North Central Soybean Research Program

Biology and control of Sclerotinia stem rot of soybean

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In the temperate north-central soybean production areas of the United States, Sclerotinia stem rot (SSR), also known as white mold of soybean, can be a significant yield-limiting disease. SSR is caused by the fungal pathogen *Sclerotinium sclerotiorum.* Combinations of management strategies have been utilized to limit losses from SSR. These include cultural practices such as reduced tillage, crop rotation, and canopy management, and chemical control provided thorough coverage and timely application. An objective in this project is to address the effect of weather and application timing on fungicide efficacy.

A major problem is that pathogenic development of *S. sclerotiorum* is complex and not well understood. We do know that *S. sclerotiorum* is able to alter the plant recognition process via the secretion of oxalic acid OA -- a key component for the fungus to cause disease,-- and possibly other molecules. The regulation of reactive oxygen species (ROS) plays a key role in this process.

One of the major sources of ROS in plants is the plasma membrane-bound NADPH oxidases. RNA sequencing of resistance and susceptible soybean lines following *S. sclerotiorum* challenge has recently confirmed the importance of soybean NADPH oxidases in disease development. We will continue to study this mechanism in soybean in order to better understand how fungicide resistance develops.

Project Objectives

1. Determine the factors affecting fungicide efficacy in the north-central states
2. Study soybean NADPH oxidases as a novel host resistance mechanism for soybean fungicide resistance
3. Develop new outreach and disease management strategies.

Reporting Period Accomplishments

Progress report October 2016
Objective 1
The second year of a fungicide efficacy study was conducted in Michigan, Iowa and Wisconsin. The trial will be repeated in 2017.

Our spray prediction model predicting apothecial presence is now available through the integrated pest information platform for extension and education (iPiPE, Fig. 1). In Wisconsin, in 14 of the 19 scouted fields, the model successfully predicted the risk of inoculum presence during the susceptible growth stages; these results were verified by the resulting presence or absence of disease. Currently, the model is exhibiting a promising 74% success rate across soybean growing systems throughout the state. The model will be further refined using high resolution results from tri-state validations.

Objective 2

Previously, we reported that a specific group of NADPH oxidases (termed GmRBOH-VI) was up-regulated during infection, particularly at the later stages of the infection process (Fig. 2). In this period, we used a BPMV VIGS system to silence this group and determine if it is required for disease development. Using the BPMV system, we were able to achieve a 45 to 65% reduction in transcript levels compared to empty vector control (Fig. 3). GmRBOH-VI silenced soybean plants were then evaluated for their response to S. sclerotiorum challenge. Five days following S. sclerotiorum inoculation BPMV-0 soybean plants showed typical SSR symptoms and began to wilt. In contrast, GmRBOH-VI silenced plants did not show any wilting symptoms (Fig. 3). In GmRBOH-VI silenced plants, lesion development was arrested shortly after reaching the main stem, and a red/dark discoloration was apparent at the edge of the lesion (Figure 5B). This is a remarkable result that shows that silencing of GmRBOH-VI genes leads to enhance resistance in soybean against S. sclerotiorum infection, and thus could be targeted to achieve durable resistance against this pathogen.

Objective 3

In January of 2016, we initiated laboratory testing, where each fungicide was first optimized for the spiral plate assay using a selected number of isolates and adjusted the concentration of each fungicide to find the most suitable deposition concentration. To date, we have characterized sensitivity of 111 isolates from Nebraska to all four fungicides (Table 2). Fungal mycelial growth inhibition to each of these active ingredients was determined in the lab. Concentration of each fungicide active ingredient needed to inhibit fungal growth was estimated for each of the 111 samples from 35 fields in this study.

Results showed some S. sclerotiorum isolates were able to grow at higher concentrations than sensitive isolates. On average, laboratory resistant isolates were able to grow at a concentration that was approximately four to six times higher than the average concentration needed to control sensitive isolates. Reduced fungicide sensitivity was most prevalent for Prothioconazole (17.1% of white mold isolates) and found in half of fields sampled in both Antelope and Holt Counties in Nebraska. The second most frequent fungicide with reduced sensitivity white mold samples was Thiophanate Methyl (5.4%), followed by Picoxystrobin (2.7%) and Boscalid (<1%).

Our goal is to complete fungicide screening for the most relevant and representative isolates, among all 436 isolates from participating states, by the end of the year.

Objective 4
Despite the generally less favorable conditions for disease, White mold developed in pockets in both dry land and irrigated production. Severely infested fields coincided with growers that are maintaining a high fertility program, either 100 lb/A starter fertilizer, or chicken or cattle manure applications. Several visits were made with affected producers involving seed salesman and industry personnel.

