MISSISSIPPI SOYBEAN PROMOTION BOARD

Evaluating fungicide efficacy, desiccant applications, and delayed harvest for soybean grain quality, 14-2022.

ANNUAL REPORT

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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.

Report of Progress/Activity

Objective 1: Evaluate the effect of early and mid-season fungicide application in combination with desiccation on seed quality.

A widely utilized seed variety (46X6) was planted on May31, 2022 to 20 foot by 4 row plots to assess seed quality with combinations of fungicide and desiccant applications. Plots were sprayed with Miravis Top fungicide starting at R3 (beginning pod) growth stage to simulate an automatic R3-R4 application implemented in many of the southern soybean production systems.

Applications of Miravis Top fungicide were sprayed at R3 growth stage (July 28, 2022) and R6 growth stage (September 6, 2022) along with a desiccant treatment at maturity (September 26, 2022). Individual rows were scheduled to be harvested to determine the effect of delayed harvest and environment over time. The first rows of the plots were harvested on Thursday October 18, 2022. Assessments include plot weight, moisture, test weight, and damage ratings to include a Phomopsis, purple seed stain and an overall damage score. The first harvest timing was completed on October 18, 2022. Due to inclement weather and harvest equipment issues only one of the four harvest timings were evaluated. Due to lack of data integrity the remaining harvest rows (3 remaining) were destroyed.

Results

No significant differences occurred between treatments with regard to overall quality for either application timing at the optimal harvest #1 timing; however, numerically more purple seed stain and a decrease in overall seed quality was observed, up to 38% and 22%, respectively in the plots treated at R3 with Miravis Top at 13.7 oz./a when compared to all other treatments (Table 1). In most cases applications with Miravis Top provided equal benefit with applications at R3 and R6 alone and in combination (Table 1). Applications of Miravis Top at R6 followed by a desiccant application at maturity provided a numerical increase of 10% when compared to the non-treated plots; however, no treatments were significantly better with regards to overall quality (Table 1).

Discussion

During 2022 *phomopsis* levels were minimal at the optimal harvest timing and overall quality was observed at acceptable levels throughout treatments. Delayed harvest timings are needed to conclude effects of treatments combined with less than optimal conditions to assess the benefit of application. In most cases all applications performed equally to the non-treated; therefore, no conclusion can be made surrounding these applications. From this one year of data there is no significant effect observed on overall seed quality due to fungicide applications alone or in combination with a desiccant. Additional research is needed to determine the effect, if any, of increased rates and timings on seed quality when harvest is delayed.

Objective 2: Evaluate desiccation application rates in reducing seed quality in delayed harvest situations.

Another season of the late season trial initiated in 2021 was established on May 4, 2022 to examine different rates of desiccants along with a fungicide application to mimic situations such as occurred in 2021 when hurricane conditions threatened on-time harvest. Growers were put in situations in which decisions had to be made to desiccate soybean to ensure harvest before conditions either destroyed fields or cause extreme harvest delays. Higher rates of desiccants were paired with fungicide applications in an earlier than optimal growth stage in simulation of occurrences happening with our local grower fields. The trial was planted to NK 549F5X soybean. Plot were established as 4 rows by 20 feet and applications began at growth stage R6. Three rates of gramoxone (a local standard and two higher rates) were used paired alone and in combination with Miravis Top fungicide. Beans were allowed to dry down and individual rows were harvested to examine quality differences. Two total harvests occurred to include an optimal and 2 weeks after to simulate delayed scenarios. Due to inclement weather and harvest equipment issues only two of the four harvest timings were evaluated. Due to lack of data integrity the remaining harvest rows (2 remaining) were destroyed.

