North Central Soybean Research Program

Seedling Diseases: Biology, Management and Education

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Progress report for 2018

Soilborne seedling and root diseases of soybean significantly reduce yields in the North Central region of the United States. Seedling diseases rank among the top four pathogen threats to soybean, because their insidious nature makes them difficult to diagnose and control. It is nearly impossible to predict when they will take a heavy toll, until it happens. The challenges and failures of managing soilborne diseases and pathogens of soybean and other crops are based in part on limitations in knowledge and methods.

This project addresses critical limitations in identifying and managing seedling diseases. Producers and industry will see benefits in the form of rapid diagnostics and management recommendations. This benefit will also help industry in their assessments in pesticides and germplasm development.

Project Objectives

1. Development and deployment of a panel of QPCR probes to identify and quantify fungal seedling pathogens of soybean
2. Curate the collection of fungal pathogens collected during the first phase of this project
3. Improve understanding of the biology of *Rhizoctonia solani* as a seedling pathogen of soybean
4. Improve understanding of the biology of *Fusarium* sp. as a seedling pathogen of soybean
5. Improve understanding of the biology of *Pythium* as a seedling pathogen of soybean
6. Evaluate the effect of multiple pathogen interactions on seedling disease
7. Impact of seed treatments on the interaction of seedling pathogens
8. Communicate research results with farmers and stakeholders

Reporting Period Accomplishments

Objective 1: *Develop and deploy of a panel of QPCR probes to identify and quantify fungal*

seedling pathogens of soybean

Phytophthora genus-specific markers that will have applicability across species and hosts have been developed and are currently being validated. In addition, preliminary *Pythium* genus specific markers have been designed and are undergoing additional testing.

We have added another universal assay targeting both Phytophtora and Pythium genera. The assay has been optimized and tested for efficiency and sensitivity. Multiplexing is still being tested for the probe panel, and if successful it will reduce the number of assays from 10 assays to 3 or less. A manuscript is under preparation entitled “A probe panel assay for the detection and quantification of seedlings pathogens in soybean fields” is under preparation and will be submitted during 2018.

Objective 2: *Curate the collection of fungal pathogens collected during the first phase of this project*

We have finished cataloguing 3000 fungal isolates that are now stored and maintained at SIU. A searchable website is under construction and will be publically available soon.

Objective 3: *Improve understanding of the biology of Rhizoctonia solani as a seedling pathogen of soybean*

Our results have expanded the collection of Rhizoctonia root and stem rot isolates, adding a total of 114 Rhizoctonia isolated from soybean fields in 2015, 2016, and 2017. Thus far, we have identified *Rhizoctonia zeae* (75), *R. solani* AG-4 (26), and 13 other *R. solani* identified as one of the following anastomosis groups: AG1-1 IA, AG-B, AG-3, AG-5, AG-K, and AG-2-1. 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.

Fungicide sensitivity is being determined for four commonly used seed treatments in soybean and corn: azoxystrobin (QoI), fludioxonil (phenylpyrrole), prothioconazole (DMI), and sedaxane (SDHI). Average EC_{50} of 41 *Rhizoctonia zeae* isolates for fludioxonil was 0.1 ppm (range: 0.03 - 0.33 ppm). Average EC_{50} of 15 *R. zeae* isolates for prothioconazole was 0.17 ppm (range: 0.08 - 4.5 ppm). Preliminary screening for azoxystrobin showed that EC_{50} of 28 *R. zeae* isolates was more than 100 ppm.

Our population analysis is also underway. We obtained GBS data for 12 *R. zeae* was obtained from Dr. O. O. Ajayi-Oyetunde and Dr. C. Bradley. Data was filtered and 2,161 loci were identified as potential candidates for developing SNP markers. Further analysis is underway to identify loci that are polymorphic for at least 25% samples. Markers developed from this data will be used for analyzing population structure of *R. zeae* isolates obtained from Nebraska.
We continue to identify common *R. solani* anastamosis groups on soybean in MN and determine if early maturity group soybean germplasms vary in response to the common isolate

types. We have continued to test new isolates of *R. solani* from fields in MN. We have determined that the AG 2-2 IIIB type is common on soybean seedlings in MN and that these isolates vary widely in aggressiveness/virulence on different soybean varieties This work is continuing and preparations are underway for field studies.

Objective 4: *Improve understanding of the biology of Fusarium sp. as a seedling pathogen of soybean*

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 $P = 0.05$.

Publication

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

Previous work has shown that approximately 25% of *F. proliferatum* isolates were found to be resistant to fludioxonil. Work with azoxystrobin has shown that approximately 12.5% of *F. proliferatum* isolates and 6% of *F. semitectum* show some level of resistance to azoxystrobin.