April 2017

Objective 1
We have now conducted studies from multiple site-years evaluating the efficacy of fungicide treatments and timings. Fungicide evaluations were conducted a total of 26 site-years. These studies were used to test eight popular fungicide classes, including 19 active ingredients, and 11 common application timings. Moderator effects of class and active ingredient were found to significantly affect yield ($P<0.0001$) (Figure 1), and application timing was found to significantly affect disease levels ($P=0.0069$) (Figure 2). Overall, these studies will help growers select cost effective fungicide programs for use in integrated management of SSR in soybean.

Our white mold prediction model was further refined using high resolution results from tri-state validations. Models predicted apothecial observations with 82-91% accuracy during the soybean flowering period (Figure 3). Furthermore, model predictions explained end-of-season disease observations in all four validation fields and would have resulted in both the reduction of fungicide applications in low-risk areas and improved timing of critical applications in high-risk fields. The models developed here will be integrated into a smartphone-based risk assessment tool.

We are preparing a manuscript for submission to Plant Disease.

Objective 2
Previously, we reported that NADPH oxidase silenced soybean plants were evaluated for their response to *S. sclerotiorum* challenge. Remarkably, we found that silenced plants showed enhanced resistance to SSR and that these genes could be targeted to achieve durable resistance against this pathogen. In this period, we expanded our screen to include other pathogens and abiotic stress. So far, we found that the NADPH oxidase silenced plants were also drought tolerant (Figure 4).

These results suggest that knocking down expression of these genes leads to increased drought tolerance, possibly by limiting oxidative damage and ultimately death of the plant imposed by elevated ROS levels during this stress. Overall, with the multiple traits associated with the silencing of these genes, we will move forward with the generation of stable transgenic plants that further tested in the field against a broad range of abiotic and biotic factors.

A manuscript describing this work has been accepted in Molecular Plant Pathology.

Objective 3
An additional 190 isolates were obtained from Michigan and Iowa from disease outbreaks and field trial plots in 2016. In the previous year, 344 isolates were obtained from Nebraska, Michigan, Iowa, and Wisconsin, bringing the total number of isolates collected to 534. Our goal is to complete fungicide screening for the most relevant and representative isolates, among all 534 isolates, by the end of the year, including isolates from field plots that received different fungicide treatments. Fungicide sensitivity of isolates under different field treatments will be compared to determine if treatments lead to shifts in sensitivity, and results published at the county-level in an online map.

Nine isolates of *S. sclerotiorum* were exposed to fungicides for a total of 12 generations and the experiment repeated. Fungicides selected for the study were azoxystrobin (QoI), pyraclostrobin (QoI), iprodione (dicarboximide), thiophanate methyl (BZI), and boscalid (SDHI). Preliminary results showed some EC50 values of isolates exposed to different fungicides were significantly increased, although there was not a consistent trend of decreased sensitivity (Figure 5).

Publications from this objective:
Amaradasa, B.S., and S.E. Everhart. 2016. Effects of sublethal fungicides on mutation rates and genomic variation in fungal plant pathogen, *Sclerotinia sclerotiorum*. PLOS ONE. 11(12): e0168079. DOI 10.1371/journal.pone.0168079

Everhart, S., and B. Amaradasa. 2016. Fungicide stress induces genome mutation in Sclerotinia sclerotiorum. Phytopathology 106:S4.169.

Amaradasa, B., and S. Everhart. 2016. Sub-lethal fungicides induce microsatellite mutation in Sclerotinia sclerotiorum. Phytopathology 106:S4.139.

Objective 4
Several visits were made with affected producers involving seed salesman and industry personnel. In addition, most winter meeting extension talks covered white mold management and updates from this project.

Manuscripts and reports:
Fall, M.L., Boyse, J.F., Wang, D., Wilbur, J.F., Smith, D.L., Chilvers, M.I. Submitted Dec 23, 2016. Case Study of an Epidemiological Approach Dissecting Historical Soybean Sclerotinia Stem Rot Observations and Identifying Environmental Predictors of Epidemics and Yield Loss. Phytopathology XXX:XX-XX

Byrne, A.M. Chilvers, M.I. 2017. Efficacy of foliar fungicides for white mold management of soybean in 2016a. Plant Disease Management Reports 11:FC030

Byrne, A.M. Chilvers, M.I. 2017. Efficacy of foliar fungicides for white mold management of soybean in 2016b. Plant Disease Management Reports 11:FC029