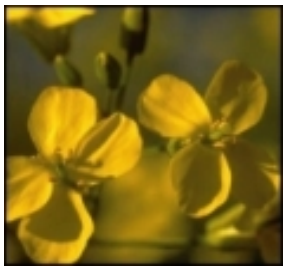
National Sclerotinia Initiative

USDA-ARS

National Sclerotinia Initiative

2022 Annual Meeting

January 19-20, 2022






USA Dry Pea & Lentil Council

National Sunflower Association

US Dry Bean Council

Northern Canola Growers Association

United Soybean Board

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Helping farmers produce a safe, nutritious and sustainable food supply

2022 National Sclerotinia Initiative Meeting

January 19-20, 2022

Virtual

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2022 National Sclerotinia Initiative Annual (Virtual) Meeting (ALL TIMES CENTRAL TIME)

January 19, 2022

ZoomGov Meeting Link for Day 1
https://www.zoomgov.com/j/1617518393

10:00 am Welcome & Introductions **– Mike Grusak, USDA-ARS, Fargo, ND**

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

Invited Presentation

10:20 am Innovations in Sclerotinia disease management – **Michael Wunsch, Jesse Hafner, Kelly Cooper, Venkatarama Chapara, Suanne Kallis, Thomas J. Miorini, Amanda Arens, Heidi Eslinger & Seth Nelson, North Dakota State University, Fargo, ND**

10:50 am Break / Small Group Discussions (10 minutes)

Sclerotinia Research Progress – Biocontrol/Management

11:00 am Unravelling the cross-kingdom function of dicer-independent tRNA-derived small RNAs **– Sushant Khandehar, University of Toledo, Toledo, OH; Shin-Yi Marzano, USDA-ARS, Wooster, OH**

11:20 am Hypovirulent mycovirus-infected strains of *Sclerotinia sclerotiorum* grow endophytically and induce host plant resistance to white mold **– Min Fu & Daohong Jiang, Washington State University, Pullman, WA; Phil Miklas & Lyndon Porter, USDA-ARS, Prosser, WA; George Vandemark & Weidong Chen, USDA-ARS, Pullman, WA**

11:40 am Hypovirulence-determinant of a genomovirus infecting plant pathogen white mold fungus *Sclerotinia sclerotiorum* identified **– Connor Pedersen & Shin-Yi Marzano, USDA-ARS, Wooster, OH**

12:00 pm Development of RNA fungicides for management of *Sclerotinia sclerotiorum* on canola **– L. del Rio Mendoza & A. Bezbaruah, North Dakota State University, Fargo, ND**

12:20 pm Small cationic cysteine-rich peptide with potent antifungal activity controls white mold in soybean **– Arnaud Thierry Djami-Tchatchou, Meenakshi Tetorya & Dilip M. Shah, Donald Danforth Plant Science Center, St. Louis, MO**

12:40 pm Lunch Break (30 minutes)

Sclerotinia Research Progress – Pathogen Biology

1:10 pm Identification and Characterization of a *Sclerotinia sclerotiorum* Effector (SsE3) that targets *Arabidopsis thaliana* fatty acid hydroxylase 2 (AtFAH2) **– Wei Wei, Liangsheng Xu & Kiwamu Tanaka, Washington State University, Pullman, WA; George Vandemark & Weidong Chen, USDA-ARS, Pullman, WA**

1:30 pm Evaluating *Sclerotinia sclerotiorum* detoxification genes as drivers of pathogenicity and as potential targets for disease control **– Nathaniel Westrick, Dandan Shao, Damon Smith & Mehdi Kabbage, University of Wisconsin, Madison, WI**

1:50 pm Breakout Discussions (20 minutes)

Sclerotinia Research Progress – Screening

2:10 pm Multi-site evaluation of white mold resistance derived from wide crosses in common bean and AmpSeq genotyping for population variation and fungicide resistance in *Slcerotinia sclerotiorum* – **R. Higgins & E. Nieot-Lopez, University of Nebraska; E. Wright & F. Gomez, Michigan State University; S.E. Everhart, University of Nebraska, University of Connecticut**

2:30 pm Searching for resistance sources to Sclerotinia white mold in wild relatives of cultivated pea (*Pisum sativum*) **– Yungchun Chen, Clare Coyne, Rebecca McGee, George Vandemark & Weidong Chen, USDA-ARS, Pullman, WA**

2:50 pm End of Day 1

January 20, 2022

9:00 am NSI Steering Committee Meeting

ZoomGov Meeting Link for Day 2
https://www.zoomgov.com/j/1608905731

10:00 am Welcome Day 2 **– Mike Grusak, USDA-ARS, Fargo, ND**

Sclerotinia Research Progress – Host Resistance

10:10 am Deciphering soybean phenylpropanoid pathway for resistance against *Sclerotinia sclerotiorum* **– Ashish Ranjan, Nick Talmo & Robert Stupar, University of Minnesota – Twin Cities, St. Paul, MN**

10:30 am Physiological and molecular basis of oxalic acid tolerance in sunflower **– William Underwood, USDA-ARS, Fargo, ND**

10:50 am Plant genetics, and soil bacteria and fungi, are associated with resistance to *Sclerotinia* – **Nolan Kane & Lara Vimarcati, University of Colorado, Boulder, CO; Brent Hulke, USDA-ARS, Fargo, ND**

11:10 am Breakout Discussion (20 minutes)

