James Buck Project Update (2019)

Two graduate students are currently working on soybean disease projects in Dr. Buck's program: Bhawana Ghimire (started Aug, 2017; Dr. Li on committee) and Bennett Harrelson (Aug, 2018; Dr. Kemerait on committee). Ms. Ghimire's investigated host colonization by the stem canker pathogen, *Diaporthe aspalathi*, determined if an in vitro toxin bioassay can be used to verify host resistance. She will finish fall of 2019. Mr. Harrison's project was directly funded by the 2019 Ga Soybean Commission award. His project update is below:

An Evaluation of *Cercospora sojina* Fungicide Resistance and Race Determination in Georgia Soybean Production

Bennett Harrelson

In 2018 and 2019, 80 isolates of *C. sojina*, or frogeye leaf spot (FLS), were collected throughout the state of Georgia from state variety testing locations and commercial soybean fields. Soybean foliage displaying symptoms associated with FLS was collected and taken back to the lab for processing. Symptomatic lesions were observed for sporulation and conidia were transferred to V8 agar plates. Isolates recovered in 2018 were screened for fungicide resistance using a restriction fragment length polymorphism (RFLP) assay to determine either isolate resistance or sensitivity to the fungicides in the class of quinone outside inhibitors (QoIs), or also known as strobilurins. *C. sojina* isolates containing the G143A mutation, that conveys resistance to QoI fungicides, produced two distinct band fragment sizes, whereas sensitive isolates produce only one fragment length when cast on agarose gels. Isolates from 2019 have been recovered from infected soybean leaves and will processed using the same methods as were the isolates from 2018. All isolate DNA recovered from 2018 and 2019 will also be sent for sequencing to confirm RFLP results.

To determine the races of *C. sojina* present in Georgia soybean production, race trials for all isolates recovered in 2018 and 2019 are being conducted. To do so, 6 differential cultivars: Davis, Hood, Lincoln, Lee, Tracy, and Blackhawk are being used to screen isolates based on their ability to infect each differential cultivar. Trials are designed using a randomized complete block design (RCBD) with three replicates and each trial being repeated. Due to space limitations, only 8 isolates are used per trial. Spore suspensions of each isolate are made and

adjusted to a standard concentration of $6x10^4$ spores/mL and sprayed on fully expanded soybean trifoliates. After inoculation, soybean plants are placed in dark humidity chambers for 24 hours. After 24 hours, plants are removed from the chambers and again sprayed with a *C. sojina* spore suspension and returned to the humidity chambers for an additional 24 hours. After the additional 24 hours, plants are returned to the greenhouse and results are recorded after 14 days, with a positive reaction producing lesions consistent with FLS and a negative reaction producing no lesions. Based on each *C. sojina* isolate's disease reaction, either positive or negative, on each of the 6 differential cultivars, a race designation for each isolate will be determined using the 'hagis' package in R.