North Central Soybean Research Program

Biology and control of Sclerotinia stem rot of soybean

Mehdi Kabbage (Project Leader) and Damon Smith (University of Wisconsin), Martin Chilvers (Michigan State University), Sydney Everhart (University of Nebraska-Lincoln), Daren Mueller (Iowa State University.

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: Investigate fungicide resistance emergence in *Sclerotinia sclerotiorum*
4. Develop new outreach and disease management strategies.

Reporting Period Accomplishments April 2018

Objective 1: Determine the factors affecting fungicide efficacy in the north-central states.

The data from the second year of a fungicide efficacy study conducted in Michigan, Iowa and Wisconsin is currently being analyzed.

We conducted a meta-analysis of North Central Sclerotinia Stem Rot trials conducted in Illinois, Iowa, Michigan, New Jersey, Minnesota, and Wisconsin from 2009 to 2016. This represents a total of 25 site-years. These trials focused on 10 common active ingredients and 7 common timings (V5 through R5). Most common active ingredients provide a reduction in Disease Severity Index, with boscalid, picoxystrobin, lactofen providing the best protection. However, when yield data were analyzed, boscalid and picoxystrobin were the best performers (lactofen likely imposed severe stress). The timing of fungicide application plays a significant role in maximizing disease reduction, and applications in the R1-R3 gave the best results in maximizing disease reduction and provided the best yield benefits. A manuscript is currently being prepared for publication.

Our spray prediction model predicting apothecial presence is now available through the integrated pest information platform for extension and education (iPiPE). 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.

Willbur, J., Fall, M., Bloomingdale, C.A., Byrne, A.M., Chapman, S., Isard, S.A., Magarey, R., McCaghey, M., Mueller, B., Russo, J., et al. (2017). Weather-based models for assessing the risk of *Sclerotinia sclerotiorum* apothecial presence in soybean (Glycine max) fields. Plant Disease. https://doi.org/10.1094/PDIS-04-17-0504-RE

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. https://apsjournals.apsnet.org/doi/10.1094/PHYTO-12-16-0446-R

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

Objective 2: Study soybean NADPH oxidases as a novel host resistance mechanism for soybean fungicide resistance

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.
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. 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.

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. 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.

Ranjan, A., Jayaraman, D., Grau, C., Hill, J. H., Whitham, S. A., Ané, J.-M., Smith, D. L. and Kabbage, M. (2017), The pathogenic development of *Sclerotinia sclerotiorum* in soybean requires specific host NADPH oxidases. Molecular Plant Pathology. Accepted Author Manuscript. doi:10.1111/mpp.12555

Objective 3: Fungicide resistance emergence in *Sclerotinia sclerotiorum*

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. 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.

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