National Sclerotinia Initiative

USDA-ARS

National Sclerotinia Initiative

2025 Annual Meeting

January 22-23, 2025






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

2025 National Sclerotinia Initiative Meeting

January 22 - 23, 2025

Table of Contents

2025 National Sclerotinia Initiative Annual Meeting
(ALL TIMES CENTRAL TIME)

January 22, 2025

7:00 – 8:00 am Breakfast (City A)

8:00 am Welcome & Introductions **– Lanie Bilodeau, USDA-ARS, Fargo, ND**

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

Sclerotinia Research Progress – Disease Management

Moderator ***Shin-Yi Marzano, USDA – ARS, Toledo, OH***

8:30 am **Correlation between minimum free energy and loop structure with the level of RNA silencing in Sclerotinia sclerotiorum –** Abdolbaset Azizi, Luis E. del Rio Mendoza, North Dakota State University, Fargo, ND

8:50 am **Exploring RNAi-based management strategies to confer plant resistance to white mold infection** - Shin-Yi Marzano, Yi-Wen Tseng, USDA-ARS, Toledo, OH

9:10 am **Identification and Evaluation of Biological Control Agents from North Dakota Soybean Fields and Evaluation of Commercial Biological Products for the Control of Sclerotinia sclerotiorum** – Madeeha Matloob, Hope Renfroe-Becton, Wade Webster, North Dakota State University, Fargo, ND

9:30 am **Silver Nanoparticles Synthesized Using Sclerotinia sclerotiorum Metabolites have Antifungal Properties against Fungal Diseases** - Nickisha Pierre-Pierre, George Vandemark, Weidong Chen, USDA-ARS, Pullman, WA

9:50 am **Systematic Evaluation of NSI Impacts on White Mold Management: A Comprehensive Analysis** - Bashir Tiamiyu, Srikanth Kodati, Sydney Everhart, University of Connecticut, Storrs, CT

10:10 – 10:30 am Break (City A)

10:30 am **Crop diversification manipulates soil microbiota for the control of Sclerotinia sclerotiorum** - Chuntao Yin, Nathan Lahr, Shannon Osborne, Michael Lehman, USDA-ARS, Brookings, SD, Weidong Chen, USDA-ARS, Pullman, WA

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

11:10 am **Identification of Brassica napus plant introductions with resistance to multiple Sclerotinia sclerotiorum isolates** - Bita Babakhani, Luis del Rio Mendoza, North Dakota State University, Fargo, ND

Sclerotinia Research Progress – Germplasm Evaluation

Moderator ***Bill Underwood, USDA-ARS, Fargo, ND***

11:30 am **Using genomics assisted breeding to advance sunflower germplasm development** – Brent Hulke, USDA-ARS, Fargo, ND

11:50 am Discussion

12:10 – 1:30 pm Lunch Break (City A)

Sclerotinia Research Progress – Gene Discovery

Moderator ***Bill Underwood, USDA-ARS, Fargo, ND***

1:30 pm **Spatial and Temporal Dynamics of Molecular Mechanisms in Canola Leaves during Sclerotinia Sclerotiorum Infection** - Hira Kamal, Weidong Chen, Kiwamu Tanaka, University of Washington, Pullman, WA; USDA-ARS, Pullman, WA

1:50 pm **Pyramiding plant-derived antifungal proteins to enhance white mold resistance -** Elizabeth Regedanz, Feng Qu, Preangka Briste, Chien-Fu Wu, Shin-Yi Marzano, USDA-ARS, Toledo, OH

2:10 pm **Enhancing the resistance to Sclerotinia by co-expressing the AAE3 and OCD1 genes** - Benjamin Merritt, Chenggang Wang, Zhonglin Mou, Jeffrey A Rollins, University of Florida, Gainesville, FL

2:30 pm **Investigating the relationship between oxalic acid tolerance and basal stalk rot resistance in sunflower** - Srushtideep Angidi, Israt Zaman, Julie Pasche, Luis del Rio Mendoza, North Dakota State University, Fargo, ND; William Underwood, USDA-ARS, Fargo, ND

2:50 pm Discussion

3:10 – 5:00 pm Break & Poster Session (City A)

5:00 – 5:30 pm Free Time

5:30 – 7:00 pm Group Dinner (City A)

<u>January 23, 2025</u>

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 **Developing Soybean Varieties with Resistance to Sclerotinia Stem Rot** - Raju Thada Magar, Feng Lin, Muhammad Salman, Jason Anandappa, Drew Mitchell, Prabjhot Kaur, Suneth Sooriyapathirana, Cuihua Gu, Paige Pickett, Randy Laurenz, Martin Chilvers, Dechun Wang, Michigan State University, East Lansing, MI

8:50 am **Improved white mold resistance in dry and snap beans through multi-site screening throughout major production areas -** Evan Wright, F.E. Gomez, Martin Chilvers; Collaborators: M. Wunsch (ND), Jim Myers (OR), Phil Miklas (WA), Juan Osorno (ND), C. Urrea (NE), K. Kmiecik (WI), Valerio Hoyos-Villegas (QC)

9:10 am **Mobilization of white mold resistance in common bean -** Phil Miklas, USDA-ARS, Prosser, WA; Jim Myers, Oregon State University, Corvallis, OR; Phil McClean, Juan Osorno, North Dakota State University, Fargo, ND

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

10:30 am **Combating Sclerotinia Stem Rot (SSR) in Canola; Pyramiding and Introgression of Resistant Genes followed by Morphological and Molecular Screening** - Md Zahangir Alam, Luis E. del Rio Mendoza, Mukhlesur Rahman, North Dakota State University, Fargo, ND

Sclerotinia Research Progress – Pathogen Biology

Moderator ***Mehdi Kabbage, University of Wisconsin, Madison, WI***

10:50 am **Detox Mechanisms and Environmental Sensing in Sclerotinia sclerotiorum** - Madeline Bondy, Nathaniel Westrick, Damon Smith, Mehdi Kabbage, University of Wisconsin, Madison, WI

11:10 am Discussion

11:30 am – 1:00 pm Lunch Break (City A)

1:20 pm **Deciphering the genetics of Sclerotinia sclerotiorum aggressiveness to develop multi-crop resistance tools** - Megan McCaghey, Hsuan Fu Wang, University of Minnesota – Twin Cities

1:40 pm **A putative virulence effector of Sclerotinia sclerotiorum identified through expressing the hypovirulent DNA virus SsHADV-1** - Wei Wei, George J Vandemark, Weidong Chen, USDA-ARS, Pullman, WA