Results

No significant difference occurred between treatments with regard to overall quality for any treatment at the optimal harvest #1 timing; however, up to 70% more purple seed stain was observed in the plots with applications made with a dessicant alone at 22 oz/a at the R7 growth stage when compared to all other treatments, although still at a minimal amount. (Table 2). With that same treatment overall seed quality was numerically, up to 44%, reduced when compared to other treatments at greater and lesser rates of desiccant. Significant difference occurred between treatments with regard to purple seed stain and overall quality for treatments at the harvest #2 timing. These data suggest greater amounts of damage occurred at R5.5 growth stage with applications of desiccant alone at the 32 oz/a rate. The treatment provided up to 90% increase in purple seed stain and up to 96% decrease in overall quality (Table 3). Reductions in pounds of seed per plot were also observed when compared to treatments at later application timings. In some instances, applications with Miravis Top provided benefit even with increased amounts of desiccant applied at all growth stages (Table 2).

Discussion

During 2022 *phomopsis* and purple seed stain ratings were at minimal levels during each of the harvest timings and overall quality was at acceptable levels. Samples that would have been taken at delayed harvest timings may have provided additional data to support previous research; however, environmental conditions did not allow for collection. From this year of data there is no significant negative effects observed on overall seed quality due to increase in desiccant application rates; however, additional research is needed to determine the effect, if any, of increased rates and timings on seed quality when harvest is delayed.

Objective 3: Determine the causal agents of reduced soybean seed quality in harvested soybean seed

Methods

Thirty randomly selected soybean seed will be selected 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

Due to lack of complete harvest these experiments have been put on hold until data can be obtained from an entire trial.

Objective 4: Determine efficacy of fungicide on reducing growth of pathogen causing seed rot in vitro.

Sample collection: *P. longicolla* was isolated from soybean seed collected from a field in Stoneville, MS in 2019 on potato dextrose agar (PDA) medium.

- Culture plates were stored for six months. Isolates were transferred to fresh PDA plates and incubated in dark at 25°C .
- After 7 days of incubation, mycelium was observed on microscopic slides at 400X magnification. Genomic DNA was extracted using a cetyltrimethylammonium bromide (CTAB) method. Internal transcribed spacer region (ITS) was amplified using ITS4 (forward) and ITS5 (reserve) primers. Purified DNA products were sent for sequencing for species identification.
- A fungicide sensitivity assay was conducted using technical grade azoxystrobin (FRAC code 11), benomyl (FRACcode1), difenoconazole (FRAC code 3), and fludioxonil (FRAC code 11).
- A plug (≈6 mm in diameter) containing mycelia was removed from the leading edge of an actively growing PL colony and placed in the center of fungicide-amended plates (0,1,5,10,20 ppm).
- The colony diameter was measured after 72 hours of incubation at 25°C and relative growth was calculated.
- The experiment was arranged in a completely randomized design with three replications.
- Data were analyzed using R studio 3.6.1

Results

- Floccose, dense, white mycelia with occasional greenish-yellow areas and large black stromata developed after 7 days of incubation at 25 °C.
- Only alpha-conidia were observed after 7days under the microscope.
- ITS sequencing confirmed the isolates to be *P. longicolla* based on the best match from the sequences in Genbank database of NCBI.
- *In vitro* efficacy of four different fungicides resulted in significant difference among the isolates ($P<0.01$).
- Relative growth varied among the isolates. Azoxystrobin and difenoconazole resulted in greater percent relative growth, whereas benomyl showed complete growth inhibition. Only isolate 3 was not complete inhibited by fludioxonil (Table 4).
- Benomyl at all concentrations tested resulted in 100% inhibition for all four isolates.
- Fludioxonil resulted in nearly 100% inhibition for all the four isolates.
- On average, azoxystrobin resulted in 85.2% growth inhibition and difenoconazole resulted in 92.0% inhibition (Fig. 1)

Discussion

- Fungicide sensitivity differed between the isolates.
- *P. longicolla* isolates recovered from soybean in Mississippi are less sensitive to azoxystrobin compared to the other fungicides evaluated.
- Benomyl and Fludioxonil were observed to inhibit growth.
- EC_{50} values were not calculated because mycelia growth didn't reach to 50%.
- Research is on-going to determine fungicide sensitivity of other fungi isolated from seed.

