National Sclerotinia Initiative

USDA-ARS

National Sclerotinia Initiative

2024 Annual Meeting

January 17-18, 2024






USA Dry Pea & Lentil Council

National Sunflower Association

US Dry Bean Council

Northern Canola Growers Association

United Soybean Board

USDA Agricultural Research Service

Edward T. Schafer Agricultural Research Center

Fargo, ND & East Grand Forks, MN

Helping farmers produce a safe, nutritious and sustainable food supply

2024 National Sclerotinia Initiative Annual Meeting
(ALL TIMES CENTRAL TIME)

January 17, 2024

7:00 – 8:00 am Breakfast (City A)

8:00 am Welcome & Introductions **– Brian Peterson, USDA-ARS, Fargo, ND**

8:10 am Welcome & Update from Plains Area **– Bryan Kaphammer, USDA-ARS, Fort Collins, CO**

8:20 am Welcome & Update from Office of National Programs **– Roy Scott, USDA-ARS, Beltsville, MD**

Sclerotinia Research Progress – Disease Management

Moderator ***Luis del Rio Mendoza, North Dakota State University***

8:30 am **Biosynthesis of Silver and Zinc Nanoparticles using Sclerotinia sclerotiorum Metabolites to Combat Fungal Pathogens –** Nickisha Pierre-Pierre, George Vandemark, Weidong Chen, USDA-ARS, Pullman, WA

8:50 am **Characterizing plant- and virus-derived proteins in important crops that enhance the resistance to white mold -** Chien-Fu Wu, Shin-Yi Marzano, Aurelie Rakotondrafara, Peihan Shu, AJ Lawrence, Yasi Kiani, USDA-ARS, Toledo, OH, University of Wisconsin - Madison

9:10 am **Double-stranded RNA targeting white mold Sclerotinia sclerotiorum argonaute 2 for disease control via spray-induced gene silencing –** Shin-Yi Marzano, USDA-ARS, Toledo, OH

9:30 am **Developing an RNA-based fungicide to manage Sclerotinia stem rot of canola -** Abdolbaset Azizi, Luis del Río Mendoza, North Dakota State University, Fargo, ND

9:50 am **Field trials of mycovirus SlaGemV1 biological fungicide sprays show promise in increasing yield and reducing white mold infection –** Connor Pedersen, Shin-Yi Marzano, USDA-ARS, Toledo, OH

10:10 – 10:30 am Break (City A)

10:30 am **Leveraging Aerial Imaging and Machine Learning to Predict White Mold in Common Bean –** John Hawkins, Michigan State University

10:50 am **Small cysteine-rich antifungal peptides as potential biofungicides for control of white mold in soybean -** Dilip M. Shah, Arnaud Thierry Djami-Tchatchou, Godwin James, Ruby Tiwari, Meenakshi Tetorya, Donald Danforth Plant Science Center, St Louis, MO

Sclerotinia Research Progress – Host Resistance

Moderator ***Weidong Chen, USDA-ARS, Pullman, WA***

11:10 pm **Role of soybean phenylalanine ammonia-lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) genes in white mold disease control -** Nick Talmo, Ji Hyun Kim, Robert Stupar, Ashish Ranjan, University of Minnesota – Twin Cities

11:30 am **Investigating the relationship between oxalic acid tolerance and basal stalk rot resistance in sunflower -** Srushtideep Angidi, Israt Zaman, Julie Pasche, Luis del Rio Mendoza, William Underwood, North Dakota State University, Fargo, ND; USDA-ARS, Fargo, ND

11:50 am ***Identification of virulence factors and their application to block Sclerotinia sclerotiorum disease development -*** *Chenggang Wang, Zhonglin Mou, Jeffrey A. Rollins*

12:10 – 1:30 Lunch Break (City A)

1:30 pm **Spatially Differentiated Gene Expression Patterns in Canola Leaves during Sclerotinia sclerotiorum Infection -** Hira Kamal, Weidong Chen, Kiwamu Tanaka, Washington State University; USDA-ARS, Pullman, WA

Sclerotinia Research Progress – Pathogen Biology

Moderator ***Sydney Everhart, University of Connecticut***

1:50 pm **A Single Laccase Acts as a Key Component of Environmental Sensing in a Broad Host Range Fungal Pathogen -** Nathaniel M. Westrick, Damon L. Smith, and Mehdi Kabbage, University of Wisconsin - Madison

2:10 pm **Development of *S. sclerotiorum* screening panels to evaluate resistance and discover conserved aggressiveness determinants across crop species -** Megan McCaghey, Hsuan Fu Wang, University of Minnesota – Twin Cities

2:30 pm **Identification and characterization pathogenicity genes affected by DNA mycovirus in *Sclerotinia sclerotiorum* -** Wei Wei, George J Vandemark, Weidong Chen, Washington State University; USDA-ARS, Pullman, WA

2:50 pm **Population genetic characterization of *Sclerotinia sclerotiorum* from USA soybean and dry bean using AmpSeq, and development of an informational survey to assess NSI impacts -** Bashir Tiamiyu, E. Nieto-Lopez, R. A. Koch Bach, S. Kodati, N. Gambhir, Sydney Everhart, University of Connecticut; Iowa State University; University of Nebraska

3:10 pm **Sclerotinia sclerotiorum SsCelp0028 protein is a cytotoxic effector contributing to virulence -** Wei Wei, Vishnutej Ellur, Nickisha Pierre-Pierre, George J Vandemark, Weidong Chen, Washington State University; USDA-ARS, Pullman, WA

3:30 – 4:00 pm Break (City A)

4:00 – 5:00 pm Poster Session (City A)

5:00 – 6:00 pm Free Time

6:00 – 7:30 pm Group Dinner (City A)

January 18, 2024

7:00 – 8:30 am Steering Committee Breakfast (The Loft)

7:00 – 8:30 am Meeting Attendee Breakfast (City A)

Sclerotinia Research Progress – Breeding

Moderator ***Jim Myers, Oregon State University***

8:30 am **A comprehensive assessment of genomic prediction models for sclerotinia stem rot resistance in soybean (*Glycine max*)** - Raju Thada Magar, Feng Lin, Muhammad Selman, Jason Anandappa, Drew Mitchell, Cuihua Gu, Randy Laurenz, Martin Chilvers, Dechun Wang

8:50 am **Identification of Brassica napus QTL for resistance to Sclerotinia sclerotiorum -** Bita Babakhani, Susan Ruud, and Luis del Río Mendoza, North Dakota State University, Fargo, ND

9:10 am **Improved white mold resistance in dry and snap beans through multi-site screening throughout major production areas -** Evan Wright, Francisco Gomez, Martin Chilvers, Michigan State University, East Lansing, MI

9:30 am **Introgression and Pyramiding of Sclerotinia stem rot disease resistant gene(s) into Canola cultivars -** Md Zahangir Alam, Luis Del Rio Mendoza, Mukhlesur Rahman, North Dakota State University, Fargo, ND

9:50-10:30 am Break (City A)

10:30 am **Using Genomic Prediction to Identify Dry Bean (Phaseolus vulgaris L.) Genotypes with Resistance to White Mold (Sclerotinia sclerotiorum Lib. De Bary) –** Jose C. Figueroa-Cerna, Jayanta Roy, Kristin Simons, Phillip McClean, Juan M. Osorno, Phil N Miklas, North Dakota State University; USDA-ARS, Prosser, WA

10:50 am **Phenotypic analysis of a MAGIC snap/dry bean population and generation of advanced lines from NAM populations -** Jim Myers, Ahmet Agir, Emma Landgraver, Joel Davis

Sclerotinia Research Progress – Breeding (Continued)

11:10 am **A QTL approach toward understanding and improving genetic resistance to white mold in common bean –** Phil Miklas, Alvaro Soler-Garzón, Jim Myers, Ahmet Agir, Emma Landgraver, Joel Davis, Jayanta Roy, A. Oladzad, Phil McClean, Jose C. Figueroa-Cerna, Kristin Simons, and Juan M. Osorno, USDA-ARS Prosser, WA; Oregon State University, Corvallis, OR; North Dakota State University, Fargo, ND

11:30 am Discussion – **Brian Peterson, USDA-ARS, Fargo, ND**

12:10 – 1:30 Lunch Break (City A)

Sclerotinia Research Progress – Breeding (Continued)

Moderator ***Jim Myers, Oregon State University***

1:30 pm **Using genomics assisted breeding to advance sunflower germplasm development -** Brent Hulke, USDA-ARS, Fargo, ND

1:50 pm **Evaluation and optimization of genomic selection for durable white mold resistance in dry bean -** Evan Wright, M.J. Irvin, Q. Song, Francisco Gomez, Martin Chilvers, Michigan State University; USDA-ARS, Beltsville, MD

2:10 pm Discussion – **Brian Peterson, USDA-ARS, Fargo, ND**

2:40 pm Break (City A)

3:00 pm Wrap Up – **Brian Peterson, USDA-ARS, Fargo, ND**

3:30 pm Departure

2024 National Sclerotinia Initiative Meeting

January 17 - 18, 2024

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Oral Abstracts

Biosynthesis of Silver and Zinc Nanoparticles using Sclerotinia sclerotiorum Metabolites to Combat Fungal Diseases

Nickisha Pierre-Pierre 1, George Vandemark 1, and Weidong Chen 1

1 USDA Agricultural Research Service, Pullman, WA 99164, USA

Funded plan of Work: Biological Control of White Mold Using the Mycovirus SsHADV-1-Infected Hypovirulent Strain DT-8 of Sclerotinia sclerotiorum

ABSTRACT:
Many fungal metabolites have great capability for forming different metal nanoparticles that can be utilized in various processes. Thousands of biologically active compounds are formed by fungal species. Some metal nanoparticles have shown their potential to act as inhibitory agents against plant pathogens. We report on the synthesis of silver nanoparticles and zinc oxide nanoparticles (AgNPs and ZnONPs) synthesized from the metabolites of the plant pathogenic fungus Sclerotinia sclerotiorum wild type WMA1. Cell free culture filtrates were combined with silver nitrate or zinc nitrate in separate experiments to synthesize nanoparticles and subsequently evaluated for their antifungal properties. This eco-friendly synthesis of nanoparticles is an alternative to the chemical method of synthesis, which involves the use of toxic surfactants. The resulting nanoparticles were characterized with UV-visible spectroscopy, transmission electron microscopy and scanning electron microscopy. The synthesized AgNPs produced a reddish brown color and the ZnONPs had a greenish chalk color. The ultraviolet visible spectroscopy peaks for the AgNP and ZnONP were 413 nm and 304 nm, respectively. The nanoparticles were also evaluated for the control of S. sclerotiorum and Botrytis cinerea on detached bean leaves. Detached bean leaves were treated with the nanoparticles at different concentrations (100 ppm, 250 ppm and 500 ppm) prior to inoculation. The synthesized nanoparticles were able to reduce disease severity on bean leaves caused by S. sclerotiorum and B. cinerea and reducing sclerotia formation in S. sclerotiorum. Disease lesion size caused by B. cinerea was reduced by 34%, 82% and 96% at concentrations 100 ppm, 250 ppm and 500 ppm, respectively. Bean leaves that were inoculated with S. sclerotiorum, had a disease reduction of 35%, 53% and 83% at concentrations of 100 ppm, 250 ppm and 500 ppm, respectively. These results have demonstrated the potential use of nanoparticles in the control of S. sclerotiorum and other plant pathogens.

