Title: **Determination of organisms affecting soybean seed quality and fungicide efficacy in reducing associated losses.**
Project # 14-2019

Annual Report

Tessie Wilkerson
662.820.0549
(twilkerson@drec.msstate.edu)

Maria Tomaso-Peterson
662.325.2593
(MariaT@pss.msstate.edu)

Rationale/Justification for research

Soybean growers in the mid-southern U.S. have suffered economic losses from reduced quality of harvested soybean seed. *Phomopsis/ Diaporthe* complex are one of the most important seed borne diseases affecting the quality of seed. Symptoms of this disease include shriveled, elongated seed which appear chalky and have reduce seed germination and emergence. Seed will also have reduced oil content and viability which will incur potential docking at the grain elevator. Seed infection is more severe when harvest is delayed and environmental conditions continue to be warm and humid during late season and harvest. Current management strategies for this disease include crop rotation with non-hosts, tillage, fungicide applications during pod-fill and resistant cultivars, although these are limited to non-existent. Additional research is needed to determine what other, if any, organisms are contributing to this reduction in seed quality whether it be a single pathogen or relationship between one or more present. The objective of this research is to provide a thorough understanding of the causes of reduced seed quality and potential management options to prevent losses due to this issue.

Report of Progress/Activity

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

Quarter 1- A small molecular laboratory has been equipped at the DREC in order to determine organisms involved in reducing quality. Seed from 2018 shelter plots have been evaluated and subjected to techniques to determine identities. Laboratory experiments using organisms isolated from prior year research trials will be established.

Quarter 2-Lab assays to determine organisms present on 2018 seed harvested from rainout shelter plots are ongoing. Molecular methods including ITS are being conducted to confirm identity in both the Stoneville and Starkville pathology labs.

Quarter 3- Lab assays to determine organisms present on 2018 seed harvested from rainout shelter plots are complete. Molecular methods including ITS were used to confirm identity in both the Stoneville and Starkville pathology labs. Seed assays using 2019 harvested seed have begun and are on-going to compare results from 2018.

An oral presentation entitled "**Identification of purported pathogen present on soybean germplasm associated with low grain quality**" summarizing molecular methods used to identify fungi present on

seed harvested from 2018 plots was presented at the Mississippi Association of Plant Pathology and Nematology/ Mississippi Entomological Association, October 21, 2019 in Starkville, MS.

Quarter 4- Seed assays using 2019 harvested seed have begun. Fungal organisms are being isolated from 10 random seed collected from each plot within each trial. Numerous subcultures are used to produce a single isolate from each fungus. Molecular techniques including PCR are being conducted to determine the identity of each individual fungus present on the seed. These results will determine the relationship if any between germplasm, and fungal organism and possible effect on seed quality.

Objective 2: Determine efficacy of fungicide on reducing growth of pathogen causing seed rot in vitro.

Quarter 1-nothing to report

Quarter 2-nothing to report

Quarter 3-nothing to report

Quarter 4-nothing to report

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

Quarter 1- A study using the same seed lines as planted under the shelters was planted adjacent to shelters to determine the role of stinkbugs in promoting seed damage.

Quarter 2- A study was planted outside of the shelters to determine the role of stinkbugs in promoting seed. Due to low emergence rates the trial was replanted. Once beans developed to R 5.5 growth stage plants were inoculated with a *Phomopsis* spore suspension on 12 September 2019. Once inoculated, insect cages were placed over 6 row feet within the plot and an economic threshold of stinkbugs pest including green, brown and red banded species were collected from natural field infestations and placed in cages for 14 days. Cages were removed after the infestation period and plants will be allowed to mature before damage assessments are recorded.

Quarter 3-Insect plots were allowed to mature and were hand harvested on December 5, 2019 and shelled using an Almaco belt thrasher. Plot weights were collected and germination tests will be conducted to determine the effect of insect damage on germination. Seed were examined for insect damage and an overall damage rating was assessed before being sent to Mid-South Grain Inspection service for an additional overall rating (DKT). Data is being analyzed for presentation at winter conferences.

Quarter 4- Yield, visual damage, and feeding injury levels were evaluated from 2019 field trials. Results suggest no differences observed between yield ($p=0.0690$), damage ($p=0.3438$), or injury level ($p=0.1324$); however, 65-414-132-1 provided up to 45% less damage when compared to the susceptible checks. Plans are being put in place for 2020 field trials. Seed has already been collected from the source so planting will be tentative during optimal planting depending on environment.

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

Quarter 1- The rainout shelters located at the Delta Research and Extension Center were repaired on 14 June 2019 which delayed planting during optimal timing for soybean. Seed for field studies was provided by Rusty Smith at USDA-ARS Stoneville, MS. These lines were selected from his nursery exhibiting the

highest potential for tolerance to seed quality issues. Commercial lines are also included as control treatments. Five field studies were planted on 2 July 2019. Two studies under the rainout shelters and two identical studies planted outside of the shelters were established to compare natural environmental conditions with conditions known to promote damage to harvested seed. Currently, agronomic observations are being collected.

Quarter 2-Plots under the constructed rainout shelters and the corresponding plots without shelters were inoculated with a Phomopsis spore suspension at growth stage R 5.5 on 5 September 2019. Plots under shelter 2 and one set of corresponding plots not under a shelter were sprayed with a common dual mode of action fungicide at approximately R7 to determine the efficacy of fungicide on reducing seed damage. To date plots under shelters have received approximately 82 hours of overhead irrigation. Harvest will be delayed to create an unfavorable harvest situation which promotes a reduction in seed quality.

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.

Quarter 3-Plots were allowed to mature and overhead irrigation was continued approximately 2 weeks post maturity to simulate delayed harvest with unfavorable weather conditions. Sheltered plants were harvested on November 7, 2019 using an ALMACO single row combine. Harvest data were collected including, plot weight, moisture, germination, and 2 damage ratings (one visual and one from seed sent to Mid-South Grain Inspection Service). Corresponding plots not under shelters were allowed to remain in the field post maturity and were harvested on November 14, 2019. Harvest data were collected identical to sheltered plots for comparison. Data is being analyzed for presentation at winter conferences.

Quarter 4-Results from the 2019 field trials suggest that 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. Plans are being put in place for 2020 field trials. Trials located under and adjacent to the shelters will be repeated to confirm results. Seed has already been collected from the source so planting will be tentative during optimal planting depending on environment.

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.