2019 Final Report: Delaware Soybean Board
Survey and Baseline Fungicide Sensitivities of Fungal Pathogens in Mid-Atlantic Soybean Production

Dr. Alyssa Koehler, Assistant Professor and Field Crops Extension Specialist, Plant Pathology
Department of Plant and Soil Sciences, University of Delaware, Georgetown, DE
akoehler@udel.edu | (302)242-9056 | @UDPlantPath

Project Overview:

Fungal pathogens can be very damaging to soybean (*Glycine max* (L.) Merrill) production, reducing both yield and quality. Environmental conditions can affect disease severity and favor the development of fungal organisms. In the Mid-Atlantic, 2018 was a very wet year with widespread disease issues that resulted in reduced seed quality as harvests were delayed. Species of the fungal pathogen *Diaporthe*, responsible for numerous diseases of soybean, including seed decay, seed rot, pod and stem blight, and stem canker were among the most problematic. 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 project aimed to survey sixty Mid-Atlantic soybean farms to collect fungal pathogens and conduct in-vitro fungicide trials to obtain baseline fungicide sensitivities of products with potential to manage disease. Project objectives included: (1) Characterize fungal pathogens in Mid-Atlantic conventional and organic soybean production and observe the frequency of isolation across farms. (2) Build a collection of isolates that can be screened for fungicide sensitivity and used in other projects to verify pathogen species using molecular protocols. (3) Identify locations with high disease pressure that may be used for future research and demonstration plots. This survey was conducted in parallel with a proposal submitted through the Maryland Soybean Board. While this proposal funded a student internship, the MSB project funded nematode soil samples at each collection site and molecular sequencing to identify the collected fungal isolates to species. 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 can affect management recommendations for variety selection

or fungicide program. As isolates were correctly identified to species through sequencing in the MSB project, this proposal screened representative isolates for in-vitro fungicide sensitivities to three products. Through this survey, locations with high disease pressure were identified for future fungicide seed treatment efficacy trials and on-farm collaborations.

Project Activities and Methods:

Partnerships were made to sample sixty field sites across DE and MD. Forty full season and twenty double crop fields were selected. Fifteen of the selected field sites were organic and forty-five were conventional production. Plant samples of root and stem tissue were collected as part of this survey. This project was ran in parallel with a project through the Maryland Soybean Board that funded a nematode soil sample at each site along with the molecular sequencing of any fungal isolates collected from the root and stem tissue.

Objective 1: Characterize fungal pathogens in Mid-Atlantic conventional and organic soybean production and observe the frequency of isolation across farms.

For this project, sixty 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 and in-vitro fungicide trials.

Objective 2: Build a collection of isolates that can be screened for fungicide sensitivity and used in other projects to verify pathogen species using molecular protocols.

Figure 1: Student intern (now graduate student) measuring samples with a digital caliper

While many fungal species were collected in this project, this objective focused on establishing fungicide baselines for *Diaporthe* sp., *M. phaseolina*, and a subset of *Fusarium* species identified from stem and root isolations. Twenty-six isolates were selected for the in-vitro fungicide assay (Figure 1). Three fungicide products available for use in soybean production to manage pod and stem blight were selected. Products included: Quadris Top SBX (azoxystrobin + difenconzaole), Trivapro (azoxystrobin + propocimazole + benzovindiflupyr), and Miravis Top (difenoconazole + pydiflumetofen). Four fungicide concentrations (0.01, 0.1, 1, and 10 ppm) were selected to establish EC_{50} (effective concentration for 50% inhibition of mycelial growth) values. Each fungicide by concentration combination was replicated three times for each isolate. Isolates were

grown on PDA for four days and then a 6.75 mm diam plug was transferred from the edge of the colony to the center of a 60 × 15 mm Petri dish containing 8 mL of fungicide-amended PDA. Cultures were incubated until hyphal growth of isolates on control plates with no fungicide reached the edge of the plate (approx. 72-96 hrs), and the diameter of colonies on each plate were measured with a digital caliber (Figure 1). Percent growth inhibition was calculated using the formula $\% \, Inhibition = (\frac{diameter\ of\ control - diameter\ of\ treatment}{diameter\ of\ control}) * 100$. ED_{50} values were calculated by linear regression of the percentage of inhibition on $\log_{10}$ transformed fungicide concentration in SAS (version 9.4).

Objective 3. Identify locations with high disease pressure that may be used for future research and demonstration plots.

Fields with higher disease pressure were noted for potential collaborations in future fungicide efficacy research trials for foliar or seed treatment products. A field was identified at the Carvel Research and Education Center for seed treatment trials in 2020. Four collaborator fields were identified for continued scouting of *Diaporthe* species in 2020.

Results and Discussion:

Sixty field sites were surveyed across DE and MD. The forty-one field sites in Delaware included samples in Sussex, Kent, and New Castle counties. The nineteen samples in Maryland included Kent, Garrett, Queen Anne's, Somerset, and Wicomico counties. Most fields had very limited fungal disease present across the field. Plants displaying symptoms were targeted for collection and fungi were isolated from plants in 40% of field sites. By mid-season, conditions were hot and dry, which favored the development of charcoal rot caused by *Macrophomina phaseolina,* which was collected in 18% of fields. Symptoms of charcoal rot often appear after flowering when conditions are hot and dry. Symptoms include patches of stunted/wilted plants with leaves that remain attached after death (Figure 2). This fungus produces tiny black survival structures called microsclerotia on the surface of taproots, stems, and through the pith. Over 50% of fields with *M. phaseolina* also had the fungus *Diaporthe* present. The field with the most visually apparent symptoms had both charcoal rot and *D. longicolla* (Figure 3). In total, *Diaporthe* species were isolated from 17% of fields, other species isolated included *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*

Figure 2: Symptoms of charcoal rot.