Contact Information: Dr. Weidong Chen, USDA- ARS, and Washington State University, Pullman, WA 99164; 509-335-9178; weidong.chen@usda.gov

Characterizing plant- and virus-derived proteins in important crops that enhance the resistance to white mold

Chien-Fu Wu, Ohio State University
Shin-Yi Marzano, USDA-ARS, Toledo, OH

Funded Plan of Work: Characterizing plant- and virus-derived proteins in important crops that enhance the resistance to white mold

ABSTRACT:
Over-expression of genes encoding putative anti-fungal activities in plants or external application provide opportunities and flexibilities to researchers for adopting genetic engineering strategies to expedite translational genomics towards plant biotic resistance enhancement. The goal of this project is to develop small proteins that exhibit broad spectrum anti-fungal activities against *Sclerotinia sclerotiorum* and deploy them as biological fungicides. To achieve this, we propose to 1) Screen the anti-fungal activity of selected host- and virus-derived molecules through transient expression in plants; 2) Establish the *Bacillus subtilis* secretory protein expression system for the expression and secretion of promising proteins as cell-free filtrate and 3) Test the efficacy of bacterial cell-free filtrates containing the expressed molecules as sprays in the greenhouse and produce transgenic plants to enhance resistance. Prediction by running a deep learning of antimicrobial peptide recognition program shows that the mycoviral replication initiator protein (REP) but not coat protein (CP) from SlaGemV1 has a high probability to be anti-fungal, so are the defensins discovered in *Nicotiana tabacum*. Agroinfiltrations and leaf assays of *N. benthamiana* to transiently express these virus- and plant-derived candidates showed significantly reduced lesion formation compared to the expression of CP ($p = 0.004$). We are in progress to clone selected genes into the *B. subtilis* expression system. The ongoing experiments are on track to allow us to validate the prediction and prioritize the protein-based antimicrobials to be further developed as peptide/ protein-based fungicides.

Contact Information - Dr. Shin-Yi Marzano, USDA-ARS, Toledo, 3050 W. Towerview Blvd, Toledo OH, 43606

Double-stranded RNA targeting white mold *Sclerotinia sclerotiorum* argonaute 2 for disease control via spray-induced gene silencing

Shin-Yi Marzano
USDA-ARS

Funded Plan of Work: Exploring RNAi-based management strategies to confer plant resistance to white mold infection

ABSTRACT:
Sclerotinia sclerotiorum, the causal agent of white mold infection, is a cosmopolitan fungal pathogen that causes major yield losses in many economically important crops. Spray-induced gene silencing (SIGS) has recently been shown to be a promising alternative method for controlling plant diseases. Based on our prior research, we focus on developing SIGS approach to control white mold by silencing S. sclerotiorum argonaute 2 (SsAgo2), a crucial part of the fungal small RNA pathway. We compared the lesion size as a result of targeting each ~500-bp segments of SsAgo2 from 5'- to 3'-end and found that targeting the PIWI/ RNaseH domain of SsAgo2 is most effective. External application of double-stranded RNA (dsRNA) suppressed white mold infection using either in vitro or in vivo transcripts was determined at the rate of 800 ng/0.2cm2 area with a downregulation of SsAgo2 from infected leaf tissue confirmed by RT-qPCR. Furthermore, magnesium/ironlayered double hydroxides (MgFe-LDH) nanosheets loaded with in vitro and in vivo transcribed dsRNA segments significantly reduced the rate of S. sclerotiorum lesion expansion. In vivo produced dsRNA targeting the PIWI/RNaseH domain of the SsAgo2 transcript showed increased efficacy in reducing the white mold symptoms of S. sclerotiorum when combined with LDH nanosheets. This approach is promising to produce a large scale of dsRNA that can be deployed as an environmentally friendly fungicide to manage white mold infections in the field.

Contact Information - Dr. Shin-Yi Marzano, USDA-ARS, shinyi.marzano@usda.gov; 405-592-0727; 3050 W. Towerview Blvd., Toledo, OH 43606

Developing an RNA-based fungicide to manage Sclerotinia stem rot of canola

Abdolbaset Azizi, Luis del Río Mendoza,
Department of Plant Pathology, North Dakota State University. Fargo, ND 58108

Funded plan of Work: Development of RNA fungicides for management of *Sclerotinia sclerotiorum* on canola

ABSTRACT:
Sclerotinia sclerotiorum is the causal agent of Sclerotinia stem rot (SSR) of canola. Currently, SSR disease is managed mainly with fungicides and crop rotations because resistant cultivars are not commercially available. This project intends to use RNA silencing techniques to develop an eco-friendly strategy to protect canola against SSR. For this, six genes from *S. sclerotiorum* were chosen, four of them, a Chitin binding domain (CBD), Mitogen-activated protein kinase (MAPK), oxaloacetate acetylhydrolase (OA), and Abhydrolase-3 (Abh), were combined to produce hairpin RNA in *E. coli* HT115. The detection of dsRNA uptake by *S. sclerotiorum* mycelia was investigated using fluorescent RNA and the confocal microscopy of *S. sclerotiorum* mycelia showed the uptake of the dsRNA of selected genes. Real-time PCR analyses demonstrated the silencing of target genes with 5 ng/µl of dsRNA of individual genes in fungal liquid culture, while detached leaf assays and greenhouse applications on canola stem and leaves revealed varying reductions in necrosis symptoms, with Abhydrolase-3 proving most effective. The application of total RNA from *E. coli* HT115 expressing hairpin RNA from four genes significantly decreased disease severity ($P = 0.01$) on plants inoculated with the highly virulent *S. sclerotiorum* isolate WM031. Treated plants exhibited lesions almost 30% smaller than those treated with Abh alone in lab and greenhouse assays. The study underscores the potential of RNAi for managing *S. sclerotiorum*-caused diseases but emphasizes the need for further research to optimize its efficacy. The research will continue by exploring the influence of RNA secondary structure on the silencing efficiency and the role of nanoparticles in hpRNAs delivery and shelf-life on plant surfaces.

Contact Information - Dr. Luis del Río, Dept. of Plant Pathology, North Dakota State University, Fargo, North Dakota, P. O. Box 6050, Fargo, ND 58108; 701-231-7073; luis.delriomendoza@ndsu.edu

Field trials of mycovirus SlaGemV1 biological fungicide sprays show promise in increasing yield and reducing white mold infection

Connor Pedersen & Shin-Yi Marzano, USDA-ARS, Toledo, OH

Funded Plan of Work: Developing gemycircularvirus-based pesticide for the control of *Sclerotinia sclerotiorum*

ABSTRACT:
Gemycircularvirus soybe1 ,species of the viral particle SlaGemV1, is a CRESS-DNA virus which infects the causal agent of white mold *Sclerotinia sclerotiorum*. Successful infection of *S. sclerotiorum* by SlaGemV1 causes a hypovirulent phenotype in the fungus along with reduced growth and sclerotia formation. Crude extract sprays of SlaGemV1 in the greenhouse could slow the growth of *S. sclerotiorum* on whole sunflower (*Helianthus annus*) and pinto bean (*Phaseolus vulgaris*) plants ($p < 0.05$). Soil application of millet colonized with SlaGemV-1-infected *S. sclerotiorum* to soybean (*Glycine max*) could improve plant growth measured by height ($p = 0.005$), leaf area ($p < 0.0001$), photochemical efficiency (Fv/Fm; $p = 0.005$), and white mold lesion growth over time. To control white mold symptoms in the field, SlaGemV1 was deployed in 2022 and 2023. In 2022 SlaGemV1-infected *S. sclerotiorum* was applied to the field in pinto plots as a hyphal homogenate spray and a millet soil inoculum. Under the spray treatment plants showed a significantly increased height and fresh weight ($p = 0.016$; 0.002) while under soil inoculum treatment plants saw a significant increase in plant height and photosynthetic efficiency ($p < 0.0001$; 0.043). In 2023, SlaGemV1 was applied to the field through a hyphal homogenate and as a crude extract with fungal debris removed after homogenization to soybean, pinto bean, and sunflower plots. Preliminary results of sunflower in the field (n=2) show a reduction in average disease incidence with crude extracted viral particles (17%) after challenged with virulent *S. sclerotiorum,* compared to the control (57%). However, planting method to accommodate mower with wide alleyways resulted in no differences in soybean and pinto bean yield due to low white mold incidence. Still, SlaGemV1-homogenate treatments suggest a potential increase in seed weight in pinto beans at harvest ($p = 0.06$). Soybean yield treated with SlaGemV-1 which had not shown white mold symptoms showed a 4.8 bushel/acre yield increase compared to heat inactivated controls. Therefore, SlaGemV1 deployed as a spray inoculum shows great promise to reduce white mold symptoms and may lead to an increase in average seed weight. In 2024, we would like to repeat the field trials without alleyways for soybean and pinto beans, and perform a replicated trial on sunflower.

Contact Information – Dr. Shin-Yi Marzano, USDA-ARS, 3050 W. Towerview Blvd, OH 43606; (419) 530-5053; shinyi.marzano@usda.gov

Leveraging Aerial Imaging and Machine Learning to Predict White Mold in Common Bean

John Hawkins, Michigan State University

Funded Plan of Work: Evaluation and optimization of genomic selection for durable white mold resistance in dry bean

ABSTRACT:

White mold disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is the major yield limiting disease of common bean (Phaseolus vulgaris L.). In the US bean industry, it causes millions of dollars in yield loss annually. White mold is a monocyclic and necrotrophic disease which causes water-soaked lesions, wilting, collapse, and bleaching of bean plants. For research purposes, white mold incidence and severity are typically scored visually by examining plants for signs and symptoms of the disease and rating severity on a 1-9 or percent scale. However, this approach is both labor intensive and subjective, with no way to correct for observer bias. Imaging-based approaches using statistical models have the potential to reduce labor and improve accuracy and repeatability in white mold scoring. Advances in the technology of uncrewed aerial systems (UAS) allow high resolution imaging of agricultural fields in multiple spectra. In this research UAS imaging of a white mold nursery in both the visible and infrared spectra at several time points from flowering to immediately before harvest was used to create a dataset of vegetation indices. This data, together with visual white mold scores, was used to train machine learning models using a random forest approach. Models were created using the caret package in the R programming language, and the initial models were fitted on a single year of data. 10-fold cross validation of the best model gave an RMSE of 1.095015 and explained 18.6% of the variance. While these models do not yet have strong predictive power, they do show promise. Further training with multiple years of data is expected to improve model performance.

Contact Information: John Hawkins, Michigan State University; hawki345@msu.edu

Small cysteine-rich antifungal peptides as potential biofungicides for control of white mold in soybean

Dilip M. Shah, Arnaud Thierry Djami-Tchatchou, Godwin James, Ruby Tiwari and Meenakshi Tetorya

Donald Danforth Plant Science Center, St Louis, MO 63132

Research Project: Exploiting small cysteine-rich antifungal peptides for management of white mold disease in soybean

ABSTRACT:
White mold caused by a necrotrophic pathogen *Sclerotinia sclerotiorum (Ssc)* results in serious economic losses of soybean yield in the US. White Mold is currently managed in soybean fields primarily through application of chemical fungicides. However, increasing resistance of *Ssc* to the single-site chemical fungicides calls for development of safe and sustainable fungicides with novel multi-site modes of action. The cysteine-rich plant antimicrobial peptides with potent antifungal activity have emerged as promising candidates for design of novel peptide-based fungicides. We are exploring the potential of *Ssc*-inhibitory peptides for development as multi-target biofungicides for management of white mold. GMA4CG_V6 is a 17-amino acid cationic antifungal peptide derived from a plant defensin MtDef4 of *Medicago truncatula*. This peptide was recently shown to exhibit potent antifungal activity against *Botrytis cinerea* and has multi-site modes of action. Antifungal activity of GMA4CG_V6 was tested against an aggressive strain *Ssc* 555 using *in vitro*, semi-*in planta* and *in planta* antifungal assays. GMA4CG_V6 exhibited potent fungicidal activity against *Ssc 555* in *vitro* with the minimum inhibitory concentration (MIC) of 24 µM. External application of GMA4CG_V6 to detached Williams82 soybean leaves, stems and pods significantly decreased lesion sizes with complete inhibition of growth or decay at concentrations between 24 and 96 µM. GMA4CG_V6 markedly reduced white mold disease symptoms when applied to detached soybean leaves, pods, and stems. Its spray application on soybean plants provided robust control of this disease. GMA4CG_V6 at sub-lethal concentrations reduced sclerotia production. It was also non-phytotoxic to soybean plants.

We have recently identified a 21-amino acid cationic peptide GMAOe1C_V1 derived from a novel olive tree antifungal defensin OefDef1. This peptide inhibited the growth of *Ssc 555 in vitro* with an MIC value of 24 µM. Spray-application of this peptide on soybean plants markedly reduced white mold disease symptoms. Our results demonstrate that small antifungal peptides have significant potential as bioinspired fungicides for management of white mold in soybean.