2:00 pm Discussion/Wrap Up

2:30 pm Departure

Abstracts

Combating Sclerotinia Stem Rot (SSR) in Canola: Pyramiding and Introgression of Resistant Genes Followed by Morphological and Molecular Screening

Md Zahangir Alam[1], Luis E. del Rio Mendoza[2] and Mukhlesur Rahman[1]

[1]Department of Plant Sciences, North Dakota State University
[2]Department of Plant Pathology, North Dakota State University

Funded Plan of Work: Introgression and Pyramiding of Sclerotinia Stem Rot Disease Resistant Gene(s) into Canola Cultivars

ABSTRACT:

Sclerotinia sclerotiorum is a soil-borne, necrotrophic fungus that could severely infect one of the most important oil crops, canola (*Brassica napus* L.), causing losses to billions of US dollars worldwide. The lack of complete and durable resistance, along with reliance on chemical fungicides, makes it necessary to identify resistant germplasm, which is laborious, time-consuming, and costly. Our objectives were to identify the SSR-resistant gene(s), introgress them into elite breeding lines, develop diverse breeding lines with enhanced SSR disease resistance, and develop molecular markers to facilitate speed breeding. To achieve this, we have identified five highly resistant lines with high seed yield potential. This was accomplished by screening about 3,300 backcross populations using the Petiole Inoculation Technique (PIT) and Stem Inoculation Technique (SIT), followed by backcrossing the surviving plants with NDOLA-2, an elite breeding cultivar developed in our lab. We have genotyped and phenotyped 438 diversified breeding lines (18,000 plants) with PIT in two greenhouse environments, identified both highly resistant and highly susceptible lines. In addition, we have phenotyped 350 diversified breeding lines (22,000 plants) using SIT in one greenhouse and five field environments and identified six highly resistant lines. We also developed and tested 57 KASP (Kompetitive Allele Specific PCR) markers, of which 12 showed strong potential for distinguishing resistant canola lines from susceptible ones. Our findings demonstrate significant progress toward developing resistant canola cultivars with high yield potential.

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Correlation between minimum free energy and loop structure with the level of RNA silencing in *Sclerotinia sclerotiorum*

Abdolbaset Azizi and Luis E. del Río Mendoza

Department of Plant Pathology, North Dakota State University, Fargo, ND.

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

Abstract:

Sclerotinia sclerotiorum (Lib.) de Bary is a major pathogen responsible for sclerotinia stem rot (SSR), leading to substantial yield losses in canola. Traditionally, its management has relied heavily on chemical fungicides. However, growing concerns over chemical use in agriculture have driven the exploration of alternative control strategies, such as RNA silencing. Previous studies by our program have shown a significant reduction in SSR disease severity when hpRNA from four *S. sclerotiorum* genes are sprayed on canola plants. This study investigated the role of RNA secondary structure, specifically minimum free energy (MFE), in silencing these four key *S. sclerotiorum* genes: Chitin binding domain, Mitogen-activated protein kinase, Oxaloacetate acetylhydrolase, Cytochrome P450, and Abhydrolase-3. Double-stranded RNA (dsRNA) and hairpin RNA (hpRNA) targeting these genes were synthesized in vitro and in vivo, respectively, to evaluate their gene-silencing efficacy. The results showed that applying 7 ng/µl of dsRNA significantly reduced the expression of all target genes, irrespective of their RNA secondary structure energy levels. In contrast, treatment with 10 ng/µl of total bacterial RNA expressing hpRNA revealed a significant ($P = 0.05$) difference in silencing efficiency between high and low MFE structures. Greenhouse experiments further demonstrated that spraying canola plants with 300 ng/µl of total bacterial RNA containing low MFE hpRNA significantly ($P = 0.05$) suppressed disease symptoms compared to controls, whereas high MFE hpRNA was less effective. Additionally, the presence of loop structures in hpRNA significantly ($P = 0.05$) enhanced gene silencing. These findings highlight the critical role of RNA secondary structure in the effectiveness of RNA silencing and underscore its importance in the design of RNA-based pathogen control strategies.

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

Identification of *Brassica napus* plant introductions with resistance to multiple *Sclerotinia sclerotiorum* isolates

Bita Babakhani and Luis del Río Mendoza, Dept. of Plant Pathology, North Dakota State University. Fargo, ND

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.). In North Dakota, it has been estimated that SSR leads to an annual decrease in canola production by an average of 0.5 % per unit increase in disease occurrence. Resistance to SSR is influenced by multiple genes that have additive effects. The quest for sources of resistance typically starts with large number of germplasms being evaluated with a single strain of the pathogen. Once multiple sources have been identified, they are evaluated against multiple isolates. This study characterizes the reaction of 16 *B. napus* plant introduction genotypes (PIs), previously identified as resistant to *S. sclerotiorum* to inoculations, with five highly virulent isolates, with the goal of identifying PIs with broader and more effective resistance. The isolates, collected from multiple hosts and states were inoculated on the plants at the flowering stage using the agar plug stem inoculation technique. The replicated trial was conducted in greenhouse conditions twice. The resulting lesions were measured seven days later. Significant differences in the reaction to inoculations were detected among PIs with one of them showing high levels of resistance to all isolates and lesions averaging 16.2 mm in length. The susceptible control had stem lesions averaging 87 mm in size. Seven other PIs had lesions of 24, 26, 28, and 29 mm, respectively and were considered moderately resistant to all isolates. The remaining PIs were resistant to some isolates but susceptible to others. Significant differences also were detected among isolates on their ability to grow and produce sclerotia on agar medium. These results will be useful to breeding programs and will contribute to further our efforts to characterize genetic resistance to this pathogen by establishing a pecking order for the development of mapping populations. At the same time, they provide an opportunity to study the genetic basis of the differential response to isolates.

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Breeding for *white mold* resistance in chickpea: prioritizing *Sclerotinia* isolates from different hosts and locations across the US Pacific Northwest based on mycelial compatibility groups (MCGs) and genomic diversity.

Miguel A. Garcia Aguirre & Douglas R. Cook, University of California, Davis, CA.