Figure 3: Charcoal rot (*M. phaseolina*) and *Diaporthe longicolla* present in the same field. Symptoms progressed throughout the season with premature senescence and dry down of the canopy.

(Figure 4). 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, only southern stem canker was identified. These results confirm the presence of Southern Stem Canker and other *Diaporthe* spp. that warrant further survey work to continue to characterize the distribution and abundance of *Diaporthe* spp. in Mid-Atlantic soybean fields. Of the 196 fungi isolated, *Diaporthe* spp. and *M. phaseolina* were the most economically important pathogens. *Sclerotinia sclerotiorum*, the causal agent of white mold, was only found in one field in far western MD (Garrett County). Other fungi including eight species of *Fusarium* and multiple free living and saprophytic fungi were isolated, but likely of secondary association and not of economic concern.

Figure 4: Symptoms of soybean stem canker.

To screen for in-vitro fungicide sensitivities, a subset of twenty-six isolates were selected. The sample set was composed of eight *M. phaseolina* isolates, two *Diaporthe aspalathi*, one *Diaporthe ueckerae*, nine *Diaporthe longicolla*, and six *Fusarium* species that included *F.* fujikouri (1), *F. armeniacum* (1), *F. graminearum* (2), and *F. oxysporum* (2). *F. oxysproum* (13% of fields) and *F. graminearum* (7% of fields) are species frequently associated with Fusarium root rot on soybean. *F.* armeniacum (2% of fields) was first reported in US soybeans in Iowa in a 2008-09 survey (Ellis et al. 2012).

F. *fujikouri* (2% of fields) was first associated with US soybeans in a Kansas survey from 2010-2012 (Pedrozo et al. 2015). These species have not been previously identified in Delaware and will be further investigated. Each of the fungicides tested in-vitro inhibited all fungi in the trial (Table 1). Among the *Diaporthe* species, *D. longicolla* was most sensitive to all of the fungicides screened. *Fusarium* species were the least sensitive to all of the fungicides screened, but none of the products tested are labeled for *Fusarium* spp. This trial provides initial baselines for in-vitro fungicide performance. The culture collection obtained through this survey will be utilized in future research to assess seed treatment products.

Table 1: In-vitro fungicide trial ED_{50} values.

		Fungicides [a]		
		Quadris Top SBX	Trivapro	Miravis Top
Group	n [b]		ED_{50} (ppm) [c]	
Macrophomina phaseolina	8	0.07	0.05	0.15
Diaporthe longicolla	9	<0.01	0.13	<0.01
Diaporthe aspalathi	2	0.17	0.13	0.23
Diaporthe ueckerae	1	0.02	0.19	0.13
Fusarium spp.	6	1.17	2.4	0.26

[a] PDA was amended with each fungicide to establish fungicide concentrations of 0.01, 0.1, 1, and 10 ppm. Isolates were transferred to the center of a 60 × 15 mm Petri dish and incubated at 22 C for 72-96 h. Each treatment was replicated three times per trial.
[b] Number of isolates from each group that were tested.
[c] ED_{50} values were calculated by linear regression of the percentage of inhibition on $\log_{10}$ transformed fungicide concentration in SAS (version 9.4).

References:

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

Ellis ML, Arias MD, Leandro LF, and Munkvold GP. 2012. First report of *Fusarium armeniacum* causing seed rot and root rot on soybean (*Glycine max*) in the United States. Plant Disease. 96(11):1693-1693.

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

Pedrozo R, Fenoglio JJ, and Little CR. 2015. First report of seedborne *Fusarium fujikuroi* and its potential to cause pre-and post-emergent damping-off on Soybean (*Glycine max*) in the United States. Plant Disease. 99:1865.

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.

Project Expenditures:

Pre-baccalaureate Student Internship (May- Late August) total		= 5,872.16
14 weeks x 3.5 days/week x 8 hrs/day x 14.00/hr.	= 5,488	
Fringe benefits 7%	= 384.16	
Materials and Supplies		= 987
Small Petri Dishes for fungicide trials	= 574	
Potato Dextrose Growth Media	= 213	
Large Petri Dishes, growth media, and additional supplies	= 200	
Travel		=1,907.50
250 Miles/wk x 14 wks @ $0.545/mile		
Total		**$8,766.66**

Research Dissemination and DSB Recognition:

-November 20, 2019: Mid-Atlantic Crop School, Ocean City, MD

-January 9, 2020: Hudson Consulting Educational Meeting, Laurel, DE

-January 15, 2020: Delaware Ag Week, Harrington, DE

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)** Characterize fungal pathogens in Mid-Atlantic conventional and organic soybean production and observe the frequency of isolation across farms. **(2)** Build a collection of isolates that can be screened for fungicide sensitivity and used in other projects to verify pathogen species using molecular protocols. **(3)** Identify locations with high disease pressure that may be used for future research and demonstration plots. In 2019, sixty field sites were surveyed. Soilborne fungal pathogens were isolated from 40% of sampled fields. Three species of the fungus *Diaporthe* were isolated from stem and root tissue across 17% of fields, along with *Macrophomina phaseolina*, the causal agent of charcoal rot, isolated from 17% of fields. A subset of twenty-six isolates were selected for in-vitro fungicide efficacy to three fungicides. All fungi screened were sensitive to all products with ED_{50} values ranging from <0.01 – 2.4 ppm. From this trial, a field was identified that will be used for soybean seed treatment efficacy trials in 2020, other field sites with *Diaporthe* species present were identified for continued survey work in 2020.

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