Contact information - Dr. Dilip Shah, Donald Danforth Plant Science Center; 975 North Warson Road St Louis, MO 63132; (314) 587-1481, dshah@danforthcenter.org

Role of soybean phenylalanine ammonia-lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) genes in white mold disease control

Nick Talmo [1], Ji Hyun Kim [1], Robert Stupar[2], Ashish Ranjan[1]

[1]Department of Plant Pathology, University of Minnesota - Twin Cities, St. Paul, MN
[2] Department of Agronomy and Plant Genetics, University of Minnesota -Twin Cities, MN

Research Project: Characterizing and bioengineering soybean phenylpropanoid pathway genes for resistance against *Sclerotinia sclerotiorum*.

ABSTRACT:
Sclerotinia stem rot (SSR) of soybean, caused by *Sclerotinia sclerotiorum,* is a broad host range fungal pathogen. In 2022, SSR was reported as the third most destructive soybean disease in the United States, leading to an estimated yield loss of nearly 12 million bushels. To date, no completely resistant soybean varieties are known, and management relies on fungicides, crop rotations, and partially tolerant varieties. One of the ways plants defend themselves is by producing antimicrobial compounds through the phenylpropanoid pathway. Previous research suggests that phenylpropanoid pathway intermediates such as cinnamic acid, ferulic acid, and caffeic acid might play a crucial role in resistance responses. In the proposed study, we aim to characterize two soybean gene families in the phenylpropanoid pathway, phenylalanine ammonia-lyase (*GmPAL*) and cinnamyl alcohol dehydrogenase (*GmCAD*), for their role in soybean resistance/susceptibility to *S. sclerotiorum*. Phenylammonia layse (PAL) and cinnamyl alcohol dehydrogenase (CAD) are key genes of the phenylpropanoid biosynthesis pathway that catalyzes the conversion of precursor phenylalanine to cinnamic acid and aldehyde moieties such as coniferaldehyde and sinpaldehyde to their alcohol derivatives, respectively. We have shown that purified cinnamic acid, coniferaldehyde, and sinpaldehyde inhibit *S. sclerotiorum* growth, while sinapyl alcohol and p-coumaryl alcohol did not show significant inhibition. We have identified eight putative GmPALs and thirteen GmCADs genes in the soybean genome using sequence similarity searches. We performed time-course (6hpi, 12hpi, 24hpi, 48hpi, and 72hpi) gene expression studies in the controlled environment using Soybean var. Williams 82, challenged with *S. sclerotiorum*. The study identified five highly upregulated *GmPALs* while four *GmCADs* were differentially regulated following *S. sclerotiorum* challenge compared to mock inoculation. Our transient overexpression in *Nicotiana benthamiana* study indicates that *GmPAL6* shows a significantly reduced lesion area compared to empty vector control. In contrast, *GmCAD9* showed a significantly larger lesion area compared to empty vector control. We are in the process of constructing high throughput virus-induced gene silencing (VIGS) of these genes using *bean pod mottle virus (BPMV)* derived vectors in soybean to validate their function further. The study will provide a potential target for developing white mold-resistant soybean lines using the CRISPR cas9 editing tool.

Contact information - Dr. Ashish Ranjan, Department of Plant Pathology, University of Minnesota - Twin Cities, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN - 55108 (612)-624-2291, aranjan@umn.edu

Investigating the relationship between oxalic acid tolerance and basal stalk rot resistance in sunflower

Srushtideep Angidi[1], Israt Zaman[1], Julie Pasche[1], Luis del Rio Mendoza[1], William Underwood[2]

[1] Department of Plant Pathology, North Dakota State University, Fargo, ND
[2]USDA-ARS, Sunflower & Plant Biology Research Unit, Fargo, ND

Funded Plan of Work: Mapping basal stalk rot resistance and oxalic acid tolerance traits in two sunflower recombinant inbred line populations.

ABSTRACT:

Oxalic acid (OA) is an important virulence factor for *Sclerotinia sclerotiorum*, causing plant cell death and contributing to disease development. Treatment of sunflower roots with OA via soil drench or hydroponic application mimics the symptoms of basal stalk rot disease caused by *S. sclerotiorum*, including wilting, leaf necrosis, stem streaking, and development of basal stem lesions. In addition to recapitulation of disease symptoms upon OA treatment, some sunflower lines with moderate to high levels of resistance to basal stalk rot exhibit tolerance to OA, suggesting that OA tolerance may contribute to stalk rot disease resistance. Sunflower inbred lines RHA 801 and HA 61 exhibit high levels of resistance to basal stalk rot and are significantly more tolerant to OA applied to roots by soil drench than the susceptible line HA 89. Recombinant inbred line (RIL) populations were developed from the crosses RHA 801 x HA 89 and HA 61 x HA 89 to facilitate mapping of quantitative trait loci (QTL) governing the basal stalk rot resistance and OA tolerance traits. Specific objectives for this project are to: 1) Map QTL associated with basal stalk rot resistance in both RIL populations; 2) Map QTL for the OA tolerance trait using the same RIL populations; 3) Compare mapped loci identified for the basal stalk rot resistance and OA tolerance traits and assess correlations between stalk rot resistance and OA tolerance among individuals of the two populations. Genotyping of the two populations using genotyping-by-sequencing is currently underway. Additionally, one replication of basal stalk rot disease evaluations has been completed for the HA 61 x HA 89 RIL population. Transgressive segregation was observed for disease response in this population, as anticipated for quantitative resistance to basal stalk rot.

Contact information – Dr. William Underwood, Sunflower & Plant Biology Research Unit, USDA-ARS Northern Crop Science Laboratory, 1616 Albrecht Blvd., Fargo, ND USA 58102-2765; (701) 239-1316, william.underwood@usda.gov

Identification of virulence factors and their application to block *Sclerotinia sclerotiorum* disease development

Chenggang Wang, Zhonglin Mou & Jeffrey A. Rollins
University of Florida, Gainesville, FL

Funded Plan of Work: Manipulating endogenous host pathways to enhance white mold resistance in Brassicaceae

ABSTRACT:

The goal of this project is to develop effective and durable disease resistance for Sclerotinia in *Brassica napus*. Overexpression of oxalyl-CoA synthetase (AAE3) or Oxalyl-CoA Decarboxylase1 (OCD1), the enzymes from the two-step oxalate catabolism pathway in *Brassica napus*, enhanced *S. sclerotiorum* resistance in Arabidopsis. We co-expressed both enzymes in the same transgenic line by crossing OX-AAE3 and OX-OCD1transgenic lines, and no higher resistance was found compared to plants overexpressing a single enzyme. These results suggest that there is no rate-limiting reaction step in the endogenous oxalate catabolism pathway but that overexpression is not sufficient for blocking oxalate accumulation. We have shifted to finding new targets for blocking disease development. Host-induced gene silencing (HIGS) and exogenous applications of dsRNA have emerged as promising crop protection technologies, and genes encoding virulence factors or essential processes are considered ideal targets for these approaches. To screen and identify early virulence factors in *S. sclerotiorum*, an RNA-seq analysis focusing on hyphal differentiation and lesion expansion was performed. From the phase I specific expressed gene profiles, 21 candidate genes were selected for gene disruption and virulence screening. Loss-of-function mutants of *Sssak1* and *Ssreg1* showed significantly attenuated virulence. SsSAK1 and SsREG1 are important components of MAPK cascade signaling. *Ssreg1* and *Sssak1* are required for compound appressoria development and initial penetration, and are considered to be phase I virulence factors. *Ssreg1* and *Sssak1* along with previously identified virulence factors are being tested as candidate targets for HIGS and exogenous applications of dsRNA.

Contact Information – Dr. Chenggang Wang, Department of Plant Pathology, University of Florida, Gainesville, FL 32611; 352-273-4620; chenggangwang@ufl.edu

Spatially Differentiated Gene Expression Patterns in Canola Leaves during *Sclerotinia sclerotiorum* Infection

Hira Kamal, Washington State University, Pullman, WA; Weidong Chen, USDA-ARS, Pullman, WA; Kiwamu Tanaka, Washington State University, Pullman, WA

Funding Plan of Work: Systems View of Pathogenesis and Host Defense Response at Specific Infection Stages of *Sclerotinia sclerotiorum*

ABSTRACT:
A polyphagous necrotrophic pathogen, *Sclerotinia sclerotiorum*, causes major diseases in numerous agronomic crops, inflicting billions of dollars in lost revenue. Developing durable resistance against this pathogen is crucial, serving as a viable alternative to chemical control measures. In this study, we aim to unveil genetic regulatory networks and metabolomic pathways associated with disease development in canola plants. Our hypothesis centers on distinct host-pathogen interactions during various infection stages, particularly at the forefront of the advancing infection, where plants and pathogens engage in a dynamic interplay, employing both wall-reinforcing and wall-weakening strategies. Polygalacturonases (PGs) are major pathogenicity factors produced in the earlier stages of pathogen infection that depolymerize pectin, the main component of the plant cell wall. In contrast, plants have evolved cell wall-associated PG-inhibiting proteins (PGIPs) as a defense mechanism against PGs. Our preliminary data, gathered from canola leaves, supports the observation that both *PGIP* and *PG* are specifically expressed at the margin area—the leading edge of the advancing infection. Notably, chlorosis is pronounced in this margin area when canola leaves were inoculated with the oxalic acid-minus mutant *S. sclerotiorum* (M202). We investigated the expression of the salicylic acid (SA) marker gene *BnPR1* and the jasmonic acid (JA) marker gene *BnPDF1.2* in necrotic, margin (chlorotic), and uninfected zones in M202-infected canola leaves. Our findings reveal distinct spatial expression patterns between the two genes, with *PR1* highly expressed mainly in the necrotic zone, while *PDF1.2* is expressed in both margin and uninfected zones. This suggests spatial crosstalk between SA and JA pathways, challenging the notion of a single defense pathway against the pathogen's varied lifestyle. Moving forward, our research will delve into identifying significant genes and metabolic pathways involved in different stages of fungal infection through a spatially resolved multi-omics study in canola leaves.

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A Single Laccase Acts as a Key Component of Environmental Sensing in a Broad Host Range Fungal Pathogen

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Research Project: Targeting essential genes in *Sclerotinia sclerotiorum* to achieve Sclerotinia stem rot resistance in soybean

ABSTRACT:
Work continued in 2023 to identify novel virulence determinants in *Sclerotinia sclerotiorum*. Secreted laccases are important enzymes on a broad ecological scale for their role in mediating plant-fungal interactions, but their function in fungal pathogenesis has yet to be elucidated. Ascomycete laccases have been primarily associated with cell wall melanin deposition, and laccase mutants in ascomycete species often demonstrate reduced pigmentation. In this study, a putatively secreted laccase, *Sslac2*, was characterized from the broad host-range plant pathogen *S. sclerotiorum*, which is largely unpigmented and is not dependent on melanogenesis for plant infection. Of the seven putative laccases in the *S. sclerotiorum* genome, *Sslac2* was the only one found to be highly upregulated during pathogenesis and was additionally found to be induced during growth on solid surfaces. Gene knockouts of *Sslac2* demonstrate wide ranging developmental phenotypes, including abolished sclerotial formation, and are functionally non-pathogenic. These mutants also exhibited indiscriminate growth behaviors and enhanced biomass formation, likely due to altered hydrophobicity and thigmotropic responsiveness. Interestingly, *Sslac2* mutants were also unable to respond to environmental cues, and accordingly unable to differentiate infection structures, respond appropriately to chemical stress, or produce key virulence determinants. Transmission and scanning electron microscopy of WT and mutant strains show apparent differences in extracellular matrix structure that may explain the inability of the mutants to respond to their environment. Targeting *Sslac2* using host-induced gene silencing significantly improved resistance to *S. sclerotiorum*, suggesting that fungal laccases could be a valuable target of disease control. Collectively, we identified a laccase critical to the development and virulence of the broad host-range pathogen *S. sclerotiorum* and propose a potentially novel role for fungal laccases in modulating environmental sensing.