Funded Plan of Work: Population genomics of Sclerotinia sclerotiorum across pulse production regions of the US Pacific Northwest

ABSTRACT:

The goal of this proposed project is to develop chickpea (*Cicer arietinum*) varieties, suited to cultivation in the Northwestern US, with resistance to *Sclerotinia* white mold caused by *Sclerotinia sclerotiorum*. Towards this end, we will screen two *Cicer* germplasm collections: (1) recombinant inbred lines derived from crosses between cultivated chickpea (*Cicer arietinum*) and its progenitor wild species, *Cicer reticulatum* and *Cicer echinospermum*, which harbor excessive genomic and phenotypic diversity, and (2) an international collection of cultivated accessions that have been the focus of disease resistance screening for other pathogens. This project leverages significant prior and ongoing work to develop genetic and genomic resources for trait dissection and introgression within chickpea and its wild progenitor species. As a first step in this newly-funded project, we have assembled and are characterizing fungal and plant germplasm. Wild x cultivated recombinant inbred lines originate from a previous USAID-NSF project, while cultivated material was obtained from ICARDA. Fungal germplasm was obtained from colleagues, collected at multiple locations over multiple years. First steps include curating fungal strains, including long-term preservation of sclerotia and their initial phenotypic and genetic characterization. Sixty strains originate from 9 locations on 5 different hosts. Among this set, we identify variation in mycelial compatibility groups, indicating underlying genetic/genomic differences. DNA has been extracted for genomic analysis, which will serve to prioritize strains for use as inoculum in disease resistance screening assays. We are also piloting plant inoculation methods used in previous studies, including (a) inoculation of dropped flowers and petioles, and (b) stem inoculation. Our goal is to have quantitative assays for disease severity, which can be used in genetic analyses (QTL in the case of major effect loci and Genomic Prediction in the case of multiple small effect loci).

Contact Information - Dr. Douglas R. Cook, Dept. of Plant Pathology, University of California, One Shields Ave 354 Hutchinson Hall, Davis, CA, 95616.; 530-754-6561; drcook@ucdavis.edu

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

Godwin James, Ruby Tiwari and Dilip M. Shah Donald

Danforth Plant Science Center, St Louis, MO 63132

Funded Plan of Work: Exploiting small cysteine-rich antifungal peptides for management of the white mold disease in soybean and canola

Abstract:

White mold disease caused by the necrotrophic ascomycete, *Sclerotinia sclerotiorum*, leads to significant economic losses in U.S. soybean production. Currently, its management relies primarily on chemical fungicides. Lack of effective genetic resistance to this disease in soybean germplasm and increasing pathogen resistance to fungicides makes white mold difficult to control. Small cysteine-rich antifungal peptides with multi-faceted modes of action possess potential for development as sustainable spray-on bio-fungicides. Previous study from our lab identified a novel family of *Oleaceae*-specific defensin genes from both wild and cultivated perennial olive trees (*Olea europaea*). One of the members, OefDef1.1 effectively suppressed the *in vitro* growth of the ascomycete fungal pathogens, *Botrytis cinerea* and three *Fusarium* species (Li et al., 2019). In this study, we have identified a truncated and modified 22-amino acid cationic peptide GMAOe1C_V1 derived from full length OefDef1.1 defensin. GMAOe1C_V1 exhibited potent *in vitro* fungicidal activity against aggressive *S. sclerotiorum* strains (*Ssc 555* and *Jc1*) with the minimum inhibitory concentration (MIC) of 24 µM. *In planta* assays demonstrated that foliar application of GMAOe1C_V1 on soybean (cv. Williams 82, inoculated with *Ssc 555*) and canola (cv. Westar, inoculated with *Jc1*) significantly reduced disease lesions at 24 and 48 µM. GMAOe1C_V1 also decreased sclerotia production of both strains at sub-lethal and lethal concentrations. Modes of action studies revealed that GMAOe1C_V1 permeabilized the fungal cell membrane and rapidly entered the hyphae. Furthermore, combining GMAOe1C_V1 with the triazole fungicide tebuconazole at sub-inhibitory concentrations additively controlled white mold in soybean *in planta*, although no additive effects were observed *in vitro*. Our results demonstrated that small antifungal peptides have significant potential as bioinspired fungicides for the management of white mold in soybean and canola.

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

E.M. Wright, F.E. Gomez, M.I. Chilvers and V. Hoyos-Villegas

Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI

Collaborators: M. Wunsch (ND), J. Myers (OR), P. Miklas (WA), J. Osorno (ND), C. Urrea (NE), K. Kmiecik (WI), V. Hoyos-Villegas (QC)

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 2024, trials were conducted in MI, ND, NE, OR, WA and QC with a total of 18 entries that included Small Red, Pinto, Slow-Darkening Pinto, Black, Navy, Great Northern and Cranberry 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.

Contact Information – Valerio Hoyos-Villegas, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, 48824; hoyosval@msu.edu

Detox Mechanisms and Environmental Sensing in *Sclerotinia sclerotiorum*

Madeline Bondy, Nathaniel M. Westrick, Damon L. Smith, and Mehdi Kabbage

Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, United States

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

Abstract:
In 2024, we continued our work addressing novel detox mechanisms and virulence determinants in *Sclerotinia sclerotiorum*. We identified both novel detox components through gene expression studies, and we continued the functional characterization of previously identified genes. The latter focused largely on a secreted laccase, playing a role in both detoxification and environmental sensing. 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 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 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. This past year, we conducted a complex carbohydrate analysis revealing the glycosyl composition and linkage analyses, and H-NMR data indicate that the laccase mutation impacts the composition and structure of the fungal cell wall polysaccharides. 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. Cryo-EM will be conducted in 2025 to provide high resolution imaging of the fungal cell well. 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 - Mehdi Kabbage, Professor and Director of Graduate Studies, University of Wisconsin – Madison, Madison, WI; email kabbage@wisc.edu

Spatial and Temporal Dynamics of Molecular Mechanisms in Canola Leaves during *Sclerotinia Sclerotiorum* Infection

Hira Kamal,[1] Weidong Chen,[2] and Kiwamu Tanaka[1]
[1]Department of Plant Pathology, Washington State University, Pullman, WA; [2]USDA-ARS, Pullman, WA

Funded Plan of Work: System View of Pathogenesis and Host Defense Responses at Specific Infection Stages of *Sclerotinia sclerotiorum*

ABSTRACT:

