North Central Soybean Research Program

Seedling Diseases: Biology, Management and Education

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Soil-borne 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. Develop and deploy 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
   a) Characterize *R. solani* anastomosis groups affecting soybean seedlings throughout the U.S.
   b) Monitor shifts in fungicide sensitivity in *R. solani* populations
   c) Identify host resistance to Rhizoctonia root rot
4. Improve understanding of the biology of *Fusarium* sp. as a seedling pathogen of soybean. Determine pathogenicity of *Fusarium* species and identify resistant germplasm
5. Improve understanding of the biology of *Pythium* as a seedling pathogen of soybean
   a) Compare pathogenicity and fungicide sensitivity of *Pythium* species across the North Central region.
   b) Evaluate the effects of low temperature stress on soybean seedling susceptibility to disease and the contribution of seed treatments
6. Evaluate the effect of multiple pathogen interactions on seedling disease
7. Determine the impact of seed treatments on the interaction of seedling pathogens

8. Communicate research results with farmers and stakeholders

Research Progress April 2019

Objective 1: Development and deployment of a panel of QPCR probes to identify and quantify fungal seedling pathogens of soybean

We are validating an isothermal RPA assay for genus level detection of *Phytophthora*. The testing of this assay will provide final information needed by diagnostic companies interested in the commercialization of the kits. The kits can be used in the field with relatively simple equipment, with detection/identification being achieved in a matter of minutes. The commercialization of this Phytophthora assay will improve the in-field diagnosis capability of CCA's and diagnostic services to improve identification of soybean seedling diseases and improved management.

We have validated the probe panel on a set of seedlings inoculated with a mix of seedlings pathogens (including *Fusarium* species and Rhizoctonia), and the assays were successful in detecting the inoculated pathogens. In the past quarter, efficiency and sensitivity of the developed assays was successfully tested on a variety of soil and root samples from diseased seedlings soybean. we have added another universal assay targeting both *Phytophtora* and *Pythium* genera.

The developed probe panel can be used for the fast identification and accurate quantification of key seedlings disease pathogens from different matrices (soil, roots and stem) and provide a powerful decision tool for farmers and researchers. 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 2019.

Objective 2: Curate the collection of fungal pathogens collected during the first phase of this project .
The website that describes each isolate collection and allows for retrieval request is under construction.

Objective 3a: Characterize *R. solani* anastomosis groups affecting soybean seedlings throughout the U.S.
We have identified *Rhizoctonia zeae* and *Rhizoctonia solani* AG-4 as the two most prevalent groups among a total of more than 100 *Rhizoctonia* isolated. Other *R. solani* identified had one of the following anastomosis groups: AG1-1 IA, AG-B, AG-3, AG-5, AG-K, and AG-2-1. In 2019, we are increasing our survey collection and will characterize these isolates using population genetic markers that are under development in Objective 3b.

In characterizing the level of pathogenicity of these isolates, we have found a surprising number of *Rhizoctonia zeae* that are pathogenic to soybean. *Rhizoctonia zeae* is an important pathogen of soybean when evaluated at higher temperatures than *R. solani*.

Objective 3b: Monitor shifts in fungicide sensitivity in *R. solani* populations
Results to date indicate that *Rhizoctonia zeae* has a broad range of fungicide sensitivity to prothioconazole, sedaxane, and fludioxonil. For example, average EC_{50} from the two prothioconazole experiments were 0.174 ppm and 0.172 ppm. Average EC_{50} from the two sedaxane experiments were 0.067 ppm and 0.065 ppm. Consistency of results demonstrated reproducibility of the experiments.

Assays for determining EC_{50} for azoxystrobin using various combinations of compounds to repress alternate oxidative pathways thus far have shown no effect and *in planta* assays in the greenhouse are currently underway. Results to date are consistent with previous studies that suggest *Rhizoctonia zeae* is completely insensitive to azoxystrobin fungicide, which is currently one of most common fungicides used due to the expected high specificity of action. The greenhouse studies will be critical for determining the appropriate chemical control recommendations.

Our population analysis is also underway. We obtained whole-genome sequence data for five *R. zeae* isolates. We identified 1,594 candidate SSR markers and designed primers for 40 candidate loci that will be screened this summer for polymorphisms using a selection of *R. zeae* isolates. It is important to identify polymorphic markers so that isolates in a population can be differentiated from each other. The genetic markers will be applied to assess population structure of isolates obtained throughout the region, which will also allow us to gain deeper insight into the biology of this relatively understudied soybean pathogen.

Objective 3c: Identification and characterization of resistance to Rhizoctonia root rot

Significant differences were detected among northern cultivars and breeding lines for reaction to *R. solani* in greenhouse and field studies, suggesting that some soybean germplasms in northern maturity groups differ in susceptibility to Rhizoctonia diseases. Additional greenhouse studies are underway and preparations are being made for field studies in 2019.