Contact Information:

Development of *S. sclerotiorum* screening panels to evaluate resistance and discover conserved aggressiveness determinants across crop species

Megan McCaghey and Hsuan Fu Wang, University of Minnesota, Twin Cities

Funded plan of work: Identifying genetic determinants of *Sclerotinia sclerotiorum* aggressiveness across crop species

ABSTRACT:
Diseases caused by *Sclerotinia sclerotiorum* continue to result in large yield losses throughout the Northern United States, and management is limited by the pathogen's wide host range, long term survival in soil, and the incomplete resistance of crops. Our work aims to provide tools for resistance screenings assays and potential targets to reduce the aggressiveness of *S. sclerotiorum* using biotechnology approaches. Through this project we aim to 1) characterize a wide range of *Sclerotinia sclerotiorum* isolates for aggressiveness and resistance screening across crop plants and 2) identify candidate pathogenicity genes that are conserved across crop plants using transcriptomics. Initial efforts have focused on characterizing isolate aggression on multiple crops and refining an isolate panel for multi-crop resistance screenings and future studies. During the growing seasons of 2022 and 2023, isolates were collected from multiple crops and locations within Minnesota. We have also collected isolates of varying observed levels of aggression from researchers in other states where *S. sclerotiorum* causes yield losses. Screenings of 28 isolates on soybean, in three experimental replicates, has resulted in a panel of seven isolates of high, med, and low aggression that can be used for soybean resistance screening in Minnesota. These seven isolates and six out-of-state isolates are being screened on dry bean and sunflower in growth chambers to identify isolates with conserved aggressiveness rankings across crop species. One experimental replicate per crop species has been completed with seven isolates at this time. The subpanel of isolates with conserved aggression across crop species will be evaluated for their efficacy for resistance screening by challenging more resistant versus susceptible varieties of the three crop species. At the end of growth chamber screenings this spring, we will conduct RNA extractions for RNAs sequencing to discover conserved aggressiveness determinants across crop species. The genes upregulated by aggressive isolates during *S. sclerotiorum* infection may be useful targets for RNAi-based management strategies.

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Identification and characterization pathogenicity genes affected by DNA mycovirus in *Sclerotinia sclerotiorum*

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Funded plan of work: Characterizing pathogenicity effectors of *Sclerotinia sclerotiorum* preferentially expressed under acidic conditions and during plant infection

ABSTRACT:
White mold disease significantly impacts the yield and marketability of key US crops. While plants possess an immune system capable of resisting infections, *Sclerotinia sclerotiorum* has evolved mechanisms to effectively suppress these host defenses. Previous studies showed that DNA mycovirus (SsHADV-1) consistently conferred hypovirulence to *S. sclerotiorum*. In order identify pathogenicity genes of *S. sclerotiorum* that are affected by the mycovirus SsHADV-1, we created stable strains expressing either the whole viral genome or the caspid protein followed by transcriptome analysis. Strains expressing SsHADV-1 exhibited a substantial loss of virulence. Furthermore, expression of the coat protein (CP) also led to a significant reduction in virulence. We conducted RNA-seq analysis to explore virulence genes that are affected by the SsHADV-1 at the transcriptome level. The comparison between SsHADV-1-expressing strains and wildtype strain WMA1 revealed 208 up-regulated and 459 down-regulated differentially expressed genes (DEGs). Gene ontology (GO) analysis unveiled distinct classifications of up- and down-regulated DEGs, encompassing biological processes, molecular functions, cellular components, and protein classes. Notably, up-regulated DEGs included genes encoding transcription regulators, while down-regulated DEGs were exclusive to defense/immunity and chaperone proteins. Based on this RNA-seq data we want to define pathogenic genes through gene knockout and overexpression assays, protein localization, and screening for interaction proteins using techniques such as Co-IP or yeast two-hybrid systems. These findings aim to contribute to a deeper understanding of *S. sclerotiorum* pathogenesis, potentially allowing the development of innovative chemical inhibitors targeting white mold diseases and the engineering of disease-resistant crops.

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Population genetic characterization of *Sclerotinia sclerotiorum* from USA soybean and dry bean using AmpSeq, and development of an informational survey to assess NSI impacts

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Research Project: Genetic variability associated with the traits of fungicide resistance and pathogenicity in *Sclerotinia sclerotiorum.*

ABSTRACT: An AmpSeq primer array of 167 sets were developed to amplify and sequence variants in SSRs, SNPs, putative pathogenicity-related loci, and genes conferring fungicide resistance (β-tubulin, Sdh complex, and cytochrome b gene). AmpSeq was applied to 178 *S. sclerotiorum* genomic DNA hierarchically sampled from diverse sources. Our AmpSeq array included 174 polymorphic SNPs (74 SNPs in 18 genes and 100 intergenic SNPs). Fasta sequences of these genes ("poly_genes_SNP.fasta") were retrieved from NCBI and were further inspected using SECRETOOL, Batch CD-Search tool, and available scientific literature to identify putative virulence/pathogenicity factors. In our preliminary AmpSeq analysis, 61 of 178 *S. sclerotiorum* (34%) had good quality sequence data in the *SdhB* gene, and 17 of those had the A11V mutation. These samples with mutations were collected from Mexico (N=12) and USA (N=5). None of the *S. sclerotiorum* samples with good quality *SdhC* sequence data contained the I22V or I31V point mutations. No sequence data for other resistance loci were present, possibly due to low sequence depth, quality control filters, or poor primer amplification. Ongoing work is focused on identifying polymorphic and reliable variants loci that can be used for downstream population genetic analysis. We also recently developed a new objective to design and deploy a nationwide survey to evaluate the impact of recent advances in the management of disease caused by *S. sclerotiorum* on NSI-focused commodity groups, including canola, dry bean pea, lentil, chickpea, soybean, and sunflower. Our mixed-methods survey questionnaire will include questions related to NSI-improved management practices, such as planting resistant cultivars, use of biological control methods, and integrated disease management practices. A focus group is currently being sought to evaluate the preliminary effectiveness of this survey, following which the full survey will be deployed.

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Sclerotinia sclerotiorum SsCelp0028 protein is a cytotoxic effector contributing to virulence

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Funded plan of work: *Sclerotinia sclerotiorum* hijacks host cell death control in infecting plant

ABSTRACT:

Sclerotinia sclerotiorum is a broad-host-range necrotrophic fungal pathogen, causing white mold and stem rot diseases in more than 600 plant species, leading to significant losses in overall yield and marketability of many important crops. Despite extensive studies on this pathogen, the functional mechanisms of the secreted proteins in *S. sclerotiorum*-plant interaction still not adequately understood. In our preliminary study, the small secreted-protein SsCelp0028 was identified by analyzing *S. sclerotiorum* transcriptome during the early infection stage and screening genes predicted to encode secreted necrosis-inducing proteins (NIPs). SsCelp0028 is highly induced at the early stages of *S. sclerotiorum* infection and induces chlorosis in the leaf apoplastic space. Confocal microscopy results showed SsCelp0028 in the apoplastic space. Analysis of SsCelp0028-deletion mutants showed that SsCelp0028 positively regulates *S. sclerotiorum* pathogenicity and tolerance to abiotic stress including cell wall integrity, salt, and oxidation. In addition, SsCelp0028 induces the expression of *Nicotiana benthamiana* defense genes related to hypersensitive response and salicylic acid/jasmonic acid/ethylene signaling pathways. Taken together, our results demonstrate that SsCelp0028 is a secreted apoplast effector critical for *S. sclerotiorum* virulence and stress tolerance, which contributes to our mechanistic understanding of host-*Sclerotinia* interactions.

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A comprehensive assessment of genomic prediction models for sclerotinia stem rot resistance in soybean (*Glycine max*)

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Funded Plan of Work: Developing Soybean Varieties with Resistance to Sclerotinia Stem Rot

ABSTRACT:
Sclerotinia stem rot (SSR), caused by *Sclerotina sclerotiorum* (Lib.), is one of the key devastating diseases of soybean in Northern United. Genetic resistance is believed to be the most effective control measure however, no complete resistant genotype has yet been developed due to the partial and complex resistance mechanism. The best way to control this disease is to use soybean varieties with partial resistance to the disease. Genomic selection based on genome-wide markers is an efficient way to select quantitative traits such as disease resistance. This study explores the genetic basis of SSR resistance through a combination of field evaluations, genomic analyses, and prediction modeling. In this study, a total of 742 advanced breeding lines developed from the Michigan State University soybean breeding program were evaluated in a naturally infected disease nursery at Montcalm, Michigan. Best linear unbiased estimator (BLUE) values were calculated to reduce the random effect of block in the field. The predictive abilities of six genomic prediction models including Baysian models (Bays A, BaysB, BaysC and BRR), GBLUP (genomic best linear unbiased prediction) and rrBLUP(ridge regression best linear unbiased prediction) were evaluated, and the impact of training, testing and cross validation population was investigated. All the prediction models showed similar prediction accuracy of which rrBLUP model showed slightly higher prediction accuracy of 0.57 at the 90:10 training and testing population ratio with 10-fold cross validation. This study underscores the potential of genomic selection as a tool for enhancing white-mold resistance in soybeans.

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Identification of *Brassica napus* QTL for resistance to *Sclerotinia sclerotiorum* and of *B. napus* plant introductions with resistance to multiple *S. sclerotiorum* isolates

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Funded Plan of Work: Improving resistance of spring canola to Sclerotinia stem rot

ABSTRACT:

Sclerotinia sclerotiorum causes Sclerotinia stem rot (SSR) on canola (*Brassica napus* L.) and other crops of economic importance to our nation. In North Dakota, each year this disease reduces canola yields at an average rate of 0.5% for every unit of incidence. This rate, however, can be greater in disease conducive environments. Several strategies can be used to control the disease but breeding for resistant cultivars has been considered a natural, friendlier, economic, and sustainable approach. Genetic resistance to this disease is quantitative and the number of quantitative trait loci (QTL) known to be associated with reaction to SSR is still relatively small. In this study, we aimed to detect and map SSR resistance QTL in a population of 288 *B. napus* doubled haploid (DH) lines derived from the cross of NEP32 and cv. Topas and to evaluate the reaction of twelve *B. napus* plant introductions considered resistant to SSR using five aggressive *S. sclerotiorum* isolates. The DH population was phenotyped in replicated greenhouse trials by inoculating the main stem of flowering plants with an agar plug containing *S. sclerotiorum* actively growing hyphal tips and measuring the length of the resulting lesions seven days later. DNA samples extracted from all lines were genotyped using a chip array containing 18k single nucleotide polymorphism (SNP) markers at SGS North America's Genomics Lab. The population had average lesion size of 9 cm and a plant mortality of 87%. Lines with average lesions < 7 cm considered as resistant therefore, line 120 was the most resistant and had an average lesion size of 1.8 cm. A genetic map with a total length of 2373.29 cM was generated with 5608 SNP markers. QTL analysis is in progress using QGene-4.4.0 software. The resistant PIs were evaluated in replicated greenhouse trials using the agar plug inoculation technique. Resulting stem lesions were measured seven days after inoculation. Two PIs were resistant to all five isolates and three other PIs were considered moderately resistant to all five isolates. Other PIs were resistant to some isolates but susceptible to others. These results will be used to establish a pecking order for mapping population development and to study gene expression during the infection process.

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Improved white mold resistance in dry and snap beans through multi-site screening throughout major production areas

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Funded Plan of Work: Improved white mold resistance in dry and snap beans through multi-site screening throughout major production areas

ABSTRACT:
The research goal of our collaborative study is to identify improved sources of resistance to white mold in adapted dry bean breeding lines using multiple disease screening sites located in major bean-production areas of the United States. Two approaches were taken to evaluate entries submitted by collaborators: a greenhouse-based straw test and field trials carried out within white mold nurseries in five locations throughout the northern U.S. and Quebec, Canada. In 2023, trials were conducted in MI, ND, NE, OR, WA and QC with a total of 18 entries that included Black, Navy, Pinto, Pink, and Light Red Kidney seed classes, along with check cultivars G122, Bunsi, and Beryl. Preliminary analysis of greenhouse and field data shows overall moderate levels of resistance. Greenhouse data indicated multiple lines performed equal to the resistant check (G122) at some locations. Further greenhouse and field data analysis are currently underway, which will provide greater insight into the genetic progress towards developing future cultivars with improved levels of both physiological resistance and architectural avoidance to white mold.