Sclerotinia stem rot, caused by polyphagous necrotrophic pathogen *Sclerotinia sclerotiorum*, poses a significant threat to crops such as *Brassica napus* (canola) worldwide. This pathogen initiates infection by subverting plant defense mechanisms, leading to tissue necrosis and degradation. Developing durable resistance to *S. sclerotiorum* offers a sustainable alternative to chemical control strategies. This study investigates the spatio-temporal genomic regulatory networks and metabolomic pathways underlying disease progression in canola plants, with a particular focus on the dynamic plant-pathogen interactions at the infection front. Notably, infection with an oxalate-deficient *S. sclerotiorum* mutant (M202) results in pronounced chlorosis at the necrotic margin (the boundary of necrotic lesions), whereas infection with the wild-type strain causes more extensive necrosis. RNA-seq transcriptomic analysis across infection stages revealed spatially distinct gene expression patterns, particularly among Light Harvesting Complex (LHC) genes involved in the xanthophyll cycle. These genes exhibited tissue-specific regulation between infections by the Wild-type and mutant *S. sclerotiorum* strains. Metabolic analysis using HPLC further identified significant shifts in xanthophyll cycle metabolites. In necrotic tissue infected by the wild-type strain, continuous conversion of violaxanthin to zeaxanthin via intermediate antheraxanthin was observed, indicating tissue acidification and oxidative stress. This was corroborated by upregulation of genes encoding violaxanthin de-epoxidase (VDE) enzyme, driving zeaxanthin accumulation and increased non-photochemical quenching (NPQ). Conversely, M202-infected margin and necrotic tissues showed elevated antheraxanthin levels instead of zeaxanthin, along with a marked reduction in neoxanthin. RT-qPCR analysis showed higher expression of genes encoding zeaxanthin epoxidase (ZEP/ABA1) and 9-cis-epoxycarotenoid dioxygenase (NCED), enzymes involved in ABA biosynthesis, in the margin tissues. This suggests ABA accumulation, potentially compromising immune responses and enhancing susceptibility to *S. sclerotiorum* infection. These findings highlight the tissue specific plant responses in the xanthophyll cycle and ABA biosynthesis, providing new insights into the molecular and metabolic mechanisms governing plant susceptibility to *S. sclerotiorum* infection.

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Pyramiding plant-derived antifungal proteins to enhance white mold resistance

Preangka Briste, Chien-Fu Wu, Feng Qu, Elizabeth Regedanz, Shin-Yi Marzano

Funded Plan of Work: Pyramiding plant-derived antifungal proteins to enhance white mold resistance

ABSTRACT:

Work was conducted since July 2024 to engineer plants to express defensin proteins using a novel virus-induced gene silencing (VIGS) vector to test the effectiveness of suppressing white mold infection. So far, we tested two sequences of defensins, Nt1.2 and 1.6 from *Nicotiana tabacum* that we published recently for transient expression. Initially, the VIGS constructs expressing these defensins were likely toxic to *E. coli* and very few clones were obtained and all had various mutations. Therefore, we inserted a plant intron in front of the Nt1.2 sequence and numerous correct clones were obtained. However, when one correct clone was used to infect *N. benthamiana*, the plants did not show any symptoms until 15 days after inoculation (the frame-shifted control started to have symptoms at 8 days post infiltration). Even at 16 dpi, only a few plants developed symptoms, suggesting antiviral. Among them, 3 plants developed severe mosaic but no necrosis, suggesting hypersensitive response (HR) that associates with systemic resistance. One plant developed systemic necrosis that eventually led to the death of the plants, and it could be incomplete HR. These symptoms are transferrable that when a leaf from one mosaic plant was homogenized and inoculated to fresh seedling, it caused severe mosaic but no necrosis in all new plants, and vice versa. We have sequenced the viral RNA collected from the 3 original mosaic plants. The viral RNA no longer contained the intron, and lost the first 51 nt (17 aa) of the SM1 insert which is a signal peptide. We are still sequencing the viral RNA obtained from the necrotic tissues. For this objective, we will use the two kinds of leaf tissues to infect soybean to determine whether they enhance soybean defense against *S. sclerotiorum*. Moreover, we also examined the in vitro antifungal activities of novel small proteins, expressed by a yeast expression system to produce sprayable cell-free filtrates. We see that Nt1.5 from *N. tabacum*, XP_022016527 from sunflower, NP_0013522088 from chickpea under pH5.5 as well as CP from SlaGemV1, AA038756 from chickpea, and XP_022016527 from sunflower under pH 6.0 have moderate antifungal activities with the mycelial growth index greater than 10.

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

Yi-Wen Tseng, Shin-Yi Marzano, USDA-ARS, Toledo, OH 43606, USA

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. After optimization in our formulation, we produced a large amount of dsRNA using *E. coli* and evaluated the efficacy of SIGS in the field in 2024 and found the approach promising to manage white mold infections with statistical significance. Therefore, an invention disclosure has been submitted to USDA following the area office's technology transfer officer suggestion. Next, we are determining the effects of perturbing SsAgo2 homeostasis by silencing an endogenous and novel miRNA of *S. sclerotiorum* using a short tandem target mimic (STTM). We transformed *S. sclerotiorum* to express STTM to block the miRNA, resulting in an upregulation of SsAgo2, suggesting the biological function of the novel miRNA playing a role in self-regulating the SsAgo2 homeostasis. In the future, we hypothesize that this novel miRNA could be weaponized to silence SsAgo2 upon infection.

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Identification and Evaluation of Biological Control Agents from North Dakota Soybean Fields and Evaluation of Commercial Biological Products for the Control of *Sclerotinia sclerotiorum*

Matloob, M.[1], Renfroe-Becton, H.[1], and Webster, R.W.[1]

[1] Department of Plant Pathology, North Dakota State University, Fargo, ND

Funded Plan of Work: Identification of Biological Control Agents in the Northern Great Plains and Evaluation of BCAs for Controlling *Sclerotinia sclerotiorum*

Abstract:
Sclerotinia sclerotiorum is a fungal pathogen resulting in significant yield and economic losses globally. In 2023, it caused a 32.3 million bushels loss in North Dakota soybean due to Sclerotinia stem rot. To improve disease management, we have been exploring biological control agents (BCAs) derived from bulk soil collected from North Dakota soybean fields as an alternative to chemical control. This study aims to isolate and characterize BCAs collected for across North Dakota. So far, 218 fungal isolates from six counties sampled in 2023 were screened for biological control activity against *S. sclerotiorum*. Preliminary results from dual culture and detached leaf assays indicate nine fungal isolates inhibit the growth of *S. sclerotiorum.* Based on molecular identification, eight belong to *Aspergillus, Clonostachys, Penicillium,* and *Fusarium* genera and one is still unknown. Some identified BCAs exude unknown compounds that hinder fungal growth while others compete for resources, suppressing *S. sclerotiorum* mycelial and sclerotial development. Liquid chromatography-mass spectrometry and other molecular analyses will be used to identify these antifungal compounds. This ongoing study shows progress in discovering and characterizing bioagents from soil ecosystems as efficient antagonists against *S. sclerotiorum*.

