**Year End Report to cover October 1, 2016 - December 31, 2017**

**Objective 1: Development and deployment of a panel of QPCR probes to identify and quantify fungal seedling pathogens of soybean (A. Fakhoury-SIU, M. Chilvers-MSU, and D. Malvick-UMN)**

Chilvers Lab: A real time PCR and an isothermal RPA assay for genus and species level detection of *Phytophthora*, *Phytophthora sojae* and *Phytophthora sansomeana* was developed and published. The real-time PCR assay is applicable for use on samples in the lab setting, and most research and diagnostic labs are familiar with this type of assay and have equipment to run the real-time PCR assay. The advantage of this assay is the ability to quantify the pathogen in soil and plant samples. For example the assay could be used to assess the ability of seed treatments to protect from *Phytophthora* infection, or assess germplasm for the ability to limit infection. The isothermal RPA assays that were developed can also be used in the diagnostic lab with the same equipment, but give a quicker turnaround of less than 20 minutes. The RPA assays also have the advantage of being conducted with much simpler equipment, allowing them to be used in the field to diagnose a plant sample in the field.

Fakhoury Lab: Several assays have been developed that appear promising alone and in combination for selective detection of a group of key fungal seedling pathogens of soybean. we have developed several probe-based assays for the detection and quantification of top 10 fungal species frequently found associated with seedling diseases in Soybean, as determined in the first phase of the USB-NCSRP project. The list of fungal species includes *F. oxysporum, F. solani*, *F. acuminatum*, *F. equiseti*, *F. graminearum*, *F. sporotrichioides*, *F. proliferatum*, *T. harzianum*, *M. elongata* and *R. solani.* In addition, a more general assay targeting all Fungi was developed and showed more specificity and accuracy than available tests.

The primer/probe sets were designed based on the intergenic spacer of the ribosomal RNA (IGS) and translation elongation factor alpha gene (EF1). The designed primer/probe assays were first tested in silico against a constructed database collected in this project. Furthermore, the specificity of the qPCR assays has been tested against at least twenty non-target fungal species, all of which turned out to be specific. All of the assays showed optimal amplification efficiency and a limit of detection below 0.1 pg of the target pathogen DNA. A manuscript summarizing our efforts is currently in preparation. Currently we are working on including several of these assays in a multiplex RT-qPCR method which would reduce time, technical variation and cross contamination while providing the same level of sensitivity observed in singleplex reactions..

**Objective 2: Curate the collection of fungal pathogens collected during the first phase of this project (A. Fakhoury-SIU)**

The Fakhoury lab has finished cataloguing 3000 fungal isolates that are now stored and maintained at SIU. Long term storage methods have been optimized. A searchable site is still under construction and will be publically available by the end of 2017. Curated sequences are currently being submitted to GENBANK. Additional bioinformatics tools and features are being tested before final release.

**Objective 3a: Characterize *R. solani*anastomosis groups affecting soybean seedlings throughout the U.S. (S. Everhart and T. Adesemoye-UNL)**

Our results have expanded the collection of Rhizoctonia root and stem rot isolates, adding a total of 52 *Rhizoctonia* isolated from soybean fields in Nebraska, with an additional 31 *Rhizoctonia* isolated in the 2017 season. Thus far, we have identified *Rhizoctonia zeae (23)*, *R. solani* AG-4 (20), *R. solani* AG-3 (2), *R. solani* AG-2 (1), *R. solani* AG 1-IB (4), and AG-B (2). Our work is further characterizing the level of pathogenicity of these isolates and has identified a surprising number of *Rhizoctonia zeae* that are pathogenic to soybean.

**Objective 3b: Monitor shifts in fungicide sensitivity in *R. solani* populations (S. Everhart and T. Adesemoye-UN)**

Fungicide sensitivity assays are currently underway for *Rhizoctonia* isolates using the plate dilution method. Screening will be done for four fungicides with different modes of action: propiconazole (DMI), fludioxonil (Phenylpyrrole), thiabendazole (TBZ), and penflufen or sedaxane (SDHI). Results from this work will use isolates previously collected in the North Central states and new isolates collected from Nebraska, to provide a comprehensive overview of sensitivity across the region.

