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.

The overreaching goal of this project is to provide farmers and consultants with concrete control measures for Sclerotinia stem rot (white mold), and improve awareness and knowledge of Sclerotinia stem rot of soybean in the north-central region. It also provides specific information on tools that can be used to introgress resistance into commercial varieties against white mold disease.

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.

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Fungicide efficacy, and the effect of weather conditions and application timing on fungicide efficacy.

Farmers continue to be challenged with filtering through marketing information on new or repurposed fungicides for management of Sclerotinia stem rot. We evaluated a standard list of fungicides (lactofen (LACT), boscalid (BOSC), picoxystrobin (PICO), prothioconazole and trifloxystrobin (PROT+TRIF), and boscalid and fluxapyroxad/pyraclostrobin (BOSC+FLUX/PYRA), fluazinam (FLUA), fluoxastrobin and flutriafol (FLUO+FLUT), prothioconazole (PROT), tetraconazole (TETR), and thiophanate-methyl (THIM)) applied at different timings (R1-R5 growth stages) in each participating state. Each location collected weather data, disease incidence and severity and yield.

An economic analysis was also conducted for each product used and the timing of application to determine the return on investment (ROI) of fungicide application at timings from R1-R5 growth stages.

Detailed apothecia scouting data and spore trapping data were also collected. The data was used to develop a first iteration prediction model for SSR to be used to make decisions on fungicide application. However additional field experiments need to be established, not only in Wisconsin, but in other states such as Michigan, Iowa, and Nebraska to expand upon the dataset and validate model iterations to develop a more robust model. Field sites were established in all participating states.

Weather information for the models used to predict SSR, was supplied by USPest.org (http://uspest.org/wea/). USPest.org generates site-specific weather data remotely using open source algorithms. They have allowed us to set up "virtual weather stations" which allow us to get site specific, modeled weather data by simply supplying global positioning system (GPS) coordinates of the research locations.

Models were run on a daily basis when the crop is at the R1-R3 growth stages. When high risk for Sclerotinia stem rot is predicted during these growth stages, fungicide was applied. Additionally, application of fungicide was performed in separate plots based on crop phenology. The crop phenology-based program consisted of applying two applications of fungicide (once at R1 and once at R3 growth stages). Apothecial germination data and spore trapping data were also collected to support and validate model iterations.

Takeaways and recommendations:

- Lactofen (LACT), boscalid (BOSC), picoxystrobin (PICO), prothioconazole and trifloxystrobin (PROT+TRIF), and boscalid and fluxapyroxad/pyraclostrobin (BOSC+FLUX/PYRA) had the lowest level of disease incidence and were the preferred chemistries in this study.

- LACT was among the more efficacious active ingredients in high disease pressure situations, and its relatively low cost compared to other products, resulted in high estimated value to the farmer. Economical disease management balances efficacy and cost; therefore, BOSC, a highly efficacious active ingredient, was not as profitable in this analysis because of its high cost.
- Canopy closure percentage of 40% and a soil temperature between 20 and 25°C are better indicators for the production of apothecia, and therefore availability of inoculum than soybean growth stage alone.
- Newer statistical methods were used to develop a model that predicts the probability of apothecia of S. sclerotiorum being present in soybean fields during the R1-R3 flowering period. The model uses site-specific, remotely-accessible weather data, and other variables such as crop development stage and canopy closure. In the 2016 and 2017 growing seasons, we found that the model predicted the presence of apothecia with 80% accuracy.
- The above model was used to develop a prediction tool in order to time fungicide applications for better efficacy of fungicides. The use of the smart phone application SPORECASTER developed in this project, will allow for precise detection of risk areas and efficient deployment of fungicides. The app turns a few simple taps on a smartphone screen into an instant forecast of the risk of apothecia being present in a soybean field, which helps growers predict the best timing for white mold treatment during the flowering period.

Other management recommendations:
White mold is best managed by an integrated approach of selecting soybean cultivars with the highest level of resistance and adjusting cultural practices to minimize environmental factors that favor disease development. This approach requires a coordinated plan that matches the level of resistance in a soybean cultivar to expected disease potential and cropping practices that influence crop canopy closure. No single tactic will completely control white mold.

White mold is a disease of high yield potential soybean production. Although several factors are believed responsible for the increased occurrence of white mold, none may be more important than management practices or environmental conditions that promote rapid and complete crop canopy closure. White mold is particularly favored by dense soybean canopies created by plantings in narrow row widths, high plant populations, early planting, high soil fertility, or other management practices that promote rapid and complete canopy closure.

The effect of row width on incidence of white mold and subsequent yield can vary by year and is strongly controlled by annual climatic conditions. Frequently, the yield advantage of narrow row widths, compared to wide widths, is expressed even though the incidence of white mold may be greater in narrow row systems. Increasing row width from a narrow row spacing (6-8”) to a medium spacing (15”) can reduce white mold infections without compromising yields. Lowering seeding rates in narrow row systems is preferable to increasing row widths to a wide row spacing.

Crop rotations that employ non-hosts result in a reduced the incidence of white mold, but some non-hosts are better than others. A preceding crop of small grain, in contrast to corn, has a greater impact on reducing the incidence of white mold. Rotation with non-hosts such as small grains resulted in fewer of apothecia formed under the soybean canopy. The population density of apothecia was greatest in moldboard plow systems compared to no-tillage systems. Fewer apothecia in no-tillage systems is a partial explanation why lower incidence of white mold is observed in no-till fields compared to fields receiving some degree of tillage.