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Deciphering the genetics of *Sclerotinia sclerotiorum* aggressiveness to develop multi-crop resistance tools

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. 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 *S. sclerotiorum* isolates for aggressiveness and resistance screening across crop plants and 2) identify candidate pathogenicity genes that are conserved across crop plants using transcriptomics. Screenings of 28 isolates on soybean, in three experimental replicates, resulted in a panel of five isolates of high, medium, and low aggression that can be used for soybean resistance screening in Minnesota. The results of these studies revealed distinct lesion sizes and disease progression on a per isolate basis ($p < 0.001$ and $p = 0.001$); MNSS4 and WISS47 exhibited lower aggressiveness than MNSS6, MNSS2, and SSPotter. Following these results, the subpanel of five MN isolates was used to challenge 4 soybean germplasm lines with known resistance levels greenhouse trials. The subpanel of isolates differentiated disease progress in highly susceptible versus moderately to highly resistant lines ($p = 0.001$). Seventeen isolates were screened on soybean, dry bean, sunflower, and canola in growth chambers to identify isolates with conserved aggressiveness rankings across crop species. Two screening per crop species have been completed. A multistate subpanel of seven isolates with conserved aggression across crop species successfully differentiated resistant and susceptible sunflower lines (HA441 and HHA89, $p= 0.001$). RNA extractions have been completed from three replicates of sunflower and soybean inoculated with one highly aggressive isolate, Xtra7, and one low aggressive isolate, WISS47. CDNA libraries will be prepared and sent for sequencing at the University of Minnesota Genomics Center. RNA sequencing can help 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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Toward Developing Resistance to White Mold in Commercial Common Bean Market Types

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Research Project: Mobilization of white mold resistance in common bean.

ABSTRACT: During 2024, multiple genetic and breeding populations were used in support of deploying resistance to white mold into common bean market types (snap, pinto, black) with favorable traits. The Snap-Dry Bean MAGIC population was characterized using the seedling straw test in 2024. A total of 942 families were evaluated in the F_6 generation. Where families were segregating for seed color, the predominant color was tested. Families were further subdivided by growth habit and traits into four populations: snap-indeterminate (168 families), snap-determinate (365), dry-indeterminate (189) and dry-determinate (220). The population showed an approximate normal distribution skewed towards resistance for disease severity, with 40 families significantly more resistant than the partially resistant checks (G122 and NY6020-4). Fifteen lines from a NAM population using WMG904-20-4 as the common parent were further subdivided by seed color into 56 lines. Fifty-four of these sublines were evaluated using the seedling straw test and all were characterized in field plots at the OSU Vegetable Research Farm in 2024. Forty-seven of the lines were not significantly different from the most resistant check (Cornell 501). The lines exhibited a range of characteristics for both seed and plant traits and as either snap or dry beans. Genomic prediction using 379 lines from the Pinto Bean MAGIC population (WM-MAGIC) showed 49% predictive ability (cross-validation). One slow-dark parent (ID14-4) in WM-MAGIC contributed to the slow dark lines WMM-556 and WMM-750 which ranked in the top-yielding group for the 2024 variety trials conducted in ND. A modified seedling straw test was developed using a 1 to 5 scale to score WM severity. A total of 583 WM-MAGIC lines were evaluated using both the standard seedling straw test (1-to-9 scale) and the modified test, with the modified approach resulting in a 15% increase in heritability. Seventy-six select pinto bean RILs were evaluated in the new white mold field nursery in Othello, WA, in 2024. Four QTL exhibited a significant effect for increased field resistance: WM1.5 (derived from VCP-13), WM2.2 (USPT-WM-12), WM3.1 (PT12-37), and WM7.7 (PT12-37). Seventeen of the 76 lines combining acceptable agronomic traits and field resistance were selected for advanced testing. The introgression of QTL originally derived from *P. coccineus* source (I9365-31) was initiated. USDA Rattler pinto and black bean Eclipse as recipient parents were crossed with resistant RIL lines R31-24 and R31-83 from R31 (Raven/I9365-31) population. Large F2 populations (n>1000) were developed and will be screened with markers developed at multiple intervals from the peak SNP bordering white mold QTL WM2.2a and WM5.4. In addition, seed color and pattern markers will be used to select lines with pinto seed types. Overall, continued progress was made toward development of populations and lines with improved resistance to white mold combined with commercial traits whilst generating, validating and employing QTL haplotypes for marker-assisted selection.

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Poster abstract

Exploring white mold resistance in snap bean for transfer to dry bean

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Research Project: Mobilization of white mold resistance in common bean.

ABSTRACT:

The Snap-Dry Bean MAGIC population was developed from an 8-way cross of six snap and two dry beans and consists of 942 families that are now in the F_6 generation. In 2023, we conducted a field trial and phenotypic characterization. To facilitate analysis, the families were subdivided by growth habit and traits into four populations: snap-indeterminate (168 families), snap-determinate (365), dry-indeterminate (189) and dry-determinate (220). This has been followed by a seedling straw test for white mold resistance in 2024. Four replicates with reps repeated over time were employed. The population showed an approximate normal distribution skewed towards resistance for disease severity. Seven-hundred twenty-one families were not significantly different from NY6020-4 for disease severity. Eighteen families show consistently high levels of resistance across reps. Leaf tissue samples were collected for DNA extraction and genotyping. A nested association mapping (NAM) population previously created using WMG904-20-4 as the common parent and crossed to Cornell 501, NY6020-5 M0070 and A195 was further evaluated. This material was characterized agronomically and for white mold reaction, and 15 lines were selected that exhibited a combination of high levels of resistance and good agronomic performance. Thirty-two lines come from the Cornell 501 cross, six from NY6020-5, seven from M0070 and 11 from A195 crosses. The lines were further subdivided by seed color into 56 lines. Fifty-four of these sublines were evaluated using the seedling straw test and all were characterized in field plots at the OSU Vegetable Research Farm in 2024. While none of lines had significantly lower disease scores than the partially resistant check (Cornell 501), 47 had significantly lower scores than the susceptible check (OSU5630). The lines exhibited a range of characteristics for both seed and plant traits and some lines have potential utility as either snap or dry beans.

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Silver Nanoparticles Synthesized Using *Sclerotinia sclerotiorum* Metabolites have Antifungal Properties against 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 (AgNPs) formed from the metabolites of the plant pathogenic fungus *Sclerotinia sclerotiorum* wild type WMA1. Cell free culture filtrates were combined with silver nitrate and subsequently evaluated for its 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 an ultraviolet visible spectroscopy peak of 413 nm. The nanoparticles were also evaluated for the control of *S. sclerotiorum* and *Botrytis cinerea* on detached bean leaves and on intact bean plants in the greenhouse. Detached bean leaves were treated with the nanoparticles at different concentrations (100 ppm, 250 ppm and 500 ppm) prior to inoculation. The synthesized nanoparticles reduced disease severity on bean leaves caused by *S. sclerotiorum* and *B. cinerea*. 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. For the greenhouse studies, the AgNPs at 250 ppm was used to treat 3-wk-to-4-wk old bean plants before fungal inoculation. Antifungal experiments conducted in the greenhouse showed a disease reduction in plants treated with the AgNPs in comparison to the inoculated control. These results have demonstrated the potential use of nanoparticles in the control of *S. sclerotiorum* and other plant pathogens.