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Introgression and pyramiding of Sclerotinia stem rot (SSR) disease resistant gene(s) into canola cultivars

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Funded Plan of Work: Introgression and pyramiding of Sclerotinia stem rot disease resistant gene(s) into canola cultivars

ABSTRACT:
Canola, *Brassica napus* L., is the second most important oil crop in the world, after soybean. Canola, characterized by trace amounts of erucic acids & glucosinolates and rich amounts of unsaturated fatty acids, is considered one of the healthiest oil crops with the potential to promote brain development in infants and prevent heart disease in adults. However, this important crop is frequently infected by the soil-borne fungus *Sclerotinia sclerotiorum*, which could cause up to 80% yield loss in case of severe infection and thus cost billions of US dollars. The lack of complete and durable resistant canola cultivars and the ineffectiveness of crop rotation and chemical fungicides necessitates the development of resistant cultivars, which is laborious, time-consuming, and costly. Our objectives are to introgress and pyramid the SSR-resistant gene(s) into elite breeding lines, to develop diversified breeding lines with enhanced SSR disease resistance, and to develop molecular markers for marker-assisted selection in the breeding program. We have generated five very strong resistant lines with very high yield potential by screening 700 BC3S2 lines with Petiole Inoculation Technique (PIT) and Stem Inoculation Technique (SIT), followed by backcrossing with NDOLA-2, an elite breeding cultivar developed in our lab. In addition, we have genotyped and phenotyped 350 diversified breeding lines (a total of 9450 plants) in three locations in North Dakota and identified six very strong resistant canola lines. To facilitate speed breeding, we also have developed and tested 47 KASP (Kompetitive Allele Specific PCR) markers based on the SNP (Single Nucleotide Polymorphism) markers associated with SSR disease resistance obtained from the genome-wide association mapping experiment in our lab. We got a few promising KASP markers with the potential to identify and distinguish resistant lines from the susceptible lines of canola. Taken together, our results indicate the rapid progress toward the development of resistant cultivars of canola with high yield potential.

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Using Genomic Prediction to Identify Dry Bean (*Phaseolus vulgaris* L.) Genotypes with Resistance to White Mold (*Sclerotinia sclerotiorum* Lib. De Bary)

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Funded Plan of Work: White mold resistance QTL: identification, interactions, and fine mapping in common bean.

ABSTRACT:
Dry bean growers in the US northern Great Plains rank white mold (Sclerotinia sclerotiorum Lib. de Bary) as the worst disease. Reliable field screening for white mold resistance is challenging, because the incidence and severity of the pathogen is influenced by several factors. A multi-parent advanced generation inter-crosses (MAGIC) population (n = 1040) was screened for white mold reaction in the greenhouse and genotyped to carry out a genome-wide association study (GWAS). In addition, the predictive ability of six genomic prediction (models for white mold resistance and the agronomic performance of resistant genotypes were evaluated under field condition. GWAS identified 15 genomic regions associated with resistance spanning across seven chromosomes. A predictive ability of 0.34 was detected for all the models. Genomic selection applied in the validation populations were able to detected from 64 to 71% of the susceptible lines. MAGIC lines WMM-556 and WMM-750 showed superior seed yield compared to the mean of the rest of resistant lines and MAGIC parental lines.

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A QTL Approach Toward Understanding and Improving Genetic Resistance to White Mold in Common Bean

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Research Project: White mold resistance QTL: identification, interactions, and fine mapping in common bean.

ABSTRACT: During 2023, multiple genetic and breeding populations were generated, phenotyped and genotyped in support of detecting new and existing QTL that control white mold (WM) resistance in common bean. Selected lines from these populations with WM resistance and favorable agronomic traits were advanced toward possible release using marker-assisted selection (MAS). A new snap bean MAGIC population, generated using six snap beans and two dry bean parents with WM resistance, was advanced to the F5 generation in 2023. There were 996 families phenotyped but only 906 were harvested due to poor emergence (dwarf lethal syndrome) or very late maturity resulting from photoperiod sensitivity. White-flowered (61%), bush types (68%), with snap pod traits (88%) predominated. Harvested seed will be used for replicated white mold trials and genotyping in 2024. A nested association mapping (NAM) of four recombinant inbred line (RIL) snap/dry bean populations was completed (MS thesis). Fifteen lines from these populations were advanced because they showed a combination of white mold resistance, favorable agronomic traits, and select QTL haplotypes. A genome-wide association study (GWAS) in a pinto bean WM-MAGIC population identified 15 genomic regions, spanning seven chromosomes associated with white mold resistance. Genomic selection models applied in the pinto WM-MAGIC population were able to detect from 64 to 71% of the susceptible lines (MS thesis). Two pinto MAGIC lines with superior resistance and seed yield were included in the 2023 National White Mold Nursery and used in crosses. Three different mapping approaches: classical, bulked segregant analysis, and Khufu *de novo* QTL-seq were compared and combined to fine map the major WM2.2 QTL in two dry bean RIL populations (Oladzad et al., 2023). This research found three independent QTL regions WM2.2a, WM2.2b and WM2.2c underpinning the 'meta' WM2.2 QTL. Integrated classical and QTL-seq mapping was used in other dry bean RIL populations to fine map the WM5.4 and WM7.5 QTL (Roy et al., 2023). A similar integrated mapping approach was used to fine map eight QTL in two pinto bean RIL populations (publication pending). A red bean RIL population, tested for WM reaction in the greenhouse in 2023, will be tested in a newly established WM field nursery in Othello, WA in 2024. Selected lines from these RIL populations are being used to improve WM resistance in the breeding programs. Overall continued progress is being made toward identifying, developing, and generating QTL linked markers for use in breeding snap and dry beans with improved resistance to white mold.

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Using genomics assisted breeding to advance sunflower germplasm development

Brent Hulke, USDA-ARS Sunflower and Plant Biology Research Unit,
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Funded Plant of Work: Using genomics assisted breeding to advance sunflower germplasm development.

ABSTRACT:

Sunflower has a complicated, but efficient breeding method that involves the hybrid breeding system. Unique aspects of sunflower biology make this breeding system a good choice, most notably the widespread gene presence/absence variation that drives heterosis, or hybrid vigor, when two genetic pools are developed, and hybrids are made by crossing between both groups. Similarly, using genomic data is complicated, because sunflower genome size varies wildly even among related lines. This is due to introgressions from closely related species and duplication/deletion of genes among breeding lines. This complicates the use of markers in breeding, but developing a successful breeding program that is able to account for structural variation amongst genomes in a breeding pool could allow for increasing the scale of breeding programs without necessarily increasing costly and time-consuming field evaluation of testcross hybrids. This is especially important for Sclerotinia trials, as they require special field facilities and difficult-to-generate inoculum.

Our work aims to reduce the amount of field work spent on evaluating Sclerotinia and Phomopsis disease (both necrotrophic diseases with similar genetic basis in resistance) by using marker resources that have been discovered and validated in genomic association studies in recent years. We are developing genomic data by resequencing a set of populations within our breeding program to develop a core set of variant calls (markers) that segregate normally (i.e. Mendelian) across the populations. These calls will be aligned with significant disease resistance markers from previous studies, and haplotypes associated with these loci defined. A second data set that has both genomic data and phenotypic data for disease and yield related traits will be used to predict the effects of these disease resistance-associated haplotypes on yield, and verify once more that they are associated with reduced disease. Based on the results of this work, tools for breeders and general recommendations will be provided to benefit the USDA sunflower breeding program and industry breeding programs.

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Evaluation and optimization of genomic selection for durable white mold resistance in dry bean

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Funded Plan of Work: Evaluation and optimization of genomic selection for durable white mold resistance in dry bean

ABSTRACT:
Dry bean production in the U.S. suffers annually from yield loss due to white mold infection. Dry bean cultivars lack high levels of genetic resistance, and progress to breed new cultivars with improved levels of resistance has been slow due to the quantitative inheritance of this trait, difficulty pyramiding resistance, and field screening dependence on the presence of the pathogen under suitable environmental conditions. Genomic prediction provides an alternative method to pyramid resistance genes by utilizing genome-wide marker coverage to predict genotypic values for quantitative traits. This study evaluated the efficiency of different genomic prediction models given the complex population structure of multiple market classes present in dry bean breeding programs. A panel of 303 Middle-American breeding lines were genotyped with 3,026 markers and evaluated for white mold in the field over two seasons. Prediction accuracy across models and subsets was moderate (0.3 - 0.36) given the population size. Furthermore, when fixed effect QTL were identified and implemented through GP + GWAS, 1-3 QTL increased prediction accuracy only modestly (0.36 - 0.4). These results indicate that genomic prediction is a promising screening tool in dry bean breeding for white mold resistance.

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Poster Abstracts

Identification of *Brassica napus* QTL for resistance to *Sclerotinia sclerotiorum* and of *B. napus* plant introductions with resistance to multiple *S. sclerotiorum* isolates

Bita Babakhani and Luis del Río Mendoza,
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Funded Plan of Work: Improving resistance of spring canola to Sclerotinia stem rot

ABSTRACT:

Sclerotinia sclerotiorum causes Sclerotinia stem rot (SSR) on canola (*Brassica napus* L.) and other crops of economic importance to our nation. In North Dakota, each year this disease reduces canola yields at an average rate of 0.5% for every unit of incidence. This rate, however, can be greater in disease conducive environments. Several strategies can be used to control the disease but breeding for resistant cultivars has been considered a natural, friendlier, economic, and sustainable approach. Genetic resistance to this disease is quantitative and the number of quantitative trait loci (QTL) known to be associated with reaction to SSR is still relatively small. In this study, we aimed to detect and map SSR resistance QTL in a population of 288 *B. napus* doubled haploid (DH) lines derived from the cross of NEP32 and cv. Topas and to evaluate the reaction of twelve *B. napus* plant introductions considered resistant to SSR using five aggressive *S. sclerotiorum* isolates. The DH population was phenotyped in replicated greenhouse trials by inoculating the main stem of flowering plants with an agar plug containing *S. sclerotiorum* actively growing hyphal tips and measuring the length of the resulting lesions seven days later. DNA samples extracted from all lines were genotyped using a chip array containing 18k single nucleotide polymorphism (SNP) markers at SGS North America's Genomics Lab. The population had average lesion size of 9 cm and a plant mortality of 87%. Lines with average lesions < 7 cm considered as resistant therefore, line 120 was the most resistant and had an average lesion size of 1.8 cm. A genetic map with a total length of 2373.29 cM was generated with 5608 SNP markers. QTL analysis is in progress using QGene-4.4.0 software. The resistant PIs were evaluated in replicated greenhouse trials using the agar plug inoculation technique. Resulting stem lesions were measured seven days after inoculation. Two PIs were resistant to all five isolates and three other PIs were considered moderately resistant to all five isolates. Other PIs were resistant to some isolates but susceptible to others. These results will be used to establish a pecking order for mapping population development and to study gene expression during the infection process.

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Introgression and pyramiding of Sclerotinia stem rot (SSR) disease resistant gene(s) into canola cultivars

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Funded Plan of Work: Introgression and pyramiding of Sclerotinia stem rot disease resistant gene(s) into canola cultivars

ABSTRACT:
Canola, *Brassica napus* L., is the second most important oil crop in the world, after soybean. Canola, characterized by trace amounts of erucic acids & glucosinolates and rich amounts of unsaturated fatty acids, is considered one of the healthiest oil crops with the potential to promote brain development in infants and prevent heart disease in adults. However, this important crop is frequently infected by the soil-borne fungus *Sclerotinia sclerotiorum*, which could cause up to 80% yield loss in case of severe infection and thus cost billions of US dollars. The lack of complete and durable resistant canola cultivars and the ineffectiveness of crop rotation and chemical fungicides necessitates the development of resistant cultivars, which is laborious, time-consuming, and costly. Our objectives are to introgress and pyramid the SSR-resistant gene(s) into elite breeding lines, to develop diversified breeding lines with enhanced SSR disease resistance, and to develop molecular markers for marker-assisted selection in the breeding program. We have generated five very strong resistant lines with very high yield potential by screening 700 BC3S2 lines with Petiole Inoculation Technique (PIT) and Stem Inoculation Technique (SIT), followed by backcrossing with NDOLA-2, an elite breeding cultivar developed in our lab. In addition, we have genotyped and phenotyped 350 diversified breeding lines (a total of 9450 plants) in three locations in North Dakota and identified six very strong resistant canola lines. To facilitate speed breeding, we also have developed and tested 47 KASP (Kompetitive Allele Specific PCR) markers based on the SNP (Single Nucleotide Polymorphism) markers associated with SSR disease resistance obtained from the genome-wide association mapping experiment in our lab. We got a few promising KASP markers with the potential to identify and distinguish resistant lines from the susceptible lines of canola. Taken together, our results indicate the rapid progress toward the development of resistant cultivars of canola with high yield potential.

