2020 Final Report: Delaware Soybean Board
Continued Survey and Characterization of Fungal Pathogens in Mid-Atlantic Soybean Production

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Project Overview:

Fungal pathogens can be very damaging to soybean (*Glycine max* (L.) Merrill) production, reducing both yield and quality. Environmental conditions can increase disease severity and favor the spread of certain pathogens. In the Mid-Atlantic, we continue to observe extreme weather events and periods of prolonged rainfall that lead to widespread fungal infection and reduced seed quality. A survey was established in 2019 to document which soilborne pathogens are most commonly observed across the region. This project identified numerous fields with Charcoal rot caused by *Macrophomina phaseolina* and fields with various diseases caused by the *Phomopsis/Diaporthe* complex. Species of *Diaporthe* are responsible for numerous diseases of soybean, including seed decay, seed rot, pod and stem blight, and stem canker. Worldwide, *Diaporthe* associated diseases are responsible for more yield and quality losses to soybean production than any other single fungal pathogen or species complex (Udayanga et al. 2015). *Diaporthe longicolla* is the causal agent of Diaporthe seed decay (syn. Phomopsis seed decay) which can reduce yield and cause quality issues with symptomatic seeds that are shriveled, cracked, and often chalky white (Hepperly and Sinclair 1978). Stem canker can include southern stem canker caused by *D. aspalathi* or northern stem canker caused by *D. caulivora*. Northern stem canker was first reported in Maryland in 1943 (Petty 1943). Since Delaware and Maryland are transition zone states, it is assumed that both northern and southern stem canker species are present in production areas. However, distribution and relative abundance of each pathogen has not been clearly established. While there are slight differences in stem canker symptomology, morphological characteristics of isolates can be highly variable and unreliable for species identification. Southern isolates have been reported to be more aggressive and to cause greater economic damage than northern isolates (Backman et al. 1985) so understanding species distribution is important for management decisions.

This proposal aimed to continue efforts from the 2019 survey by targeting Mid-Atlantic soybean farms with history of disease based on surveys and correspondence facilitated by the 2019 proposal. Project objectives included: 1) Build a collection of isolates from Mid-Atlantic soybean fields with history of soilborne fungal disease. 2) Observe the frequency of isolation within and across farms and characterize fungal isolates to species using molecular protocols. 3)

Share research findings through extension events and use findings to inform future management trials. Twenty-eight fields were surveyed and diseased plants were observed in all fields. Certain fungal species can be difficult to separate by morphology alone and molecular tools offer a way to confirm proper identification. Knowing the correct identity of a pathogen is important for management recommendations regarding variety selection or fungicide program. This project provided a foundation of isolates that will be screened for aggressiveness in greenhouse and field trials in 2021.

Project Activities and Methods:

Partnerships were made to sample 28 field sites across DE and MD. Plant samples of root and stem tissue were collected as part of this survey.

Objective 1. Build a collection of isolates from Mid-Atlantic soybean fields with history of soilborne fungal disease.

For this project, 28 soybean fields across DE and MD were identified for sampling. Scouting for symptomatic plants was conducted and isolations were made from root and stem tissue as appropriate. Symptomatic tissue was cut into 2 cm pieces, surface disinfested with 10% sodium hypochlorite for 2 min, and rinsed with sterile distilled water. Plant pieces were then placed onto potato dextrose agar (PDA) and incubated at room temperature (21°C) for 1 week. Isolates were transferred to fresh potato dextrose agar (PDA) to obtain pure cultures that were maintained in the lab for fungal collections.

Figure 1: Lexi Kessler, MS student supported by this project scouting plants at the beginning of the season

Objective 2. Observe the frequency of isolation within and across farms and characterize fungal isolates to species using molecular protocols.

Pure culture isolates were obtained and hyphal tissue was amplified using Phire Plant Direct Master Mix to amplify DNA though polymerase chain reaction (PCR). Amplified DNA was submitted for sequencing to identify fungi to species.

Objective 3. Share research findings through extension events and use findings to inform future management trials.

Findings from this project were shared through the University of Delaware's Weekly Crop Update which reaches over 700 growers, consultants, and stakeholders and provides a platform to discuss disease concerns and other production issues. Data was also discussed at the 2020 Mid Atlantic Crop School and 2021 Delaware Ag week Agronomy Day.

Results and Discussion:

Twenty-eight field sites were surveyed across DE and MD resulting in a total of 341 unique fungal culture isolations. The 20 field sites in Delaware included samples in Sussex and Kent counties. The 8 fields in Maryland included Kent, Queen Anne's, Somerset, and Wicomico counties. Frequent rainfall drove fungal disease in much of lower DE and Eastern Shore MD. Numerous fields had widespread symptoms of chlorotic leaves that lead to accelerated dry down and maturity of plants (Figure 1). In many cases this symptom set was thought to be Sudden Death Syndrome (SDS) caused by *Fusarium virguliforme*, but sampling and fungal culturing identified *Diaporthe longicolla* as the primary fungus recovered in all fields. Soybeans have a limited number of ways to express stress and symptoms from *Diaporthe* and SDS can be very similar. SDS is known for chlorosis between veins, while *Diaporthe* can cause splotchier chlorosis. A diagnostic chart was prepared for the Weekly Crop Update to help farmers distinguish symptoms of potential soybean disease (Figure 3).