These are previous trial data. Due to lack of complete harvest these experiments have been put on hold until data can be obtained from an entire trial. Additional data will be generated from trials established in 2023.

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 "**Fungicide sensitivity of *Phomopsis longicolla* from harvested soybean grain**" was presented at the Southern Soybean Disease Workers meeting, March 2-3, 2022 in Pensacola, Florida.

Table 1. Copes soybean trial, harvest 1, 10-18-2022, Stoneville, Mississippi [x].

TRT	WEIGHT	MOISTURE	TEST WEIGHT	PHOMOPSIS	PSS	OVERALL QUALITY
Non-treated	4.6	9.8	48.8	0.0	1.0	2.0
Miravis Top 13.7 Fl oz/a NIS 0.25% v/v (R3)	4.4	10.2	48.1	0.0	1.3	2.3
Miravis Top 13.7 Fl oz/a NIS 0.25% v/v (R3) Miravis Top 13.7 Fl oz/a	4.7	9.3	50.0	0.3	0.8	2.0

NIS 0.25% v/v (R6)						
Miravis Top 13.7 Fl oz/a NIS 0.25% v/v (R6)	4.1	9.4	48.6	0.3	0.8	2.0
Miravis Top 13.7 Fl oz/a NIS 0.25% v/v (R3) Desicant	4.6	9.5	49.3	0.3	0.5	1.8
Miravis Top 13.7 Fl oz/a NIS 0.25% v/v (R6) Desicant	4.7	7.5	48.5	0.3	0.8	2.0
Miravis Top 13.7 Fl oz/a NIS 0.25% v/v (R3) Miravis Top 13.7 Fl oz/a NIS 0.25% v/v (R6) Desicant	4.4	9.4	49.5	0.0	0.8	1.8
MSE	0.2	2.8	4.6	0.1	0.4	0.6
CV	10.9	18.1	4.4	257.1	78.6	38.2
P	0.6835	0.4610	0.8907	0.7788	0.7690	0.9685

Table 2. Oops soybean trial, harvest 1, 10-3-2022, Stoneville, Mississippi [x].

TRT	WEIGHT	MOISTURE	TEST WEIGHT	PHOMOPSIS	PSS	OVERALL QUALITY
NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	2.8 b-e	8.8	49.8	0.0	0.5	1.5
NIS 0.25% V/V + DESSICANT 22 FL OZ/A	1.9 e	8.3	50.6	0.5	0.8	2.3
NIS 0.25% V/V + DESSICANT 32 FL OZ/A	2.3 de	8.5	48.9	0.3	0.8	2.0
NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	3.4 abc	7.9	49.1	0.0	0.3	1.3
NIS 0.25% V/V + DESSICANT 22 FL OZ/A	3.7 ab	8.7	49.6	0.5	0.8	2.3
NIS 0.25% V/V + DESSICANT 32 FL OZ/A	3.0 a-d	8.4	48.8	0.0	0.5	1.3
NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	3.9 a	8.8	48.4	0.3	0.5	1.8
NIS 0.25% V/V + DESSICANT 22 FL OZ/A	4.0 a	8.2	50.4	0.3	0.3	1.5
NIS 0.25% V/V + DESSICANT 32 FL OZ/A	3.9 a	9.2	47.8	0.0	0.3	1.3
NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	2.4 cde	8.4	49.9	0.3	0.8	2.0
NIS 0.25% V/V + DESSICANT 22 FL OZ/A	3.8 ab	8.0	51.4	0.3	1.0	2.3
NIS 0.25% V/V + DESSICANT 32 FL OZ/A	3.6 ab	8.3	50.0	0.0	0.5	1.5

Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	2.8 b-e	9.2	49.1	0.3	0.5	1.5
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 22 FL OZ/A	1.9 e	7.9	51.2	0.0	0.5	1.5
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 32 FL OZ/A	2.1 de	8.5	50.0	0.0	0.3	1.3
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	3.6 ab	8.8	48.3	0.8	0.5	2.3
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 22 FL OZ/A	3.9 a	8.2	50.5	0.3	0.3	1.5
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 32 FL OZ/A	3.4 abc	8.1	51.6	0.0	0.0	0.8
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	2.5 cde	9.3	48.3	0.5	0.8	2.3
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 22 FL OZ/A	3.9 a	7.6	52.2	0.0	0.5	1.5
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 32 FL OZ/A	3.9 a	8.8	49.2	0.0	0.5	1.5
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	3.6 ab	7.7	52.0	0.3	0.5	1.8
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 22 FL OZ/A	3.9 a	8.7	49.6	0.0	0.3	1.3
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 32 FL OZ/A	3.8 a	8.5	49.1	0.5	0.3	1.8
MSE	0.5	1.2	6.3	0.1	0.3	0.6
CV	22.2	13.1	5.1	193.6	115.5	48
P	<0.0001	0.8505	0.5663	0.1825	0.8254	0.4110

[x] Test was planted at the Delta Research and Extension Center, Stoneville, MS.

Table 3. Oops soybean trial, harvest 2, 10-18-2022, Stoneville, Mississippi [x].

TRT	WEIGHT	MOISTURE	TEST WEIGHT	PHOMOPSIS	PSS	OVERALL QUALITY
NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	4.8 b	9.2	51.8	0.0	0.8 ab	1.8 abc
NIS 0.25% V/V + DESSICANT 22 FL OZ/A	3.9 b	9.2	51.0	0.3	0.8 ab	2.0 ab
NIS 0.25% V/V + DESSICANT 32 FL OZ/A	3.8 b	9.0	51.0	0.3	1.0 a	2.3 a
NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	7.4 a	9.8	49.4	0.3	0.0 c	1.0 b-e
NIS 0.25% V/V + DESSICANT 22 FL OZ/A	7.4 a	10.2	48.2	0.0	0.5 abc	1.0 b-e
NIS 0.25% V/V + DESSICANT 32 FL OZ/A	7.1 a	9.4	50.6	0.0	0.0 c	0.5 de
NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	7.9 a	10.5	47.6	0.0	0.0 c	1.0 b-e
NIS 0.25% V/V + DESSICANT 22 FL OZ/A	8.1 a	9.4	50.4	0.0	0.3 bc	1.0 b-e
NIS 0.25% V/V + DESSICANT 32 FL OZ/A	8.1 a	9.5	50.7	0.0	0.0 c	0.3 de
NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	8.5 a	10.9	46.1	0.0	0.5 abc	1.3 a-d
NIS 0.25% V/V + DESSICANT 22 FL OZ/A	7.5 a	9.8	49.3	0.0	0.3 bc	0.8 cde
NIS 0.25% V/V + DESSICANT 32 FL OZ/A	8.5 a	10.2	48.9	0.0	0.0 c	0.5 de
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	5.3 b	9.8	49.8	0.0	0.5 abc	1.3 a-d
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 22 FL OZ/A	4.2 b	9.6	50.5	0.3	0.3 bc	1.8 abc
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 32 FL OZ/A	4.9 b	10.5	47.8	0.3	0.8 ab	2.0 ab
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	7.6 a	10.1	49.5	0.0	0.0 c	0.5 de
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 22 FL OZ/A	7.1 a	10.0	49.5	0.0	0.0 c	0.0 e

Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 32 FL OZ/A	7.9 a	9.8	49.2	0.0	0.8 ab	1.8 abc
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	8.5 a	29.7	50.4	0.0	0.5 abc	1.0 b-e
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 22 FL OZ/A	8.0 a	10.0	49.4	0.0	0.5 abc	1.3 a-d
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 32 FL OZ/A	8.5 a	10.2	48.9	0.3	0.5 abc	1.8 abc
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 10.7 FL OZ/A	7.8 a	10.2	49.0	0.0	0.3 bc	1.0 b-e
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 22 FL OZ/A	8.0 a	10.0	49.6	0.0	0.3 bc	1.0 b-e
Miravis top 13.7 FL OZ/A + NIS 0.25% V/V + DESSICANT 32 FL OZ/A	8.1 a	10.0	49.7	0.3	0.3 bc	1.3 a-d
MSE	1.3	68.8	5.1	0.1	0.2	0.6
CV	16.1	77.5	4.6	371.1	124.7	66
P	<0.0001	0.5166	0.2422	0.7922	0.0229	0.0035

[x] Test was planted at the Delta Research and Extension Center, Stoneville, MS.
[y] Means followed by the same letter(s) within a column are not significantly different according to Fisher's Protected LSD (P = 0.05).[x].

Table.4. Average relative growth of four *Phomopsis longicolla* isolates growing on fungicide amended media at different concentrations.

Isolate	Azoxystrobin					Difenoconazole					Fludioxonil					Benomyl				
Dose	% relative growth					% relative growth					% relative growth					% relative growth				
(ppm)	0	1	5	10	20	0	1	5	10	20	0	1	5	10	20	0	1	5	10	20
Isolate																				
1	100	20.7	16	11.7	13.2	100	15.8	6.3	2.1	0	100	0	0	0	0	100	0	0	0	0
2	100	19.5	26.6	33.1	11.9	100	10.8	5.6	5.4	0	100	0	0	0	0	100	0	0	0	0
3	100	35.2	21	16.2	9.5	100	22.9	17.6	9	7.8	100	25	10.3	10	0	100	0	0	0	0
4	100	33.1	24.4	12.4	9.6	100	28.2	14.3	11	0	100	0	0	0	0	100	0	0	0	0

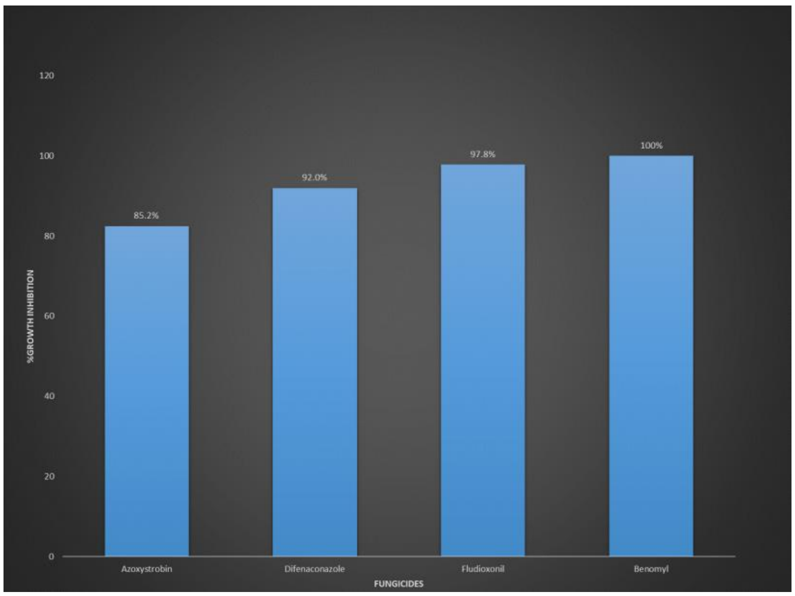

Table 2. Mean differences among the isolates of two fungicides.

Relative growth	Azoxystrobin	Difenoconazole
Isolate1	b	b
Isolate2	a	a
Isolate3	ab	ab
Isolate4	ab	ab

Fig.1. Percentage growth inhibition of four isolates growing at different fungicides