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Developing Soybean Varieties with Resistance to Sclerotinia Stem Rot

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Funded Plan of Work: Developing Soybean Varieties with Resistance to Sclerotinia Stem Rot

ABSTRACT:

Sclerotinia stem rot (white mold), caused by *Sclerotinia sclerotiorum*, is one of major yield limiting disease in soybean. In 2022, this disease caused nearly 20 million bushel yield loss in Northern United States. To improve soybean resistance to white mold, the Michigan State University soybean breeding program has established a steady breeding pipeline for selecting and releasing elite soybean varieties with high resistance to white mold. In 2023, a total of 165 lines, including 55 elite lines and 110 advanced breeding lines, were evaluated in our naturally infested white mold disease nursery at Montcalm, Michigan. The disease severity index (DSI) is determined by assessing the number of infected plants, utilizing a rating scale ranging from 0 to 3. The DSI is ranged from 0 to 100, with an average of 47.16. Furthermore, we explored the application of unmanned aerial vehicles (UAV) indices to measure white mold severity. We found significant negative correlation -0.48 between DSI and average green leaf index. In 2023, a variety E18638T with resistance to white mold was released for commercialization.

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Evaluation and optimization of genomic selection for durable white mold resistance in dry bean

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Funded Plan of Work: Evaluation and optimization of genomic selection for durable white mold resistance in dry bean

ABSTRACT:
Dry bean production in the U.S. suffers annually from yield loss due to white mold infection. Dry bean cultivars lack high levels of genetic resistance, and progress to breed new cultivars with improved levels of resistance has been slow due to the quantitative inheritance of this trait, difficulty pyramiding resistance, and field screening dependence on the presence of the pathogen under suitable environmental conditions. Genomic prediction provides an alternative method to pyramid resistance genes by utilizing genome-wide marker coverage to predict genotypic values for quantitative traits. This study evaluated the efficiency of different genomic prediction models given the complex population structure of multiple market classes present in dry bean breeding programs. A panel of 303 Middle-American breeding lines were genotyped with 3,026 markers and evaluated for white mold in the field over two seasons. Prediction accuracy across models and subsets was moderate (0.3 - 0.36) given the population size. Furthermore, when fixed effect QTL were identified and implemented through GP + GWAS, 1-3 QTL increased prediction accuracy only modestly (0.36 - 0.4). These results indicate that genomic prediction is a promising screening tool in dry bean breeding for white mold resistance.

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Biosynthesis of Silver and Zinc Nanoparticles using Sclerotinia sclerotiorum Metabolites to Combat Fungal Diseases

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Funded plan of Work: Biological Control of White Mold Using the Mycovirus SsHADV-1-Infected Hypovirulent Strain DT-8 of Sclerotinia sclerotiorum

ABSTRACT:
Many fungal metabolites have great capability for forming different metal nanoparticles that can be utilized in various processes. Thousands of biologically active compounds are formed by fungal species. Some metal nanoparticles have shown their potential to act as inhibitory agents against plant pathogens. We report on the synthesis of silver nanoparticles and zinc oxide nanoparticles (AgNPs and ZnONPs) synthesized from the metabolites of the plant pathogenic fungus Sclerotinia sclerotiorum wild type WMA1. Cell free culture filtrates were combined with silver nitrate or zinc nitrate in separate experiments to synthesize nanoparticles and subsequently evaluated for their antifungal properties. This eco-friendly synthesis of nanoparticles is an alternative to the chemical method of synthesis, which involves the use of toxic surfactants. The resulting nanoparticles were characterized with UV-visible spectroscopy, transmission electron microscopy and scanning electron microscopy. The synthesized AgNPs produced a reddish brown color and the ZnONPs had a greenish chalk color. The ultraviolet visible spectroscopy peaks for the AgNP and ZnONP were 413 nm and 304 nm, respectively. The nanoparticles were also evaluated for the control of S. sclerotiorum and Botrytis cinerea on detached bean leaves. Detached bean leaves were treated with the nanoparticles at different concentrations (100 ppm, 250 ppm and 500 ppm) prior to inoculation. The synthesized nanoparticles were able to reduce disease severity on bean leaves caused by S. sclerotiorum and B. cinerea and reducing sclerotia formation in S. sclerotiorum. Disease lesion size caused by B. cinerea was reduced by 34%, 82% and 96% at concentrations 100 ppm, 250 ppm and 500 ppm, respectively. Bean leaves that were inoculated with S. sclerotiorum, had a disease reduction of 35%, 53% and 83% at concentrations of 100 ppm, 250 ppm and 500 ppm, respectively. These results have demonstrated the potential use of nanoparticles in the control of S. sclerotiorum and other plant pathogens.

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Developing an RNA-based fungicide to manage Sclerotinia stem rot of canola

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Funded plan of Work: Development of RNA fungicides for management of *Sclerotinia sclerotiorum* on canola

ABSTRACT:
Sclerotinia sclerotiorum is the causal agent of Sclerotinia stem rot (SSR) of canola. Currently, SSR disease is managed mainly with fungicides and crop rotations because resistant cultivars are not commercially available. This project intends to use RNA silencing techniques to develop an eco-friendly strategy to protect canola against SSR. For this, six genes from *S. sclerotiorum* were chosen, four of them, a Chitin binding domain (CBD), Mitogen-activated protein kinase (MAPK), oxaloacetate acetylhydrolase (OA), and Abhydrolase-3 (Abh), were combined to produce hairpin RNA in *E. coli* HT115. The detection of dsRNA uptake by *S. sclerotiorum* mycelia was investigated using fluorescent RNA and the confocal microscopy of *S. sclerotiorum* mycelia showed the uptake of the dsRNA of selected genes. Real-time PCR analyses demonstrated the silencing of target genes with 5 ng/µl of dsRNA of individual genes in fungal liquid culture, while detached leaf assays and greenhouse applications on canola stem and leaves revealed varying reductions in necrosis symptoms, with Abhydrolase-3 proving most effective. The application of total RNA from *E. coli* HT115 expressing hairpin RNA from four genes significantly decreased disease severity ($P = 0.01$) on plants inoculated with the highly virulent *S. sclerotiorum* isolate WM031. Treated plants exhibited lesions almost 30% smaller than those treated with Abh alone in lab and greenhouse assays. The study underscores the potential of RNAi for managing *S. sclerotiorum*-caused diseases but emphasizes the need for further research to optimize its efficacy. The research will continue by exploring the influence of RNA secondary structure on the silencing efficiency and the role of nanoparticles in hpRNAs delivery and shelf-life on plant surfaces.

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Wheat Rhizosphere-derived Bacteria Enhance Soybean Against *Sclerotinia sclerotiorum*

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ABSTRACT:
Soybean [*Glycine max* (L.) Merr.] is an important oilseed crop with a high economic value. However, the damaging soybean diseases, *Sclerotinia* stem rot (white mold) caused by the fungus *Sclerotinia sclerotiorum* (Lid.) de Bary, and soybean root rot caused by *Fusarium* spp., are major constraints to soybean production in the Great Plains. Microbes with antagonistic activity are a promising option to control soybean diseases with the advantage of being environmentally friendly and sustainable. In this study, 61 bacterial strains isolated from wheat rhizospheres were used to examine their antagonistic abilities against *S. sclerotiorum* and *F. graminearum*. Six bacterial strains inhibited the growth of *F. graminearum* and two *Pseudomonas spp.* significantly inhibited the growth of *S. sclerotiorum* in the dual-culture assay. These bacterial strains were identified as *Chryseobacterium ginsengisoli, C. indologenes, Pseudomonas poae,* two *Pseudomonas spp.,* and *Delftia acidovorans* by 16S rRNA gene sequencing. Further greenhouse tests found that two *Pseudomonas* spp. protected soybean plants from leaf damage and collapse after being infected by *S. sclerotiorum*. And *C. ginsengisoli* and *C. indologenes* reduced soybean *Fusarium* root rot disease. The study provides potential antagonists for management of soybean white mold.

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Exploring plant defense protein polygalacturonase-inhibiting proteins (PGIPs) for resistance to Sclerotinia sclerotiorum white mold

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Funded Plan of Work: Exploring defense proteins to improve plant resistance to Sclerotinia white mold

ABSTRACT:

Polygalacturonase inhibiting proteins (PGIPs) on plant cell wall inhibit pathogen polygalacturonases (PGs). Previously we identified ad characterized two new PGIPs (Capgip3 and Capgip4) in chickpea (Cicer arietinum). Our analysis of all four chickpea PGIPs showed that CaPGIP1, CaPGIP3, and CaPGIP4 proteins contain N-terminal signal peptides, ten leucine rich repeats (LRR)s like other legume PGIPs. But CaPGIP2 lacked a signal peptide, more than half of the LRRs, and other characteristics of a typical PGIP and therefore cannot be classified as a true PGIP. Transient expression of chickpea PGIPS in Nicotiana benthamiana leaves followed by microscopy showed that all chickpea PGIPS except CaPGIP2 are located to the cell wall or membrane, whereas Capgip2 is found in the cytoplasm (endoplasmic reticulum). We cloned the chickpea PGIPs from cultivar Dwelley and expressed and isolated their recombinant proteins in yeast Pichia pastoris in order to investigate the efficacy of their PG-inhibitory activity. The recombinant proteins of all five S. sclerotiorum polygalacturonases (SsPG1 to 5) were also expressed in Pichia pastoris. Enzyme assays revealed that only CaPGIP3 and CaPGIP4 were capable of inhibiting SsPG1 and SsPG5, with CaPGIP4 showing the strongest inhibition activity. To further study the effect of CaPGIP in disease resistance, CaPGIP overexpression transgenic Nicotiana tabacum lines were generated. The transgenic lines were purified through advancing the generations. Their expression in the transgenic lines were confirmed using RT-PCR and antibody detection. Their effects on Sclerotinia infection are being assessed. These findings will allow us to design experiment to investigate the interaction of CaPGIP with Sclerotinia sclerotiorum PGIP INactivating Effector (SsPINE1).

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Identification and characterization pathogenicity genes affected by DNA mycovirus in *Sclerotinia sclerotiorum*

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Funded plan of work: Characterizing pathogenicity effectors of *Sclerotinia sclerotiorum* preferentially expressed under acidic conditions and during plant infection

ABSTRACT:
White mold disease significantly impacts the yield and marketability of key US crops. While plants possess an immune system capable of resisting infections, *Sclerotinia sclerotiorum* has evolved mechanisms to effectively suppress these host defenses. Previous studies showed that DNA mycovirus (SsHADV-1) consistently conferred hypovirulence to *S. sclerotiorum*. In order identify pathogenicity genes of *S. sclerotiorum* that are affected by the mycovirus SsHADV-1, we created stable strains expressing either the whole viral genome or the caspid protein followed by transcriptome analysis. Strains expressing SsHADV-1 exhibited a substantial loss of virulence. Furthermore, expression of the coat protein (CP) also led to a significant reduction in virulence. We conducted RNA-seq analysis to explore virulence genes that are affected by the SsHADV-1 at the transcriptome level. The comparison between SsHADV-1-expressing strains and wildtype strain WMA1 revealed 208 up-regulated and 459 down-regulated differentially expressed genes (DEGs). Gene ontology (GO) analysis unveiled distinct classifications of up- and down-regulated DEGs, encompassing biological processes, molecular functions, cellular components, and protein classes. Notably, up-regulated DEGs included genes encoding transcription regulators, while down-regulated DEGs were exclusive to defense/immunity and chaperone proteins. Based on this RNA-seq data we want to define pathogenic genes through gene knockout and overexpression assays, protein localization, and screening for interaction proteins using techniques such as Co-IP or yeast two-hybrid systems. These findings aim to contribute to a deeper understanding of *S. sclerotiorum* pathogenesis, potentially allowing the development of innovative chemical inhibitors targeting white mold diseases and the engineering of disease-resistant crops.