Of the three Kansas *F. oxysporum* isolates tested against azoxystrobin so far, all three (100%) are resistant to azoxystrobin. More work is needed on *F. oxysporum* in Kansas in this regard. Isolates of other *Fusarium spp.,* including *F. graminearum, F. thapsinum, F. tricinctum*, and *F. verticillioides* did not appear to be resistant to this fungicide active ingredient, although more isolates of these species need to be tested.

Although *Fusarium proliferatum* has showed potential to cause soybean seedling and root rots, its influence and conditions necessary to negatively affect seed quality still uncertain and underexplored. The objectives of this study were to evaluate the aggressiveness of *Fusarium proliferatum* and their inoculum potential on soybean seed quality. Screening for aggressiveness was conducted using laboratory and greenhouse assays. Eight *F. proliferatum* isolates were used and the results, from all of parameters tested, were compared with mock-inoculated controls. Overall, all of *F. proliferatum* isolates tested significantly decreased seed germination in laboratory ($p < 0.001$) assays. In greenhouse assays, most *F. proliferatum* isolates tested were also able to reduce seed vigor ($p < 0.001$) when compared with mock-inoculated control.

Four *F. proliferatum* isolates were used to study the influence of inoculum potential treatment and its interaction with aggressiveness on soybean seed quality using the rolled-towel assay. There was a highly and significant interaction between the isolate aggressiveness and the inoculum potential treatment used on the percentage of artificially inoculated seeds ($p < 0.001$). Overall, the effects of seedborne *F. proliferatum* isolates on soybean seed germination decreases as the inoculum potential in contact with the seeds decreases. No significant reduction of seed germination was observed when soybean seeds were treated with low inoculum potential (2.5 x 10^1 conidia ml-1) with both moderate and highly pathogenic isolates, suggesting that *F. proliferatum* has the potential to reduce soybean seed quality, depending on the aggressiveness and inoculum potential of the pathogens present in seeds.

Objective 5: *Improve understanding of the biology of Pythium as a seedling pathogen of soybean*

Peer-reviewed papers accepted pending revision:

1. Serrano, M., McDuffee, D. and **Robertson, A.E.** XXXX. Seed treatment reduces damping-off caused by *Pythium sylvaticum* on soybeans subjected to periods of cold stress. Can. J. Pl. Path. (accepted pending revision).
2. Serrano, M. and **Robertson, A.E.** XXXX. The effect of cold stress on damping off of soybean caused by *Pythium sylvaticum*. Plant Dis. (accepted pending revision).

Presentations:

Robertson, A.E. 2018. Soybean Seed Treatments. ISUEO Crop Advantage Series: Waterloo. January 2018.

A manuscript describing fungicide sensitivity of four species from across the NC region is in progress.

Manuscripts describing guidelines for accurate fungicide sensitivity estimation and the high-throughput fungicide sensitivity assay were submitted for publication.

See: Significant influence of EC50 estimation by model choice and EC50 type - *Plant Disease, December 2017*

A manuscript describing the high-throughput fungicide sensitivity assay is being revised for resubmission to Phytopathology. An additional manuscript describing Pythium and Phytophthora species sensitivity to mefenoxam and ethaboxam is being written.

Objective 6: *Evaluate the effect of multiple pathogen interactions on seedling disease*

The following paper was submitted for review:

Lerch, E. and Robertson, A.E. XXXX. Effect of co-inoculations of *Pythium* and *Fusarium* species on seedling disease development of soybean. Can. J. Pl. Path.

Objective 7: Impact of seed treatments on the interaction of seedling pathogens

Fewer modifications were introduced to isolate the effect of each pathogen and in combination on plant health. Fusarium species were evaluated individually and under interaction in the following scheme: A; B; C; A+B; A+C; B+C and A+B+C (whereby A= *F. oxysporum*; B= *F. proliferatum*; C= *F. sporotrichioides)*. Root length, surface area and projected area data were collected for each inoculation scheme.

Our results have shown that *Fusarium proliferatum* to be more aggressive than the other two species *Fusarium oxysporum* and *F. sporotrichioides* based on root morphology and pathogen density. On the other hand, *F. oxysporum*, and *F. proliferatum* data suggested that they have an additive (synergistic) effect when causing root rot on soybean.

Objective 8: *Communicate research results with farmers and stakeholders*
Factors to Consider Before Using a Soybean Seed Treatment. *Crop Protection Network: CPN 4003, 2018.*

Research leads to a better understanding of Rhizoctonia solani on soybean - *SRII Research Highlight, November 2017*