



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. 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

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 - We have sent *Pythium* sequence data and DNA samples to collaborators Dr. Frank Martin (USDA) and Dr. Tim Miles (CMBU) for the development of *Pythium* genus specific marker. We have also continued to work with these collaborators in the validation of the isothermal RPA assay for genus level detection of Phytophthora. The testing of this assay will provide information to the diagnostic companies for commercialization of the kits. Which would allow wider deployment and use of this type of assay which can be used in the field with relatively simple equipment.

Fakhoury lab – we have added another universal assay targeting both Phytophthora 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. 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. Currently, efficiency and sensitivity of the developed assays is also being tested on soil and root from diseased seedlings soybean. 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 (A. Fakhoury-SIU)

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. (S. Everhart and T. Adesemoye-UNL)

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 zea* (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 zea* that are pathogenic to soybean.

This survey has provided novel information, which shows that *Rhizoctonia zea* (*Waitea circinata* var. *zea*) is an important pathogen of soybean, which has not been well studied. We have identified *Rhizoctonia zea* and *Rhizoctonia solani* AG-4 as the two most prevalent groups in Nebraska, among a total of more than 100 *Rhizoctonia* isolated. Our work is further characterizing the level of pathogenicity of these isolates and *has identified a surprising number of Rhizoctonia zea* that are pathogenic to soybean. We are investigating this further and if confirmed, it will be a major finding. Different methods of isolation of *Rhizoctonia* and growth media were developed or evaluated, which will enable standardized approaches to future studies on this pathogen. Thus far, we have presented information to growers about conditions that may favor *Rhizoctonia* infection as well as information on disease management.

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

Fungicide sensitivity assays are nearly complete, with results from the entire first experimental replicate showing that *Rhizoctonia zea* has a broad range of fungicide sensitivity to prothioconazole, sedaxane, and fludioxonil. However, these results also suggest that *Rhizoctonia zea* is completely insensitive to azoxystrobin fungicide, which is currently one of most common fungicides used due to the expected high specificity of action. A greenhouse study will be used to determine if these results are repeatable for the fungicide applied *in planta*.

Our population analysis is also underway. We obtained isolates of *Rhizoctonia zea* from Dr. O. O. Ajayi-Oyetunde and Dr. C. Bradley, collected from several North Central states. We obtained whole-genome sequence data for five isolates and are currently identifying polymorphic SSR loci to characterize the population structure of these populations across the region. These markers will be used to assess population structure of isolates obtained throughout the region.

Publications in 2018:

1. Adesemoye, A. O. 2018. Root and Soilborne Diseases Update. CropWatch July 2, 2018.
2. Adesemoye, A. O. 2018. Soilborne and early seedling pathogens and delayed planting in corn and soybean. CropWatch May 3, 2018.
3. Gambhir, N., S. Everhart, S. Kodati, & A. Adesemoye. 2018. Fungicide Resistance: Risk and Management. SoybeanNebraska Mag., Spring 2018, Page 22.
4. Kodati, S., A. Adesemoye, N. Gambhir, & S. Everhart. 2018. Rhizoctonia Diseases in Soybean. SoybeanNebraska Magazine, Spring 2018, Page 23.

Posters Presented in 2018:

5. Gambhir, N., Kodati, S., Adesemoye, A.O., and Everhart, S.E. 2018. Fungicide sensitivity of *Rhizoctonia spp.* isolated from soybean fields in Nebraska. Poster at ICPP Meeting in Boston, MA.
6. Kodati, S. and Adesemoye, A. O. 2018. Emerging understanding of the pathogenesis of *Rhizoctonia zea* in row crops. ICPP-APS Joint Conference holding August 1 to 5 in Boston, MA.