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Enhancing the resistance to Sclerotinia by co-expressing the AAE3 and OCD1 genes

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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* through manipulating endogenous host pathways. The oxalic acid secreted by *Sclerotinia sclerotiorum* is essential to colonize and produce disease symptoms in *Brassica napus* and other Brassicaceae species. A two-step oxalate metabolic pathway is present in Brassicaceae species. First, oxalyl-CoA synthetase (*AAE3*) ligates oxalate and CoA to form oxalyl-CoA, and then Oxalyl-CoA Decarboxylase1(*OCD1*) catalyzes oxalyl-CoA into formyl- CoA and CO2. Interestingly, *AAE3* and *OCD1* genes are induced by oxalate and Sclerotinia infection in Arabidopsis, and *aae3* mutants in Arabidopsis show increased susceptibility to Sclerotinia. We cloned the *BnAAE3* and *BnOCD1* genes from *Brassica napus* and created transgenic lines overexpressing *BnAAE3* and *BnOCD1*, respectively. 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 created a new vector to simultaneously overexpress *BnAAE3* and *BnOCD1* in a single transgenic line and have included sequences encoding signal peptides for both genes. Both single gene expression lines and double AtAAE3/AtOXC1 gene lines have been isolated. All lines are being selfed to create homozygous lines. Resistance phenotypes upon challenge with *S. sclerotiorum* will be performed on the homozygous lines. If these proof-of-concept experiments are successful, we plan to enhance durable disease resistance in *B. napus* by CRISPR mutagenesis of *BnAAE3* and *BnOCD1* gene promoters to increase gene expression, the level of endogenous oxalate metabolism and disease resistance.

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Assessing Sclerotinia Stem Rot Resistance Across Early Maturity Soybean PI Lines

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Funded Plan of Work: Evaluation of Soybean Resistance to Sclerotinia Stem Rot

ABSTRACT:
Sclerotinia sclerotiorum, the causal agent of Sclerotinia stem rot (SSR), is a devastating fungal pathogen that led to over 11 million bushels of loss in northern US soybeans in 2022. While utilizing resistant cultivars can effectively limit SSR, no commercial varieties have been found to offer complete resistance. In this study, we screened 125 soybean accessions from the USDA germplasm collection, with different maturity groups (ranging from 000 to I). Four previously established check lines (Dwight, 51–23, SSR51 and 52-82B) were used, as suggested by Webster et al. (2021). The greenhouse experiment was conducted in a randomized complete block design. The plants were inoculated at the V5 growth stage with mycelium plugs from a highly aggressive isolate of *S. sclerotiorum* (WI-20) using the cut-petiole method. The inoculated plants were placed in the misting chambers to maintain 100% relative humidity for 10 days. Lesion length was monitored, and data were collected at 5, 7, and 10 days post-inoculation. Area under the disease progress curve (AUDPC) values were generated from this data. While all PI lines showed disease symptoms, the level of resistance varied significantly among them ($P < 0.001$). A total of 33 lines demonstrated higher resistance compared to the previously established resistant check, 52-82B. Additional lines are currently being screened, and the resistant lines will undergo further evaluation using multiple isolates with varying levels of aggressiveness. This research can aid in establishing new check lines for effectively screening early-maturity soybean lines against *Sclerotinia sclerotiorum*.

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Elucidating Organic Acid-Responsive Pathways in *Sclerotinia sclerotiorum*

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Abstract:

The increased demand and soaring prices for organic soybean products has driven a 73% increase in its production acreage over the past decade. However, pests and diseases, particularly white mold disease caused by *Sclerotinia sclerotiorum* (*S.s.*), pose significant challenges to organic production due to limitations on allowable fungicide application and variable soil properties. In the US Midwest, livestock manure is extensively applied as a soil amendment to enhance nutrient availability and to support soil health. Among soil amendment organic acids (OA) constituents, humic acid (HA) and fulvic acid (FA) are the principal water-soluble fractions that have been extensively studied for their roles in promoting plant growth and affecting soilborne disease pressure. In fact, many commercial soil amendments are rich in these two organic acids. Thus, we have started to investigate the effect of HA and FA on soybean white mold pathosystem that could alter the disease pressure, particularly in livestock manure supplied soybean cultivation. In our study, we used 33 diverse isolates collected from South Dakota that we characterized based on their mycelial growth, melanin pigmentation, sclerotia production and responsiveness to OA on amended PDA. We expected to find a general decrease of isolate fitness upon increase in the concentration of OA as shown in for other fungal pathogens in published studies, however to our surprise we found FA and HA gradients have differential response on these *S.s.* isolate ($r=3$, $n=6$) mycelial growth ($p<0.05$, Tukey's HSD) indicating a genetic basis of OA responsiveness. From these isolates, five distinct groups (3E, Y5, 2A, 2C, 22B) were selected for treatment with a gradient of OA concentrations (FA/HA: 20, 30, 40, and 60 mg/L) and a mock control. ImageJ analysis of data on mycelial growth, sclerotia number, and its size identified that the treatment concentration does show effect on growth responsiveness and sclerotia production ($p<0.05$, Tukey's HSD) among these isolates. Notably, at 30 mg/L of FA, isolate Y5 showed a negative correlation for sclerotia number, whereas 22B exhibited a positive correlation. Similar trends were observed for sclerotia number across all the FA concentration for both isolates, although in nonlinear fashion. Thus, it is evident that *S.s.* isolate genetic diversity influences their OM responsiveness. We aim to identify the underlying contrasting genetic components influencing OA responsiveness in *Sclerotinia sclerotiorum*, contributing to a deeper understanding of this important soilborne pathogen in organic amended production systems.