Biological control of white mold has also been researched. Sclerotia can be parasitized by several fungi and these fungi have been investigated as candidates for commercialization. Contans® WG is a commercial biological control product labeled for the control of Sclerotinia sclerotiorum in agricultural soils. Contans® has shown promise as a biological control agent and a potential alternative for chemical fungicides to control white mold. In Wisconsin, the best and most economical times for application are during preplanting or post-harvest on the stubble of a previously diseased crop. The time between the application of Contans® WG and the typical onset of disease should be as long as possible.

Development of genetic resistance against white mold.
Genetic resistance is the most desirable form of control against any plant pathogen.

Unfortunately, commercial soybean varieties with adequate levels of resistance against white mold are currently not available. We have identified a genetic component in soybean that can be manipulated to achieve an adequate level of resistance against this disease.

Our results show that a group of genes controlling soybean NADPH oxidase production are specifically induced in the plant following *S. sclerotiorum* infection, with peak expression at the later stages of the infection process. Thus, it appears that *S. sclerotiorum* may be co-opting the soybean ROS machinery to its benefit, by modulating the expression of host NADPH oxidases.

We used Virus Induced Gene Silencing (VIGS) to turn off, or "silence", this group of genes to see if they are required for disease development. The VIGS system is an established technique in soybean molecular genetics used to study the function of genes, particularly the function of disease resistance genes and defense genes involved in plant-microbe interactions. Using the VIGS system, we were able to achieve a 45 to 65% reduction in transcript levels in silenced plants compared to the control. The silenced soybean plants were then evaluated for their response to *S. sclerotiorum* challenge. Five days following inoculation with the pathogen, the control plants showed typical SSR symptoms and began to wilt. In contrast, the silenced plants did not show any wilting symptoms and developed normally. This is a remarkable result that shows that silencing of a specific group of soybean NADPH oxidase genes leads to markedly decreased ROS production and enhanced resistance in soybean against infection by *S. sclerotiorum.* These genes provide a potential target for the generation of SSR-resistant soybean lines.

We expanded our screen of silenced plants to include other pathogens and abiotic stress.

Surprisingly, we found that these plants were also drought tolerant — possibly because decreased ROS production would limit the oxidative damage and ultimately death of the plant imposed by excessive ROS levels during drought stress. We have know generated transgenic soybeans with the described characteristics, and we are excited to evaluate the efficacy of these new lines in the field against a broad range of diseases and abiotic stresses.

Takeaways and recommendations:

- We have identified a group of genes in soybean that can be manipulated to provide resistance against white mold.
- Targeting the same genes in soybean confers drought tolerance, thus making it feasible and viable to use a transgenic strategy to address both of these issues.
- Transgenic plant were produced as part of this grant, and will be assayed in the field against wide range of biotic and abiotic stresses
- The same transgenic approach should be pursued in commercial varieties to test these varieties and eventually release them for public use.

Fungicide resistance emergence in *Sclerotinia sclerotiorum*

Broad sampling throughout the soybean producing region was achieved by collecting a minimum of 10 samples from ten field locations in each represented state. GPS coordinates of each field site was recorded for reporting of fungicide sensitivity in each county.

Fungicide sensitivity of each isolate was determined using high-throughput fungicide resistance assays in the Everhart Lab. This was performed in a specialized instrument capable of creating a gradient of fungicide concentrations on media in an oversized Petri plate (150 mm). Fungicide sensitivity (EC50) for each isolate was determined for fungicides with different modes of action (QoI, MBC, SDHI, etc). Appropriate controls and technical replications were included.

Our work also characterized the effects of sub-lethal fungicide exposure on resistance emergence and genome evolution. *S. sclerotiorum* was an ideal model system for this study because of its noted long-term stability in genetic variation and also due to the availability of a fully annotated and optically mapped, published genome.

Our approach was to generate a panel of fungicide exposed *S. sclerotiorum* isolates, to pre-screen for small and large genomic changes using SSR and AFLP genetic markers, and submit selected isolates for whole-genome sequencing to examine extent of genomic change.

Fungicides used in this study represented all commercial fungicides currently in use for control of disease caused by *S. sclerotiorum* in the U.S. and represent chemicals with different modes of action: Azoxystrobin and Pyraclostrobin (QoI's), Iprodione (DMI), Thiophanate methyl (MBC), and Boscalid (SDHI). Sub-lethal fungicide exposure utilizes the AutoPlate 5000, a specialized instrument that creates a radial gradient of fungicide concentration Petri plate containing PDA. Each isolate is inoculated onto the fungicide gradient and myclial growth from the 50-100% inhibition zone is collected for the next generation, which is repeated a total of 12 times.

Takeaways and recommendations:

- At this point, evidence suggest that field populations of S. sclerotiorum have not developed widespread resistance to the common fungicides used.
- Results of the current study conclusively show in vitro sublethal fungicide stress induces mutations in the S. sclerotiorum genome, where future studies using whole-genome sequencing may shed more light on genomic damage specific to each class of fungicide and used to examine fungicide-resistant isolates for evidence of such mutagen exposure.
- Results were broadly disseminated and reported directly to growers
- Whole genome sequencing was performed on isolates exhibiting genomic change (as indicated by SSR / AFLP analysis conducted) and those with induced resistance. Results of SSR/AFLP analysis were submitted for publication.
- Compilation of fungicide sensitivity data and trends was performed. Bioinformatic pipelines will be developed for genomic data analysis.