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Sclerotinia sclerotiorum SsCelp0028 protein is a cytotoxic effector contributing to virulence

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Funded plan of work: *Sclerotinia sclerotiorum* hijacks host cell death control in infecting plant

ABSTRACT:

Sclerotinia sclerotiorum is a broad-host-range necrotrophic fungal pathogen, causing white mold and stem rot diseases in more than 600 plant species, leading to significant losses in overall yield and marketability of many important crops. Despite extensive studies on this pathogen, the functional mechanisms of the secreted proteins in *S. sclerotiorum*-plant interaction still not adequately understood. In our preliminary study, the small secreted-protein SsCelp0028 was identified by analyzing *S. sclerotiorum* transcriptome during the early infection stage and screening genes predicted to encode secreted necrosis-inducing proteins (NIPs). SsCelp0028 is highly induced at the early stages of *S. sclerotiorum* infection and induces chlorosis in the leaf apoplastic space. Confocal microscopy results showed SsCelp0028 in the apoplastic space. Analysis of SsCelp0028-deletion mutants showed that SsCelp0028 positively regulates *S. sclerotiorum* pathogenicity and tolerance to abiotic stress including cell wall integrity, salt, and oxidation. In addition, SsCelp0028 induces the expression of *Nicotiana benthamiana* defense genes related to hypersensitive response and salicylic acid/jasmonic acid/ethylene signaling pathways. Taken together, our results demonstrate that SsCelp0028 is a secreted apoplast effector critical for *S. sclerotiorum* virulence and stress tolerance, which contributes to our mechanistic understanding of host-*Sclerotinia* interactions.

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Towards identification of population diversity of *Sclerotinia sclerotiorum* in South Dakota for resistance breeding

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ABSTRACT:

South Dakota (SD) ranks among the top ten soybean producing states in the United States. White mold, also known as Sclerotinia stem rot caused by fungus Sclerotinia sclerotiorum, is a major problem in soybeans grown in US Midwestern states including South Dakota. This disease has been frequently reported in SD soybean fields, particularly in farms using limited crop rotations, irrigation, or manure applications, raising growing concerns among our stakeholders. Thus, we initiated a systematic approach to assess the status of white mold pathogen diversity in SD with a long-term goal to identify available resistance in current soybean germplasm. We conducted a statewide disease survey in the 2023 growing season and 33 representative isolates were retrieved from multiple locations. These isolates were checked for their morphological characteristics, growth pattern, sclerotia formation and melanin pigmentation on Potato Dextrose Agar plates. High Molecular Weight DNA is used for molecular marker-based species confirmation and elsewhere. Five of these isolates grew relatively faster than others. Moreover, two of these isolates exhibited more melanin pigmentation upon visual observation. Leaf assay was performed on three different crops viz., sunflower, soybean, and pennycress to generate initial virulence data for the isolates. Currently we are assessing the pathogen aggressiveness on different soyabean germplasm including William82 as standard check. Additionally, we have selected a panel of 400 diverse soybean accessions with available SoySNP50K BeadChip genotypic data in SoyBase for a Genome-Wide Association Study. Screening of germplasm in the greenhouse is ongoing using selected SD white mold isolates to identify resistance QTL in the tested germplasm. Our two-prong approach, i.e. dissecting the white mold pathogenomics as well as tapping the genomic diversity in the host germplasm moves us closer to our research goal to better understand this disease and support our farming communities.

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Strategic Plan

United States Department of Agriculture

Research, Education & Economics

Agricultural Research Service

Northern Plains Area

January 2016

Version 1.0

National Strategic Plan for the Sclerotinia Research Initiative

Integrated Research for Disease Management in Sunflower, Canola, Dry Bean, Pea & Lentils and Soybean

2017 to 2021

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Executive Summary

Vision Statement: An integrated research approach is needed to guide effective development of diagnostic technologies, disease management systems, genomic resources, and crop germplasm exhibiting durable resistance to *Sclerotinia sclerotiorum*. Strategic deployment of these resources will help sustain global food security through increased competitiveness of U.S. canola, pea, lentil, chickpea, common bean, soybean, and sunflower producers.

Process & Development of the Strategic Plan for the National Sclerotinia Research Initiative - 2017-2021: On January 20-22, 2016, approximately 60 scientists and stakeholders with knowledge of the fungal pathogen, *Sclerotinia sclerotiorum* participated in an annual workshop hosted by the United States Department of Agriculture's Agricultural Research Service (ARS) in Minneapolis, MN. ARS, the National Sunflower Association, the U.S. Canola Association, the USA Dry Pea and Lentil Council, the U.S. Dry Bean Council, and the United Soybean Board co-organized this program. Participants reviewed annual research accomplishments and peer assessment of program performance toward that targeted improved understanding and management of Sclerotinia disease in canola, dry edible beans, peas & lentils, soybean, and sunflower. A summary (pending addition of 2015 data) of program performance against priorities of the USDA ARS National Sclerotinia Research Initiative (NSI) strategic plan for 2013 to 2017 follows:

NSI Program Performance 2013-2017

Sclerotinia Initiative Research Progress Evaluation

	2013	2014	2015	2016	2017
	number of accomplishment citations				
Total Accomlishments	43	58			
Total Milestones	79	79			
Achievement Rating (%)	54.4	73.4			
Total Projects	21	22			
Accomplishments / project	2.0	2.6			
Total Publications	266	283			
Germplasm/Varieties released	41	9			

Achievement Rating: # cited accomplishments/ # published milestones *100

ARS leadership considered stakeholder input in the overall assessment of the NSI program performance and in determining the research needs of industry. Reviews of ARS projects associated with NSI also were conducted by the Office of Scientific Quality Review to ensure relevance, quality and performance in meeting goals of ARS national programs. USDA-ARS leadership of NSI included:

Dr. Roy Scott, National Program Leader, Office of National Programs, Beltsville MD
Dr. John McMurtry, Area Director, Northern Plains Area, Ft. Collins, CO
Dr. William Kemp, Agricultural Administrator, Red River Agricultural Research Center, Fargo ND

Stakeholder and scientists worked in concert during the 2015 calendar year to gather and develop input for highest research priorities for the next 5-years. These data were compiled for final edits by stakeholders and scientists during break-out sessions the annual NSI meeting in January, 2016.

There was consensus that genetic as well as management solutions to the Sclerotinia problem were attainable. This optimism was largely due to NSI scientist applications of advances in molecular biology to characterize genes involved in Sclerotinia resistance genomic data of soybean, dry bean, *Medicago truncatula*, the pathogen and other plant genomes. Implementation of molecular tools in breeding programs and the availability of genome sequence resources has greatly enhanced gene discovery and characterization of NSI data through on-line genomic research tools.

Three goals plus performance measures and milestones were agreed upon for the NSI Strategic Plan 2017 to 2021. The following individuals lead teams that developed and edited this Strategic Plan:

Goal 1: Germplasm Resources & Translational Genomics--Phil Miklas, USDA ARS, Prosser WA
Goal 2: Pathogen Biology & Mechanisms of Resistance---- Jim Steadman, Univ. Nebraska, Lincoln, NE
Goal 3 Disease Management & Crop Production---------- Michael Wunsch, ND State Univ., Fargo ND

This strategic plan encompasses the breadth of research disciplines necessary to better understand the disease and to provide significant management options for the affected producers across the U.S. To achieve the strategic goals and research objectives, this plan emphasizes achievements that hinge on teamwork throughout the Sclerotinia research community. All actions and results will be attained in a manner that is both inclusive and open to public scrutiny.

Background

Sclerotinia sclerotiorum, the most important species of *Sclerotinia,* has an unusually large host range of over 400 plant species in numerous families. This fungus causes diseases known as white mold, Sclerotinia stem rot, wilt or stalk rot, or Sclerotinia head rot on a wide variety of broadleaf crops. It commonly causes economic yield loss in dry edible beans, sunflower, soybean, canola, pea and lentils. Many other crops also are susceptible such as alfalfa, potato, peanut, mustard, safflower, flax, borage, crambe, buckwheat, chickpea, lupine, faba bean and numerous vegetables such as lettuce and carrots. The pathogen is found in diverse environments from southern to northern climates and in different agricultural systems under both dryland and irrigated conditions. Although found primarily as a pathogen in the field, it can also be a problem under storage conditions for some crops. The success of this pathogen and its demonstrated ability to adapt to a wide range of conditions can be largely attributed to its aggressive mode of pathogenesis and to the production of specialized multicellular developmental structures for survival and dispersal. Improved knowledge of population structure, ecological types, virulence diversity, germination factors, pathogenicity factors, and advances in molecular biology are needed to develop effective control methods for the numerous diseases caused by this pathogen.

The collective annual economic loss attributed to Sclerotinia damage in the five crops participating in the ARS National Sclerotinia Research Initiative has been as high as $482 million. Specifically, annual losses for each of the crops have been as high as $100 million for sunflowers; $300 million for soybean; $46 for dry edible beans; $24 million for canola; and $12 million for pulse crops. The disease is a serious threat to the future of the confection sunflower, where quality is a significant concern. Diseased seeds can't always be separated in cleaning and processing resulting in bitter tasting seeds which are rejected by consumers.

The primary survival (overwintering) structure of *S. sclerotiorum* is the sclerotium. A sclerotium is a hard resting structure consisting of a light colored interior portion called a medulla and an exterior black protective covering called the rind. The rind contains melanin pigments which are highly resistant to degradation, while the medulla consists of fungal cells rich in beta glucans and proteins. The shape and size of sclerotia depend on the host and where they are produced in or on infected plants. The Sclerotinia disease cycle begins when sclerotia germinate after overwintering in soil. Sclerotia may undergo carpogenic germination which results in the production of a small mushroom called an apothecium and ascospores which are ejected into the environment. The pathogen produces oxalic acid and numerous enzymes that break down and degrade plant tissue. Disease development is favored by moisture and moderate temperatures of 15 to 25 C.

Another method of germination is myceliogenic, where sclerotium produces mycelium. This is common in the disease cycle in Sclerotinia wilt of sunflower. Most other Sclerotinia or white mold diseases of dry edible beans, soybean, canola and sunflower head rot are initiated by carpogenic germination and infection of above ground plant parts by ascospores. Few studies have quantified sclerotia survival in the field. Microbial degradation is the principal reason for a decline in populations of sclerotia. Many fungi, bacteria and other soil organisms parasitize or utilize sclerotia as carbon sources. Crop rotations allow the natural microbial population to degrade sclerotia. Two important fungal parasites involved in the natural degradation of sclerotia are *Coniothyrium minitans* and *Sporidesmium sclerotivorum.* Both may become biocontrol agents for sclerotia.

The effect of tillage on survival of sclerotia is poorly understood. Fungicides have been used with some success in dry edible bean and canola. Crop rotation continues to be used for certain crops such as sunflower where inoculum densities in the soil play a major role in disease development. Most Sclerotinia diseases are not controlled by host resistance. However, moderate levels of host resistance in dry edible beans and soybean have been used in integrated control programs.

The *National Strategic Plan for the Sclerotinia Initiative 2017-2021* provides the research community with a foundation for a comprehensive and integrated research approach toward these problems. The performance measures outlined in this plan are relevant to the current needs of US agriculture. The plan defines the actions that will be taken to solve these problems, describes what is promised or will be

produced, assigns accountability for the work to be accomplished, and provides a mechanism for peer review and assessment of research progress.

National Sclerotinia Research Initiative Strategic Plan (2017 to 2021)

Crop Germplasm Resources & Translational Genomics

Goal 1: Characterize genetic diversity and facilitate transfer of useful genes among germplasm resources to achieve higher levels of field resistance against a wide range of aggressive *Sclerotinia sclerotiorum* isolates.