Presentations in 2018:

7. Everhart, S.E. and Adesemoye, A.O. 2018. An update on the project presented during the 2018 Crop Production Clinics at the Sandhills Convention Center, North Platte, NE on January 11, 2018.
8. Gambhir, N., Kodati, S., Adesemoye, A.O., and Everhart, S.E. 2018. Fungicide sensitivity of *Rhizoctonia zea* from soybean and corn in Nebraska. Presentation at International Rhizoctonia Workshop: *Rhizoctonia at crossroads: research advances and challenges*, Boston, MA.
9. Kodati, S. and Adesemoye, A.O. 2018. Diversity and pathogenicity of *Waitea circinata* on row crops. Presentation at International Rhizoctonia Workshop: *Rhizoctonia at crossroads: research advances and challenges*, Boston, MA.
10. Presentation during 2018 Nebraska Crop Management Conference at the Younes Conference Center, Kearney, NE on January 24 and 25, 2018.

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

rot (D. Malvick-UMN)

The primary goals of this project are to identify common anastomosis groups (AG) of *R. solani* on soybean seedlings in Minnesota and determine if early maturity group (MG) soybean germplasms vary in response to *R. solani*. Based on our sampling, the predominant anastomosis group of *R. solani* infecting soybean in Minnesota is AG 2-2 IIIB. Our greenhouse studies have determined that isolates in this AG group vary in aggressiveness on soybean seedlings. Fungicide sensitivity studies are underway with multiple isolates. In 2017, significant differences were detected among MG 1.5-2.0 cultivars and breeding lines for final plant population and yield in field studies in southern Minnesota, indicating that soybean cultivars and breeding lines differ in susceptibility to Rhizoctonia diseases. The field studies were repeated with the same varieties and breeding lines in 2018 in southern MN, and the study was expanded to include earlier maturity (~MG < 1) cultivars and lines at a site in northern Minnesota. Field conditions (warm and moist soil) were very favorable for Rhizoctonia root rot at both field sites this year, which created unusually severe disease pressure that killed most plants. Harvest was delayed due to wet weather and final field data from 2018 has not yet been analyzed.

Scientific Presentations

1. C. M. Floyd, P. Sharma, A. K. Chanda, and D. K. Malvick. 2018. Soybean varieties and breeding lines differ in susceptibility to Rhizoctonia root and stem rot. Presented at the Annual Meeting of the North Central Division of the American Phytopathological Society in Fargo, ND. June 2018.

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

Presentation:

1. Mathew, F. M. 2018. Improve understanding of the biology of *Fusarium* sp. as seedling pathogen of soybean. Meeting for Seedling Disease of Soybean (NCSRP and USB) Research Group, Excelsior Springs, MO. September 11-12, 2018.

Objective 2: Screening soybean germplasm (performed between April and September 2018).

1. Two accessions significantly less susceptible to *F. graminearum*; three to *F. proliferatum* and one to *F. subglutinans*.
 - a. PI 592907C was significantly less susceptible to *F. graminearum* and *F. proliferatum*
 - b. PI 361090 was significantly less susceptible to all the three pathogens
2. None of the accessions were significantly less susceptible to *F. sporotrichioides*

Publication –

1. Okello, P., and Mathew, F. M. XXXX. Screening soybean germplasm for resistance to *Fusarium graminearum*, *F. proliferatum*, *F. subglutinans* and *F. sporotrichioides* Plant Health Progress (*Manuscript in preparation* for submission by January 2019).

Objective 3: Cross-pathogenicity of species of *Fusarium* causing disease on soybean and corn (performed between April and September 2018).

1. On soybean, *Fusarium proliferatum* isolates from corn caused the greatest disease severity. Significant differences in disease severity were observed among soybean and corn isolates within *F. graminearum*, and *F. proliferatum*. However, significant differences in RTE were not observed among soybean and corn isolates within *F. oxysporum*, *F. subglutinans*, *F. equiseti-incarnatum* complex, *F. solani*, and *F. acuminatum*.
2. On corn, significant differences in root rot severity were observed among soybean and corn isolates within *F. graminearum*, *F. subglutinans*, and *F. proliferatum*. However, significant differences in disease severity were not observed among soybean and corn isolates within *F. oxysporum*, *F. equiseti-incarnatum* complex, and *F. acuminatum*.
3. The results indicate that the seven species of *Fusarium* are important root rot pathogens of corn and soybean and that the corn-soybean rotation may not help with the management of *Fusarium* root rot.