**Objective 3c: Identification and characterization of resistance and tolerance to Rhizoctonia root rot (D. Malvick-UMN)**The Malvick lab has been investigating resistance and tolerance to Rhizoctonia root rot in northern soybean germplasms in greenhouse and field trials.  Replicated field and greenhouse studies were conducted to demonstrate that different soybean cultivars and breeding lines respond differently to*R. solani*in survival, growth, and yield. Varieties and lines responded with significant differences in plant height and stand count in the greenhouse and with significant differences in stand count and yield in the field for inoculated vs. noninoculated treatments. We have continued to refine and evaluate greenhouse inoculation methods, and our results suggest that different inoculation methods are more effective than others at detecting resistance/tolerance to R. *solani .*

**Objective 4a: Pathogenicity of *Fusarium* species and identify resistant germplasm (F. Mathew-SDSU)**

Screening soybean germplasm (performed in March to August 2017) = For *F. graminearum*, of the 160 soybean genotypes that were screened for resistance, the fungus caused significantly shorter lesions on 15 genotypes when compared to the susceptible check at *P* = 0.05.For *F. proliferatum*, of the 227 genotypes that were screened for resistance, the fungus caused significantly shorter lesions on 85 genotypes belonging to maturity group 0 and 43 genotypes belong to maturity group I compared to the susceptible check at *P* = 0.05. For *F. sporotrichioides*, of the 115 soybean genotypes, the pathogen did not cause significantly shorter lesions on any of the genotypes when compared to the susceptible check at *P* = 0.05. For *F. subglutinans*, the pathogen caused significantly shorter lesions on 21 genotypes when compared to the susceptible check at *P* = 0.05.

Cross-pathogenicity of *Fusarium* causing disease on soybean and corn (performed in November 2017) = Seven *Fusarium* species were identified causing disease on corn, among which *Fusarium graminearum* and *Fusarium oxysporum* were most frequently recovered (≥20%). In the greenhouse pathogenicity study, significant differences in aggressiveness (*P* ≤ 0.05) was observed among the *Fusarium* species with *F. graminearum* (FG13) and *F. acuminatum* (FA8) being the most aggressive. The cross-pathogenicity experiment is in progress to determine the effect of *Fusarium* isolates from corn on soybean.

Publication =

Okello, P. N., and Mathew, F. M. 201X. Interaction between *Fusarium*and soybean cyst nematode on soybean (*Glycine max* L.). Plant Dis. (PDIS-10-17-1570-RE; *Accepted*16-Dec-2017).

Outreach =

Byamukama, E., Strunk, C., Tande, C. and Mathew, F. 2017. Sudden death syndrome of soybean confirmed in South Dakota. Online, iGrow Published September 2017.

Okello, P., Osborne, S., Kleinjan, J., and Mathew, F. 2017. Evaluating fertilizer effect on the interaction between *Fusarium proliferatum* and soybean cyst nematode on soybean. American Phytopathological Society Annual Meeting, San Antonio, TX. August 5-August 9, 2017 (Poster).

Okello, P., Osborne, S., and Mathew, F. 2017. Effects of N-P-K fertilizer rates on the interaction between *Fusarium virguliforme* and soybean cyst nematode on soybean. American Phytopathological Society Annual Meeting, San Antonio, TX. August 5-August 9, 2017 (Poster).

Byamukama, E. Mathew, F. Strunk, C. and Tande, C. 2017. Scout for root rots in soybean. Online, iGrow Published June 2017.

**Objective 4b. Improve understanding of the biology of *Fusarium* sp. as seedling pathogen of soybean (K. Little-KSU)**

*Fusarium* spp. are one of the most important pathogen groups on soybeans, their identity and frequency in seeds as well as their importance as seedborne pathogens remains unclear. Thus, the objectives of this work was to characterize: i) the identity and frequency of *Fusarium* spp. present within 408 soybean seed samples in the state of Kansas during three growing seasons 2010. 2011 and 2012; and ii) to test the pathogenicity of the most commonly encountered seedborne Fusarium spp. on soybean seeds and seedlings under growth chamber and greenhouse conditions using artificially inoculated seeds. A semi-selective medium (PCNB) was used for Fusarium isolation. Identification was based upon morphological characters and PCR. The influence of Fusarium spp. on soybean seed germination and vigor was assessed by pathogenicity assays in laboratory and greenhouse. The three-year screening effort showed that 33% of the seed samples analyzed contained Fusarium spp. at some level. Nine Fusarium species were identified among the infected seed samples. Fusarium semitectum was the most frequently encountered species, followed by *F. proliferatum*, *F. verticillioides,* *Fusarium acuminatum*, *F. equiseti*, *F. thapsinum*, *F. fujikuroi*, *F. oxysporum*, and*F. graminearum* were least frequently observed in infected soybean seeds. Regarding pathogenicity, only soybean seeds artificially inoculated with *F. proliferatum*,*F. graminearum*, *F. fujikuro*i, F. oxysporum, and F. thapsinum significantly decreased seed germination (p > 0.001) and vigor (p > 0.001) when estimators of seed quality were compared with mock-inoculated control. No significant reductions of seed quality were observed for seeds artificially inoculated with F. semitectum, F. verticillioides, F. acuminatum, and F. equiseti. Understanding the relationship between pathogenic *Fusarium* spp. and soybean seeds will contribute to improvements for seed health testing methods, and ensure global food security, quality and production.