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A Comprehensive Evaluation of Genomic Prediction Models for Selecting Sclerotinia Stem Rot Field Resistance in Soybean (*Glycine max*)

Raju Thada Magar, Feng Lin, Muhammad Salman, Jason Anandappa, Drew Mitchell, Prabjhot Kaur, Suneth Sooriyapathirana, Cuihua Gu, Paige Pickett, Randy Laurenz, Martin Chilvers, and Dechun Wang, Michigan State University

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

Abstract:
Sclerotinia stem rot (SSR), or white mold, caused by *Sclerotinia sclerotiorum* (Lib.), is a major yield-limiting disease in soybeans across the Northern United States. Genetic resistance remains the most effective control strategy; however, no fully resistant genotypes have been developed due to the partial and complex nature of resistance mechanisms and challenges in phenotyping. Genomic selection, leveraging genome-wide markers, offers an efficient approach for selecting quantitative traits like disease resistance. In this study, 962 advanced breeding lines from the Michigan State University soybean breeding program were evaluated in a naturally infected disease nursery and genotyped using 6K genome-wide markers. Spatial trends in the field were corrected using two-dimensional P-spline smoothing. The predictive abilities of six genomic prediction models—Bayesian methods (Bayes A, Bayes B, Bayes C), GBLUP (genomic best linear unbiased prediction), and rrBLUP (ridge regression best linear unbiased prediction) were assessed. The impact of training, testing, and cross-validation populations was also examined. All models demonstrated comparable prediction accuracy, with GBLUP achieving the highest accuracy (0.72) at an 80:20 training-to-testing population ratio with 10-fold cross-validation. These findings highlight the potential of genomic selection for improving white mold resistance genotype selection in soybeans.

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Developing Soybean Varieties with Resistance to Sclerotinia Stem Rot

Raju Thada Magar, Feng Lin, Muhammad Salman, Jason Anandappa, Drew Mitchell, Prabjhot Kaur, Suneth Sooriyapathirana, Cuihua Gu, Paige Pickett, Randy Laurenz, Martin Chilvers, and Dechun Wang, Michigan State University

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

Abstract:

White mold, caused by *Sclerotinia sclerotiorum*, is a significant disease impacting soybean production in the Northern United States. The Michigan State University soybean breeding program has prioritized the development of soybean cultivars with resistance to white mold. Over the years, more than 1,500 advanced lines have been evaluated in naturally infested disease nursery and resistance resources. In 2024, 193 advanced breeding lines were evaluated as part of ongoing efforts. Crosses between resistant germplasm and high-yielding varieties are being consistently made, with 9 new cross combinations were made in 2024, while progenies from earlier crosses have been advancing through various generations. Advance yield trials (AYTs) are being conducted at seven locations across Michigan's major soybean production to evaluate agronomic performance. Lines demonstrating both white mold resistance and acceptable yield potential will be selected for release as varieties or germplasm. Furthermore, advanced lines have been continuously genotyped using 6K single nucleotide polymorphism (SNPs) genome-wide markers, and the resulting data are being utilized to develop genomic selection models and conduct genome- wide association studies (GWAS) to accelerate breeding efforts.

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Systematic Evaluation of NSI Impacts on White Mold Management: A Comprehensive Analysis

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Funded Plan of Work: Genetic variability associated with the traits of fungicide resistance and pathogenicity in *Sclerotinia sclerotiorum.*

ABSTRACT:
The National Sclerotinia Initiative (NSI) is one of just a few pathogen-specific funding programs in the United States with the aim to use research to solve the devastating effects of white mold disease on seven important crops: chickpeas, dry bean, dry peas, lentils, soybean, and sunflower. Through the NSI-funded research, there have been remarkable advancements, such as releasing lines with tolerance or resistance and improving management recommendations. Since its inauguration in 2002, no study has evaluated the impact of this program. To address this, we proposed a study to quantify user awareness and adoption of available disease management resources and identify gaps in knowledge or resources that should be the target of future research, outreach, and education. To achieve this, we designed a nationwide survey to evaluate the impact of recent advances in managing disease caused by *S. sclerotiorum* on NSI-focused commodity groups. Our mixed-methods questionnaire included questions related to NSI- improved management practices, such as planting resistant cultivars, biological control methods, and integrated disease management practices. The survey was deployed from Dec. 3 - 18, 2024, via Qualtrics to 1340 people in the U.S., and 171 (12.8% response rate) completed the survey. The majority of the respondents are researchers and extension professionals, with 50% having over 10 years of familiarity with the disease. From our results, most respondents agreed that white mold has been of great concern in the last five years, and they ranked resistant varieties and disease forecasting as the most important advancements in managing white mold. Cooperative Extension and outreach education, publications, and conferences were also identified as the key sources of information for white mold management. Additionally, respondents reported that the greatest barriers to the adoption of technological advancement in white mold management are consumer concerns about GMOs and the labor needed to implement such technologies. Collectively, these results can be used to identify priorities for funding and alternative management practices.

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Population genetic characterization of *Sclerotinia sclerotiorum* from soybean and dry bean using AmpSeq

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Funded Plan of Work: Genetic variability associated with the traits of fungicide resistance and pathogenicity in *Sclerotinia sclerotiorum.*

ABSTRACT:
Sclerotinia sclerotiorum is one of the most destructive fungal pathogens, infecting many plant species worldwide. Management of this pathogen relies on the coordinated use of fungicides and resistant host cultivars with other control measures. Still, the effectiveness of these methods requires knowledge of the genetic variability and structure of the pathogen. We developed an array of primers to generate Amplicon sequence data. 167 primer sets were designed 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). The AmpSeq was applied to 178 *S. sclerotiorum* genomic DNA hierarchically sampled from diverse sources. Variant calling analysis was performed using the GATK pipeline to give a total of 2,313 variants, which were filtered using *the vcfR* package. From our preliminary results, 26 of 178 *S. sclerotiorum* (14%) had good-quality sequence data in the *SdhD* gene and 21 SNP sites across more than 160 isolates in the *β-tubulin* gene. At the same time, work is ongoing to detect the mutations. None of the *S. sclerotiorum* samples with good quality *SdhC* sequence data contained any variants and, as a result, no mutations. Ongoing work is focused on identifying more polymorphic and reliable variants loci that can be used for downstream population genetic analysis. These results will be useful, especially for understanding the population structure and genetic variability of this economically important pathogen and for further understanding of the genetic basis of pathogenicity.

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

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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. Recombinant inbred line (RIL) populations were developed by crossing stalk rot resistant and OA tolerant inbred lines lines RHA 801 and HA 61 to stalk rot susceptible and OA sensitive line 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. Phenotyping of the HA 61 x HA 89 population for stalk rot resistance was completed in 2024 and a single replication to evaluate OA tolerance for this population was recently completed. Evaluation of stalk rot resistance for the HA 89 x RHA 801 population is currently underway and genotyping of the two populations using genotyping-by- sequencing is ongoing. Transgressive segregation for stalk rot disease response was observed for the HA 61 x HA 89 population, as anticipated for quantitative resistance to basal stalk rot, and several RILs were identified with very high levels of BSR resistance that may be candidates for future release of germplasm. Completion of all phenotyping and genotyping efforts for the HA 61 population is expected in 2025, with completion of the RHA 801 population phenotyping anticipated by spring 2026.