Publication –

1. Okello, P., and Mathew, F. M. XXXX. Seven species of *Fusarium* from soybean and corn are pathogenic on the two hosts. *Plant Health Progress* (Manuscript submitted on 03-Oct-2018; PHP-10-18-0056-RS).

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

Symptomatic and asymptomatic soybean seedlings were collected from the twelve environments located in central and eastern Kansas where the bulk of production occurs. Total fungi and *Fusarium* spp. were collected from seedling roots at the V2 stage. Inter- and interfield diversity measurements showed overall negative relationships between seedling-associated fungal species richness and dominance, and diversity and dominance.

In an effort to characterize environmental parameters associated with certain seedling-associated *Fusarium* spp. with soybean seedling roots, air temperature, precipitation, wind speed, soil temperatures (2" and 4"), solar radiation, and evapotranspiration were collected from each environment. As a result, differences in favored environmental conditions could be determined for particular root-associated species. Frequency of *Fusarium acuminatum* and *F. reticulatum* on soybean seedling roots was positively correlated to increased air temperature. However, *F. solani* and *F. fujikuroi* were negatively correlated to air temperature, indicating that they preferred cooler temperatures for seedling colonization.

This result was further confirmed as the same results for these species were evident when 2" and 4" soil temperatures were related to species frequencies. Peak isolation frequencies for *F. oxysporum* and *F. solani* occurred from environments where temperatures ranged between 45 to 69F and 44 and 69F, respectively. In addition to favoring cooler temperatures, these two species increased in frequency from environment where higher levels of precipitation occurred accompanied by less evapotranspiration during seedling development. On the other hand, *F. acuminatum*, which was found in abundance on soybean seedling roots, appeared to prefer higher temperatures in air and soil, and higher levels of evapotranspiration (ET_o). It is possible

that his species takes advantage of stressed seedlings, a situation that would certainly occur under higher soil temperatures and ETo. In a 15 day period, when ETo exceeded approximately 3.8 inches, *F. acuminatum* isolation frequencies were significantly higher than ETo were less than this value.

Further, higher wind speeds, which can also create stress for seedlings, were correlated to higher isolation frequencies of *F. proliferatum*. In this case, average daily windspeeds of 8 mph or greater, were associated with the highest recovery of *F. proliferatum*. This too could be due to plant stress or damage, a situation of which *F. proliferatum* could take advantage.

Further, Fusarium isolates per seedling values were calculated from environments with differences in watering regime (dryland vs. irrigated), soil type, tillage, and crop rotation. For *F. proliferatum*, dryland environments, sandy- and silty-loam soils, and corn rotations yielded marginally significantly greater numbers of *F. proliferatum* isolates per seedling ($P < 0.10$). Tillage did not have a significant effect upon *F. proliferatum* isolation, but numerically greater levels of this pathogen were isolated from seedlings under conventional tillage regimes. Also, there is a positive relationship ($r = +0.481$, $P = 0.0816$) between seedling root length and *F. proliferatum* isolation across the environments tested. This may be due to seedling stage. However, the underlying reason for this relationship is not clear."

Publications (in progress):

1. Pedrozo, R., and Little, C.R. #####. Fusarium proliferatum inoculum potential affects soybean seed and seedling quality. Plant Disease: *In preparation*.
2. Pedrozo, R., Fenoglio, J., Waite, N., and Little, C.R. #####. Fludioxonil resistance in diverse seedborne Fusarium isolates from Kansas. Plant Health Progress: *In preparation*.

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

Our 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.

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. (in press)
2. Serrano, M. and Robertson, A.E. XXXX. The effect of cold stress on damping off of soybean caused by *Pythium sylvaticum*. Plant Dis. 102: 2194-2200.

Chilvers Lab: 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 (A. Robertson and G. Munkvold-ISU)

Revisions are being made to the following paper that was submitted:

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 (A. Fakhoury and J. Bond-SIU)

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.

In a new experiment, we are working on developing an artificial media system to visualize root architecture and development as effected by seedling pathogens in a 3D view. The media contains all the required nutrients and the pictures will be analyzed using a MATLAB script. The assay will allow us to document closely using 3D imaging the modes of pathogen infection, spread and interaction with other pathogens in presence of the root system.

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