PM 1.1: Identify new sources of resistance in plant germplasm. USDA & International Germplasm Collections are a valuable and virtually untapped source of genes that could mediate effective resistance to *S. sclerotiorum* in canola, pea, lentil, chickpea, common bean, soybean and sunflower cultivars.

Milestones & Deliverables:

- Improved phenotypic methods for identifying & validating DNA markers for *S. sclerotiorum* resistance in accessions in USDA & World germplasm collections.
- Molecular cytogenetic systems for developing comparative genomic hybridization and single nucleotide polymorphism (SNP) arrays to facilitate germplasm genotyping.
- A comprehensive association of genotypic x phenotypic features among germplasm accessions and wild crop relatives to identify useful sources of resistance to *S. sclerotiorum*.

PM 1.2: Use of interspecific resources to transfer resistance genes into cultivated plant germplasm. Transfer of resistance genes via interspecific and other wide crosses often is constrained by genetic incompatibilities or other problems resulting in non viable progeny. Undesirable genes may accompany the introgression of beneficial genetic variation for Sclerotinia resistance from unadapted and wild species in modern variety production.

Milestones & Deliverables:

- Ability to evaluate utility and track the chromosomal location and expression profile of DNA segments introgressed from interspecific crosses to related breeding lines.
- Improved genetic methods for combining useful genes for resistance to Sclerotinia stem rot from unadapted sources to agronomic lines of canola, chickpea, lentil, pea, soybean and/or sunflower
- Determine the utility of novel resources such as alien chromosome addition stocks for enhancing resistance to Sclerotinia stalk-rot & head-rot derived from wild annual and perennial species of sunflower.

PM 1.3: Generate high-density genetic maps with validated markers for quantitative trait loci (QTL) that confer resistance to Sclerotinia. Validated DNA markers from genome-wide-sequencing and/or exome-capture help annotate genetic maps of existing variation among recombinant-inbred lines or haplotypes for resistance genes. Micro-array technologies enable custom designed chips with

marker sets that facilitate pre-breeding efficiency.

Milestones & Deliverables:

- Highly inbred bi-parental breeding lines and special populations generated in canola, common bean, pea, soybean, and sunflower for identification of QTL associated with Sclerotinia resistance from diverse sources.
- High-resolution genetic and consensus maps of resistance QTL based on annotation with validated markers generated from genome-wide association (GWAS) mapping, exome maps, haplotype maps and/or linkage analysis.
- Transcriptomic, proteomic and metabolomic annotation of genomic sequences in QTL associated with resistance to Sclerotinia diseases
- Characterization of candidate genes involved in biological mechanisms for resistance, such as: oxalic acid oxidase.
- A commodity-based gene atlas with a comprehensive list of all expressed genes, alternative splice products, identification of co-regulated genes and gene networks
- Discovery of transcription factors and elements of gene regulation that mediate expression of disease resistance genes.
- Effective use of genome editing technologies to genetically modify genomic regions in ways that enhance resistance to Sclerotinia diseases or determine candidate gene function
- Identification of allele-specific gene markers within QTL that influence Sclerotinia-host plant interactions
- Improved arrays of validated markers to facilitate screening germplasm resources and expedite marker-assisted-selection in canola, chickpea, common bean, lentil, pea, soybean, and sunflower breeding programs.
- Development and testing of agronomic crop germplasm transformed with putative anti-fungal genes or RNA interfering constructs for reaction to white mold.
- Centralized databases that connect DNA sequences to linkage groups, chromosomes, QTL, candidate genes, polymorphisms and phenotypic traits

PM 1.4: Pyramid white mold resistance in plant germplasm and release germplasm/cultivars with enhanced resistance. Germplasm resources seldom contain all identified favorable alleles for defense against Sclerotinia. QTL from multiple sources must be combined into single lines to enhance overall resistance. Translational genetics will help determine which of the marked genes for disease resistance are most important for use in breeding enhanced germplasm and cultivars.

Milestones & Deliverables:

- Use of allele specific markers and high-throughput phenotyping methods to facilitate pyramiding genes that mediate resistance to Sclerotinia diseases.

- Canola, chickpea, lentil, and pea lines with resistance to Sclerotinia and a broad

portfolio of desirable agronomic traits developed and released.

- Breeding lines and cultivars of pinto and other bean market classes released with broadly effective resistance pyramided from diverse sources - Andean, Middle American, and secondary gene pools (*P. coccineus*), in combination with desirable agronomic traits.
- Establish disease nurseries for characterizing field and greenhouse resistance to all pathogenic forms of Sclerotinia in common bean, soybean and sunflower.
- Soybean breeding lines with Sclerotinia resistance from multiple sources of resistance as verified by QTL-linked markers, including high yield, and resistance to other diseases or insects.
- Commercial & experimental release of sunflower lines exhibiting both Sclerotinia head rot and stalk rot resistance.
- Advanced backcross populations in sunflower and MAGIC populations in common bean used to identify, validate and fine map QTL identified from exotic sources including interspecific populations.

Pathogen Biology & Mechanisms of Resistance

Goal 2: Understand *Sclerotinia sclerotiorum* biology and development

PM 2.1: Characterize migration/population structure and ecological variability of genotypes. The genotypic basis for genetic variability in *S. sclerotiorum* populations within North America is not well characterized. Identifying ecological types within populations will provide an understanding of how disease develops and survives in agro-ecosystems.

Milestones & Deliverables:

- Understanding the interaction of pathogen with environmental factors such as temperature and light.
- Identification of biotypes with resistance to new fungicide chemistry
- Characterization of the genetics of fungicide resistance
- Characterization of ecological types in the population.
- Associate traits in *Sclerotinia* with specific genetic markers.

PM 2.2: Characterize virulence/aggressiveness within the population, identify isolates for use in screening, and monitor durability of host resistance. Differences in virulence exist within pathogen populations, but relation of the variation to pathogen genotype and host range is poorly understood. Physiological characteristics may be important to disease development and pathogenesis. Standard methods will be developed to describe virulence/ aggressiveness in the pathogen. Host

specificity and the range of virulence/ aggressiveness of collections from different hosts and environments will be tested to determine impact on partial resistance.

Milestones & Deliverables:

- Documented reactions of a broad spectrum of isolates on new sources of host resistance.
- Diverse collection of isolates with a broad spectrum of aggressiveness and other characteristics
- Identification of new sources of host resistance using a new set of aggressive isolates
- Criteria for testing virulence/aggressiveness on specific hosts and tissue types.

PM 2.3: Identify environmental and genetic factors involved in myceliogenic and carpogenic germination of sclerotia. Factors like plant exudates are involved in the myceliogenic germination, whereas moisture and temperature are important in carpogenic germination. The biological mechanisms and genetic control of sclerotial germination are not precisely understood. The role of soil microorganisms, other than mycoparasites, in the sclerotia-sphere also may impact the germination process and help identify points in the cycle where germination can be disrupted.

Milestones & Deliverables:

- Identification of host factors that may enhance myceliogenic germination.
- Genetic control and required environmental conditions governing the processes of myceliogenic and carpogeneic germination
- Determination of common and unique genetic events that lead to carpogenic germination in different *Sclerotinia* spp.

PM 2.4: Identify genes that are functional at specific growth and infection stages of Sclerotinia. The genome sequence of *Sclerotinia sclerotiorum* is now available. Gene discovery in *Sclerotinia* will be accelerated by effective means of studying functional genes at specific growth and infection stages, host-pathogen interactions, or under specific environmental conditions. Comparing *S. sclerotiorum* with related *Sclerotinia* spp. will provide insight into factors and mechanisms that limit host ranges of *S. minor* and *S. trifoliorum*, and will in turn help us better understand the mechanisms involved with the extremely wide host range of *S. sclerotiorum*.

Milestones & Deliverables:

- Transcriptomic, genomic, and metabolomics data bases for growth stage-specific genes and infection-related genes from both host and pathogen.
- Improved gene annotation using transcriptomic data.
- Genetic control of differential infection processes of the *Sclerotinia* spp. in response to different host plants

PM 2.5: Identification and verification of candidate genes involved in Sclerotinia pathogenicity. Profiling transcriptomes of *Sclerotinia* in interactions with various host plant tissues would allow identification of pathogen and host gene expression patterns and will provide further clues as to key factors for pathogenicity and defense. Universal mechanisms exist in organisms to inactivate target genes with interfering RNA molecules to prevent them from being translated into functional proteins. RNAi approaches in *Sclerotinia* will be standardized and widely available.

Milestones & Deliverables:

- Development and maintenance of relevant natural and derived culture collections for use in phenotypic association.
- Transcriptome profiling approaches for a variety of gene targets and high through put functional analyses.
- Promoters useful for expressing RNAi constructs during infection (e.g., plant-inducible promoters).
- Inventory of genes potentially involved in pathogenesis recovered from mutagenesis and transcriptome profiling.
- Functional verification of candidate genes using a systems biology approach to gene silencing and quantitative expression assays.

Disease Management & Crop Production

Goal 3: Broaden knowledge of *Sclerotinia sclerotiorum* epidemiology and improve disease management strategies

PM 3.1: Optimize fungicide application programs. Efforts will identify fungicides, concentrations and application methods that provide best control of *Sclerotinia* in canola, soybean, common bean, pea, lentil, chickpea and sunflower.

Milestones & Deliverables:

- A region-wide collection of *S. sclerotiorum* isolates to establish a baseline of fungicide sensitivity
- Identification of the economic return of fungicide applications relative to timing of disease onset.
- Updated management guides for growers on use of fungicides for disease management
- New spraying technologies that improve fungicide performance by enhancing canopy penetration, plant coverage, and fungicide deposition
- Determine most effective timing of fungicide applications relative to canopy closure after blooming

PM 3.2: Develop bio-control alternatives for disease management. Activities will focus in the evaluation of already available commercial bio-control agents, like *Coniothyrium minitans*. Additional surveys and screening exercises will identify new antagonists of *S. sclerotiorum* and optimal application

Milestones & Deliverables:

- Identification of application strategies that will maximize the efficacy of currently available biocontrol agents for control of *S. sclerotiorum*
- Identification of novel antagonists of *S. sclerotiorum* and assessment of their efficacy in field trials
- Updated management guides for growers on use of biofungicides for disease management

PM 3.3: Develop disease-warning systems to optimize management of *S. sclerotiorum*. Disease-warning systems based on epidemiological associations between environmental conditions and cultural practices help optimize fungicide use for control of *S. sclerotiorum* in canola, dry bean, sunflower, soybean, and pulse crops.

Milestones & Deliverables:

- Epidemiological information on disease development to support precision agriculture programs for disease control
- Models that calculate risk of disease development as functions of leaf wetness duration and temperature, and risk of apothecia formation as function of soil moisture conditions
- Effect of tillage practices on Sclerotinia survival
- Economic loss models based on plant density at time of disease onset
- Definition of risk levels to guide crop-specific fungicide selection decisions

PM 3.4: Optimize cultural practices for disease management.
The impact of common cultural practices on disease development will be evaluated through field experiments emphasizing crop rotation schemes, variety/hybrid selection, planting dates, etc. Use of precision agriculture technology will help optimize disease management.

Milestones & Deliverables:

- Collated disease management information with distribution to growers through print media, internet postings and extension publications
- Quantified impact of irrigation scheduling on apothecia development and Sclerotinia disease dynamics with application to irrigation scheduling for optimized crop yields where Sclerotinia is an important limiting factor.
- Assessment of the relative importance of initial Sclerotinia infection from ascospores relative to secondary spread of Sclerotinia from diseased plants to

adjacent healthy plants when stems of diseased plants are girdled by the disease, lodge, and become in direct contact with adjacent healthy plants.

Appendix

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Pullman, Washington
Prosser, Washington
Fargo, North Dakota
Urbana, Illinois
Ames, IA

Universities/Institutions

North Dakota State University
University of Nebraska, Lincoln
Michigan State University
Oregon State University
University of Idaho
Ohio State University
Colorado State University
Agriculture & Agri-Food Canada

Commodity Organizations

US Dry Pea & Lentil Council
National Sunflower Association
United Soybean Board
US Dry Bean Council
U.S. Canola Association