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A putative virulence effector of *Sclerotinia sclerotiorum* identified through expressing the hypovirulent DNA virus SsHADV-1

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

Abstract:

Sclerotinia sclerotiorum, which causes white mold, is a significant disease affecting crops like soybeans and canola, leading to substantial economic losses. Currently, no crop germplasm has been identified with complete resistance to *S. sclerotiorum*. The primary method for controlling white mold is the application of chemical fungicides. A previous study showed that the mycovirus SsHADV-1 reduces virulence in *S. sclerotiorum* and performed effectively in field trials as commercial fungicides. However, the pathogenic mechanism of SsHADV-1 in *S. sclerotiorum* remains unclear. To investigate further, we inserted the DNA virus SsHADV-1 into the Sclerotinia genome and obtained *S. sclerotiorum* strains that stably express SsHADV-1. Using these novel Sclerotinia strains, we performed RNA-seq analysis. The results showed that compared to the wild- type strain during infecting the host plant, the SsHADV-1 strain significantly altered the expression of genes related to plant cell wall-degrading enzymes, metabolism, and effector-like small secretory proteins. In-depth analysis of 67 small secretory proteins revealed one protein of interest, which we designated as SsDV-28. The SsDV-28 knockout mutants showed significantly reduced disease symptoms on the host plant. Although it remains unclear whether SsDV-28 is secreted by *S. sclerotiorum* and enters the plant, plants expressing SsDV-28 showed increased susceptibility to *S. sclerotiorum*. Using yeast two-hybrid screening, we identified eight candidate plant genes that interact with SsDV-28. In the next step, we will use yeast two-hybrid, BiFC (bimolecular fluorescence complementation), and co-IP (co-immunoprecipitation) techniques to further analyze protein-protein interactions and explore the role of plant proteins interacting with SsDV-28 during *S. sclerotiorum* infection.

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Exploring plant PGIP-based defense mechanisms to improve chickpea resistance to *Sclerotinia* white mold

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Funded plan of work: *Exploring defense proteins to improve plant resistance to Sclerotinia white mold*

Abstract:
Sclerotinia sclerotiorum attacks plants by secreting cell wall degrading enzymes (CWDEs), which disrupts the plant cell wall for nutrient access. Pectin-degrading polygalacturonases (PGs), a major enzyme group of CWDEs, are secreted at the early stages of the infection process. Plants developed polygalacturonase inhibitory proteins (PGIPs) that can interact with and inhibit PGs to trigger plant immunity. However, *S. sclerotiorum* has evolved PGIP-inactivating effector 1 (SsPINE1) protein that binds to plant PGIPs and disrupts their interactions with PGs, thereby impairing plant's defense. This mechanism has been demonstrated in the model plant *Arabidopsis thaliana*. Chickpea (*Cicer arietinum*), an important economic legume, currently lacks varieties resistant to *S. sclerotiorum*, posing a significant threat to its yield. In this study, we cloned three chickpea *PGIP* genes (*CaPGIP1, CaPGIP3*, and *CaPGIP4*) from the cultivar 'Dwelley' and five *PG* genes (*SsPG1, SsPG2, SsPG3, SsPG5*, and *SsPG6*) from *S. sclerotiorum* WMA1 strain, to explore the interactions between CaPGIPs and SsPGs in order to better understand the PGIP-based defense mechanism. At the same time, we cloned the *SsPINE1* from the *S. sclerotiorum* to investigate whether SsPINE1 binds to the chickpea CaPGIPs and weaken their inhibitory ability against PGs. Interestingly, during the cloning of *SsPG6*, we found a variation in its sequence compared to the NCBI database, with an additional six amino acids (Trp-Ala-Cys-Ile-Met-Asn) at the C-terminal. Additionally, we are working to clone the promoter sequences (~1000 bp upstream of the start codon ATG) of *CaPGIPs*, but facing challenges due to unstable repetitive sequences in the promoter regions. To address this, we are amplifying shorter promoter sequences and using Stbl2 competent cells for cloning the unstable inserts. This research aims to elucidate the PGIP-based defense mechanism in chickpea at both the protein and gene levels, laying a solid foundation for breeding resistant chickpea varieties.

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Crop diversification manipulates soil microbiota for the control of *Sclerotinia sclerotiorum*

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Funded Plan of Work: Crop diversification manipulates soil microbiota for enhancing soybean resistance to *Sclerotinia sclerotiorum*

ABSTRACT:

Work was conducted in 2024 to evaluate the legacy effect of crop diversification on soybean response to *Sclerotinia sclerotiorum* infection. The bulk soils were collected from the soybean phase in a replicated field experiment evaluating different crop diversifications established in the year 2000 at the Eastern South Dakota Soil and Water Research Farm in Brookings, South Dakota. *S. sclerotiorum* susceptible soybean cultivar 'Williams 82' was grown in the collected field soils and challenged with *S. sclerotiorum* SS140 in the growth chamber. We found that soybean leaf damage was significantly lower in soybean grown in soils preceded by corn than those grown by winter wheat, with a minor effect of cover crop integration. The rhizosphere microbiota of soybean grown in the collected field soils and challenged with *S. sclerotiorum* will be characterized in 2025. Moreover, we conducted a baiting study using sclerotia and sterile soil as traps to explore microbes associated with sclerotia in the soils collected from five different crop rotation combinations in the same long-term crop rotation field trial. We found that the dominated bacterial phyla captured by sclerotia were Firmicutes, followed by Proteobacteria and Bacteroidetes. The dominated fungal phyla were Ascomycota, followed by Mucoromycota and Mortierellomycota. The microbiota captured by sclerotia were significantly different from those captured by sterile soil, and sclerotia captured a subset of plant-beneficial bacteria and plant growth-promoting bacteria. Further, we isolated a total of 427 bacteria from the buried sclerotia. About 54% of 48 tested bacteria inhibited the growth of *S. sclerotiorum* at various levels *in vitro*. The antagonistic activities of the isolated bacterial strains will be examined *in vitro* and in plants in 2025.

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

Table of Contents

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

Collaborators & Organizations

Advisory Committee

Roy Scott
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Greg Varner
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USDA Agricultural Research Service locations

Ft. Collins, Colorado
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