The predominant anastomosis group of *R. solani* that we have detected infecting soybean in MN is AG 2-2 IIIB, and these isolates vary in aggressiveness on soybean seedlings. Fungicide sensitivity studies ae underway with multiple isolates, and the isolates vary in sensitivity to different fungicidal groups. Studies are ongoing to further characterize fungicide sensitivity and cultivar reaction to diverse isolates using different methods assay methods. This project is providing results needed to improve management of this disease.

Objective 4a: Pathogenicity of *Fusarium* species and identify resistant germplasm

Eight soybean accessions (PI437949, PI438292, PI612761A, PI438094B, PI567301B, PI408309, PI361090 and P188788) were observed to be significantly less susceptible to *F. graminearum* when compared to Williams 82 and Asgrow 1835. The eight accessions may be used in breeding programs as sources of resistance to *F. graminearum* for development of resistant soybean cultivars, which the soybean growers can use to protect yield

Publications:

- Okello, P. N., Petrovic, K., Singh, A. K, Kontz, B., and Mathew, F. M. 201X. Characterization of species of *Fusarium* cause root rot of soybean (*Glycine max* L.) in South Dakota, USA. Can. J. Plant Pathol. XX: 000-000. (*Manuscript in preparation* for submission by June 2019).

- Okello, P. N., and Mathew, F. M. 2019. Cross pathogenicity studies show South Dakota isolates of *Fusarium acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* from either soybean or corn are pathogenic to both crops. Plant Health Prog. 20: 44-49.

Objective 4b.
Improve understanding of the biology of *Fusarium* sp. as seedling pathogen of soybean
Soybean seedling-borne Fusarium proliferatum sensitivity to azoxystrobin.

F. proliferatum isolates obtained from soybean seedlings in Kansas show a range of reactions to azoxystrobin, a common active ingredient in seed treatment fungicides. Approximately 14% of *F. proliferatum* isolates tested in Kansas appear to have some level of tolerance to the fungicide. EC50s range from 1.2 to 3.0 ug of a.i./ml for the tolerant isolates and <0.01 to 0.33 ug a.i./ml for the sensitive isolates, which is log order(s) less concentration required to reduce growth for the sensitive isolates.

Screening soybean germplasm for resistance to seedling disease.
In past updates, we have reported on soybean screening using different species of *Fusarium*. Now, we have pursued screening of a wide range of germplasm with *F. proliferatum* in order to find entries some level of general resistance to this seedling pathogen. During the course of this work we have developed (and are refining) a seedling quality scale (S.D.S.) as a means to estimate seedling disease severity and general seedling health.

Screening of a subset of germplasm from the K-State soybean breeding program has shown that 9/115 genotypes are resistant (>4 S.Q.S.) to the pathogen (although these must re-tested to ensure that they are not "escapes"). A total of 39/115 genotypes showed an intermediate reaction (2 to 4 S.Q.S.). And, the remaining 67/115 were susceptible (< 2 S.Q.S.).

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

Research evaluating the effect of cold stress on soybean seedling disease caused by *P. sylvaticum* (see peer-reviewed manuscripts submitted below) did not take into account soil moisture. Preliminary trials were done in the growth chamber to include soil moisture. At high soil moisture, emergence of soybean was reduced. Inoculation with *P. sylvaticum* further reduced emergence.

The following papers regarding our work have been published:
1. Serrano, M. and Robertson, A.E. 2018. The effect of cold stress on damping off of soybean caused by *Pythium sylvaticum*. Plant Dis. 102: 2194-2200
2. Serrano, M., McDuffee, D. and Robertson, A.E. 2018. Seed treatment reduces damping-off caused by *Pythium sylvaticum* on soybeans subjected to periods of cold stress. Can. J. Pl. Path. https://doi.org/10.1080/07060661.2018.1522516

A manuscript describing the high-throughput fungicide sensitivity assay has been accepted pending minor revisions. The assay has been taught to a number of other labs to aid in speeding the process of fungicide sensitivity testing, and will be widely available once the manuscript is published. An additional manuscript describing *Pythium* and *Phytophthora* species sensitivity to mefenoxam and ethaboxam has also been accepted pending minor revisions, this manuscript clearly demonstrates the need for multiple chemistry seed treatments for the management of mixed oomycete populations, this data will be of value to farmers in making seed treatment decisions.

The high-throughput fungicide assay will enable improved monitoring for fungicide resistance amongst oomycete (Pythium and Phytophthora species), which aids in ensuring seed treatments in use are effective. The study of fungicide sensitivity of oomycetes to ethaboxam and mefenoxam demonstrates

the need for both chemistries when control of a broad spectrum of oomycetes is necessary, and this information can potentially be used to further prescribe seed treatments based on a knowledge of causal organisms.

Objective 6: Evaluate the effect of multiple pathogen interactions on seedling disease
The following paper was accepted for publication:
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. Rhizosphere soil tightly attached to roots and rhizome were collected 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
The soybean seed treatment efficacy table was updated in March 2019:
https://cropprotectionnetwork.org/resources/publications/fungicide-efficacy-for-control-of-soybean-seedling-diseases