42	3.36	2.87
p-value	0.8908	0.0455	0.0347	0.2093

[a] Breeding line presented in bold indicates a commercial line serving as a check. The single line denoted with shaded cells indicates the line was previously determined to be resistant to Phomopsis seed decay.

Table 4. Harvest data (g/plot) and grain quality evaluations as visual damage (%) from fungicide treated soybean plots conducted in Stoneville, MS.

	Plot weight (g)		Visual damage (0-10 scale)	
Breeding line [a]	**2019**	**2020**	**2019**	**2020**
Asgrow 4232RR2Y	**449.39 b**	**351.9 abc**	**7.33**	**5.0 bcd**
Credenz CZ3841LL	**305.4 bcd**	**290.7 a-e**	**6.33**	**4.7 b-e**
Progeny 4211	**293.06 bcd**	**358.9 ab**	**7.67**	**5.7 ab**
SS93-6181	120.15 d	348.1 abc	4.33	5.7 ab
10061-236-11	124.03 d	374.0 a	5.67	4.3 b-e
10076-121-11	135.44 d	246.2 b-f	5.33	4.7 b-e
11030-541-210	140.42 d	216.4 def	4	5.5 b
11030-541-24	193.5 cd	262.7 a-f	5.33	3.7 b-f
11030-541-26	228.62 bcd	219.2 def	6	5.3 bc
11030-541-28	232.91 bcd	250.3 b-f	5.67	2.5 ef
11030-541-29	237.57 bcd	224.6 def	6	3.7 b-f
11043-224-81	243.95 bcd	228.6 def	5.33	4.3 b-e
11043-224-91	245.77 bcd	311.0 a-e	6.67	4.7 b-e
11043-225-72	267.52 bcd	329.2 a-d	7.33	3.0 c-f
65-414-132-1	284.2 bcd	245.6 b-f	7.67	2.7 def
DB06X06-093	285.87 bcd	244.0 c-f	7	8.0 a
LD06-7620	291.36 bcd	172.0 f	8	6.0 ab
LG03-4561-14	302.49 bcd	240.2 c-f	5.33	4.7 b-e
Pella 86	335.03 bcd	248.3 b-f	6.67	4.7 b-e
PI 587982A	381.1 bc	239.0 c-f	6.33	3.7 b-f
PI 594619	694.73 a	207.5 ef	8	1.7 f
Mean	275.83	266.52	6.29	4.53
LSD (α=0.05)	232.7	114.29	3.57	2.47
p-value	0.0083	0.0267	0.6528	0.0064

[a] Breeding line presented in bold indicates a commercial line serving as a check. The single line denoted with shaded cells indicates the line was previously determined to be resistant to Phomopsis seed decay.

Table 5. Harvest data (g/plot) and grain quality evaluations as visual damage (%) from stinkbug infested soybean plots conducted in Stoneville, MS.

	Plot Weight (g)		Visual Damage (0-10)	
Breeding line [a]	2019	2020	2019	2020
Asgrow 4232RR2Y	449.39	240.2 c-f	7.33	4.7 b-e
Credenz CZ3841LL	305.4	248.3 b-f	6.33	4.7 b-e
Progeny 4211	293.06	290.7 a-e	7.67	4.7 b-e
SS93-6181[a]	120.15	172.0 f	4.33	6.0 ab
10061-236-11	124.03	239.0 c-f	5.67	3.7 b-f
10076-121-11	135.44	348.1 abc	5.33	5.7 ab
11030-541-210	140.42	207.5 ef	4	1.7 f
11030-541-24	193.5	358.9 ab	5.33	5.7 ab
11030-541-26	228.62	351.9 abc	6	5.0 bcd
11030-541-28	232.91	329.2 a-d	5.67	3.0 c-f
11030-541-29	237.57	245.6 b-f	6	2.7 def
11043-224-81	243.95	311.0 a-e	5.33	4.7 b-e
11043-224-91	245.77	262.7 a-f	6.67	3.7 b-f
11043-225-72	267.52	228.6 def	7.33	4.3 b-e
65-414-132-1	284.2	219.2 def	7.67	5.3 bc
DB06X06-093	285.87	224.6 def	7	3.7 b-f
LD06-7620	291.36	216.4 def	8	5.5 b
LG03-4561-14	302.49	250.3 b-f	5.33	2.5 ef
Pella 86	335.03	374.0 a	6.67	4.3 b-e
PI 587982A	381.1	246.2 b-f	6.33	4.7 b-e
PI 594619	694.73	244.0 c-f	8	8.0 a
p-value	0.0083	0.0267	0.6528	0.0064

[a] Breeding line presented in bold indicates a commercial line serving as a check. The single line denoted with shaded cells indicates the line was previously determined to be resistant to Phomopsis seed decay.