**Objective 5: Improve understanding of the biology of *Pythium* as a seedling pathogen of soybean (A. Robertson-ISU and M. Chilvers-MSU)**

At ISU, a growth chamber experiment with two cold stress temperatures (4ºC and 10 ºC), two cold stress timings (24 and 96 hours after planting), three cold stress durations (24, 48 and 96 hours), and two levels of seed treatments (Intego SuiteTM and untreated) was done in cups inoculated with *P. sylvaticum* or a non-inoculated control. Emergence was reduced when the pathogen was present compared to the non-inoculated controls. In untreated seed, cold stress duration reduced emergence, but no difference in timing of cold stress and cold stress temperatures (4ºC and 10 ºC) were detected. The seed treatment improved emergence, reduced root rot severity, and increased shoot weight. There was no significant effect of seed treatment on root weight. Data from this study confirm cold stress soon after planting may increase the risk of reduced crop stands and suggests seed treatments effectively protect seedlings when adverse conditions are expected soon after planting. Two manuscripts describing these studies were prepared and submitted for peer review.

A high-throughput fungicide sensitivity assay for oomycetes (*Pythium* and *Phytophthora*) was also developed and a manuscript was submitted for publication, the manuscript is currently being revised. The advantage of the assay is the ability to screen many isolates for fungicide sensitivity or fungicides for their efficacy, as opposed to the current poison plate assays which are more labor and resource intensive. The assay has been utilized to assess the fungicide efficacy of mefenoxam and ethaboxam across 84 oomycete species collected from diseased soybean seedlings. Graduate students and technicians were trained from Iowa State in use of the protocol.

Published abstracts and presentations**:**

Serrano, M. and Robertson, A. E. 2017. The effect of cold stress on damping-off of soybean caused by Pythium sylvaticum. (Abstr.) Phytopathology 107:S5.1.

Lerch, E. and Robertson, A.E. 2017. Co-inoculation of Pythium sylvaticum and Fusarium oxysporum on soybean seedling disease development. (Abstr.) Phytopathology 107:S5.173

Serrano, M. and Robertson, A.E. Using seed treatments to manage soybean seedling disease. Iowa Soybean Association On-Farm Research Conference, Feb 2017 (~150 attendees)

**Objective 6: Evaluate the effect of multiple pathogen interactions on seedling disease (A. Robertson and G. Munkvold-ISU)**

A cup assay was used to evaluate the effect of the interaction between *Pythium sylvaticum, P. irregulare, Fusarium graminearum and F. oxysporum* on soybean seedling disease development under controlled environment conditions. Cups were inoculated with either *Pythium* alone, *Fusarium* alone, or co-inoculated with both genera. Non-inoculated cups were used as a control. Preliminary data analysis showed seedling disease was more severe in cups inoculated with *Pythium* compared to those inoculated with *Fusarium*. No difference in disease development was observed for the *Pythium* species tested, but more severe disease development occurred on seedlings inoculated with *F. graminearum* compared to those inoculated with *F. oxysporum*.

**Objective 7: Impact of seed treatments on the interaction of seedling pathogens (A. Fakhoury and J. Bond-SIU)**

At SIU, a greenhouse experiment has been established to test the effect of seed treatments on seedling pathogens. In a first experiment, *Fusarium oxysporum*, *Rhizoctonia solani* were used to inoculate the soil using infected sorghum seeds. Next, treated seeds were planted and covered with a thin layer of soil. Roots were collected 3 weeks after planting and qPCR assays are currently being conducted to quantify each pathogen.

In a second experiment, Fusarium species were selected since they were the most common pathogens isolated and their density was significantly higher. Fusarium species included *F. sporotrichioides*, *F.* oxysporum, and *F. proliferatum* isolates. A dual plate assay was conducted to test for competition and antagonism between these species. The dual pate assays confirmed an antagonism between *F. oxysporum*, *F. proliferatum*, and *F. acuminatum.*

Following, we carried a second test using different media (Weak PDA and Oatmeal Agar). The results were consistent, showing non-environmental dependence of the interaction between these species.

The pathogenicity of each pathogen was next tested separately in a greenhouse setting Subsequently, numerous permutations have been conducted, including pairwise and total combinations of the selected isolates. Disease scores and vigor ratings were taken at 10, 18 and 28 days. No difference in root rot scores and vigor were observed between the different permutations of the selected 3 fusaria. Rhizosphere soil tightly attached to roots and rhizome were collectedf **End of Project Final Report**

for quantitative PCR. At a later stage of this set of experiments, fungicide seed treatments will be incorporated as an additional variable affecting the interaction between the different isolates and soybean

**Objective 8:** **Communicate research results with farmers and stakeholders (K. Wise-UK and others)**

Albert and Kiersten met with the CPN group in Chicago in October and drafted a short communication piece summarizing some of Carl Bradley's work on *Rhizoctonia solani* AG groups. The draft is in review now, and we hope to distribute it the SRII website and media outlets in early November.  Once this draft is final, we will also distribute to the co-PIs to show them an example of the types of papers/outputs we are hoping to summarize from this project.