Figure 2: Soybean field with widespread symptoms of fungal disease

In this survey, plants displaying symptoms were targeted and fungi were isolated from plants in 82% of field sites. Primary pathogens observed were *Diaporthe* spp. and *Macrophomina phaseolina*. Other fungi including *Fusarium* and multiple free living and saprophytic fungi were isolated, but likely of secondary association and not of economic concern. The season stayed wet and development of charcoal rot caused by *M. phaseolina* was limited, accounting for only 4% of isolates. Symptoms of charcoal rot often appear after flowering and include patches of stunted/wilted plants with leaves that remain attached after death. This fungus produces tiny black survival structures called microsclerotia on the surface of taproots, stems, and through the pith. The dominant pathogen recovered was *Diaporthe* species, which were isolated from all 82% of fields with fungi recovered. As seen in previous years, there were cases of coinfection with *M. phaseolina* and *Diaporthe.* Over 80% of fields with *M. phaseolina* also had *Diaporthe* present. Symptoms of *Diaporthe* in field samples included chlorosis, wilted leaves, stems with lines of black fungal structures, lesions along the stem, or internal zone lines (Figure 4). Species isolated included *D. longicolla* (pod and stem blight and seed decay), *D. aspalathi* (southern stem canker) and *Diaporthe ueckerae*, a species recently associated with soybean (Udayanga et al. 2015). Stem canker can include southern stem canker (*D. aspalathi*) or northern stem canker caused by *D. caulivora*. Since Delaware and Maryland are transition zone states, it is assumed that both northern and southern stem canker species are present in production areas. However, distribution and relative abundance of each pathogen has not been clearly established. In the 2019 survey, a small proportion of southern stem canker isolates were identified, but the primary species isolated from canker and stem symptoms was *D. longicolla.* Although *D. longicolla* has historically been associated with seed decay, the 2020 survey confirmed that we are observing *D. longicolla* as the dominant species responsible for lesions and stem blight in our region. Results from 2019-2020 survey efforts have identified the widespread presence of *D. longicolla*, as well as the minor appearance of Southern Stem Canker and a new *Diaporthe* spp. In many cases, these *Diaporthe* fungi have been confused or misdiagnosed as SDS. Further research is warranted to improve understanding of how these species vary in aggressiveness and to quantify the extent of yield loss caused by these pathogens.

	Diaporthe species	**Sudden Death Syndrome**	**Brown Stem Rot**
Leaves	Leaves stay attached.	Leaves fall off, can look like deer browsing.	Leaves stay attached.
Symptom	 Foliar symptoms from stem canker/pod and stem blight caused by *Diaporthe* species (Photo: A. Koehler)	 Foliar symptoms from SDS caused by *Fusarium virguliforme* (Photo: J. Pollok)	 (Photo:https://cropprotection network.org/resources/ articles/diseases/brown-stem-rot-of-soybean)
Sign	 Small black dots (pycnidia) may be in rows on stem tissue (Photo: A. Kessler)	 Blue sporodochia at the base of a soybean plant with SDS. Blue will often fade to white after exposure to air, so check soon after pulling up the plant. (Photo: J. Pollok)	none
Taproot	 Split stem has limited discoloration and may have zone lines (Photo: A. Koehler)	 Browning on either side of the interior (Photo: J.Pollok). Roots	 Browning in the pith (Photo: N. Gregory). BSR does not affect roots.

Figure 3: Diagnostic chart for soilborne soybean diseases prepared to Delaware weekly crop update.

Figure 4: Symptoms of *Diaporthe.* Lines of black fungal structure (Left). Zone lines within a stem (Top right). Canker that has caused a soybean plant to snap and break (bottom left).

References:

Backman PA, Weaver DB, Morgan-Jones G. 1985. Soybean stem canker: An emerging disease problem. Plant Disease. 69:641-648.

Hepperly PR, Sinclair JB. Quality losses in Phomopsis-infected soybean seeds. 1978. Phytopathology. 68(12):1684-1687.

Petty, MA. 1943. Soybean disease incidence in Maryland in 1942 and 1943. Plant Disease Reports. 27:347-349.

Udayanga D, Castlebury LA, Rossman AY, Chukeatirote E, Hyde KD. 2015. The *Diaporthe sojae* species complex: Phylogenetic re-assessment of pathogens associated with soybean, cucurbits and other field crops. Fungal Biology. 119(5):383-407.

Proposed Budget:

Graduate Student Stipend (2 months)	=$4,890.79
2 months of annual $26,677	=$4,446.17
Fringe Benefits 10%	=$444.62
Materials and Supplies	=$1,401
Petri Dishes	=$215
Potato Dextrose Growth Media	=$175
DNA Extraction Reagents	=$435
PCR Reagents	=$576
Sequencing	=$840
Travel	
100 Miles/wk x 14 wks @ $0.545/mile	= $763
Total Proposed Budget	**=$7,894.79**

Research Dissemination and DSB Recognition:

-November 18, 2020: Mid-Atlantic Crop School, Virtual

-January 20, 2021: Delaware Ag Week, Virtual

Public Summary:

Soilborne pathogens can reduce soybean yield and quality. Limited research has been conducted in recent years to characterize and identify problematic fungal pathogens to species. Project objectives included: 1) Build a collection of isolates from Mid-Atlantic soybean fields with history of soilborne fungal disease. 2) Observe the frequency of isolation within and across farms and characterize fungal isolates to species using molecular protocols. 3) Share research findings through extension events and use findings to inform future management trials. In 2020, twenty-eight field sites were surveyed. *Diaporthe longicolla* was the dominant pathogen identified in 2020. From this trial, an isolate collection was established that will be used in greenhouse and field screening trials in 2021.

Please contact Alyssa Koehler (akoehler@udel.edu) with any additional questions