Sclerotinia Research Progress – Breeding

11:30 am Genomic prediction for dry bean resistance against *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of white mold – **Molly Irvin & Evan Wright, Michigan State University, East Lansing, MI; Qijian Song, USDA-ARS, Beltsville, MD; Francisco E. Gomez, Michigan State University, East Lansing, MI**

11:50 am A QTL approach toward understanding and improving genetic resistance to white mold 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**

12:10 pm Lunch Break (30 minutes)

12:40 pm Improving resistance of spring canola to Sclerotinia stem rot – **L. del Rio Mendoza, M. Rahman & S. Ruud, North Dakota State University, Fargo, ND**

1:00 pm Introgression and pyramiding of Sclerotinia stem rot disease resistant gene(s) into canola cultivars – **Md Zahangir Alam, Jayanta Roy, Luis del Rio Mendoza & Mukhlesur Rahman, North Dakota State University, Fargo, ND**

1:20 pm QTL mapping of Sclerotinia head rot resistance introgressed from the wild perennial *Helianthus maximiliani* species into cultivated sunflower – **Zahirul I. Taulkder, North Dakota State University, Fargo, ND; William Underwood, Christopher G. Misar & Gerald J. Seiler, USDA-ARS, Fargo, ND; Xiwen Cai, USDA-ARS, Lincoln, NE; Xuehui Li, North Dakota State University, Fargo, ND; Lili Qi, USDA-ARS, Fargo, ND**

1:40 pm Enhancing soybean for resistance to Sclerotinia stem rot – **Dechun Wang, Martin Chilvers & Feng Lin, Michigan State University, East Lansing, MI**

2:00 pm Break / Small Group Discussions (15 minutes)

2:15 pm Meeting wrap-up and Plans of Work questions

2:30 pm End of Day 2

Abstracts

Innovations in Sclerotinia disease management

Michael J. Wunsch[1], Jesse Hafner[1], Kelly Cooper[2], Venkatarama Chapara[3], Suanne Kallis[1], Thomas Miorini[1], Amanda Arens[3], Heidi Eslinger[2], and Seth Nelson[2]

[1]North Dakota State University Carrington Research Extension Center
[2]North Dakota State University Robert Titus Research Farm, Oakes
[3]North Dakota State University Langdon Research Extension Center

Research Project: Research funded by the North Dakota Soybean Council and the USDA Specialty Crop Block Grant Program.

ABSTRACT:

Multi-year, multi-location field studies conducted in eastern North Dakota indicate that white mold management in soybeans can be sharply improved by calibrating spray droplet size relative to canopy closure and suggest that it may be possible to achieve significant reductions in Sclerotinia head rot in sunflowers by using bees to vector the fungal biological control agent *Clonostachys rosea*. The impact of fungicide droplet size was tested in Carrington and Oakes, ND from 2017 to 2020 with a single application of the fungicide boscalid (270 or 392 g a.i./Ha) made at the R2 growth stage. Testing was conducted with TeeJet extended-range flat fan nozzles (Spraying Systems Co.; Glendale Heights, IL) and Combo-Jet flat-fan nozzles (Wilger Corp.; Lexington, TN) and a tractor-mounted sprayer equipped with a pulse-width modulation system (Capstan AG; Topeka, KS). Spray volume was 140 l/Ha, and pulse width was modified to maintain a constant driving seed and constant spray volume across nozzles differing in output. Testing was conducted on multiple soybean varieties differing in canopy architecture. Soybeans were seeded to rows 35.6 or 53.4 cm apart, overhead irrigation was utilized to facilitate disease pressure, and white mold was assessed by evaluating a third to two-thirds of the plants in each plot individually for white mold severity. Optimizing fungicide droplet size relative to canopy closure maximized white mold control and conferred an average 72% increase in the yield gain conferred by fungicides. The impact of honeybee-vectored *C. rosea* on management of Sclerotinia head rot in non-oil sunflowers was tested as a function of distance from the bee hive at three locations in 2019 and 2020. Automated dispensers were used to deposit a commercial formulation of *C. rosea* (BVT Inc.; Mississauga, Ontario) at the exit of hives, and perforated pollination bags that permit flow of air and humidity were placed over sunflowers to exclude bees from the non-treated control. Across six studies, exposure to bees carrying *C. rosea* conferred an average 51% reduction in Sclerotinia head rot, 14% increase in sunflower yield, and 53% reduction in sclerotia contamination of the grain. In five of six studies, no reduction in disease control was observed with increased distance from the bee hives in the 202 to 237 m lengths evaluated. Follow-up testing is in progress to confirm the results in sunflowers and to quantify any impact the pollination bags may have on head rot and sunflower yield and quality.

Contact information – Dr. Michael Wunsch, North Dakota State University Carrington Research Extension Center, P.O. Box 219, 663 Hwy. 281 N., Carrington, ND 58421; (701) 652-2951, michael.wunsch@ndsu.edu.

Unravelling the cross-kingdom function of dicer-independent tRNA-derived small RNAs

Sushant Khandehar[1], Shin-Yi Marzano[1,2]

[1]Department of Biological Sciences, University of Toledo, Toledo, OH
[2]USDA-ARS, ATRU, Wooster, OH (Satellite group at Toledo, OH)

Research Project: Developing environmentally friendly fungicides for managing white mold

ABSTRACT:

In *Sclerotinia sclerotiorum*, abundant tRNA-derived small RNAs (tRFs) are produced. They potentially belong to a new class of virulence effector because we previously reported two very tRFs at larger size of 28-30 nt that had no fungal targets detected based on high throughput sequencing of rapid amplification of cDNA-ends, *i.e.* degradome. Therefore, we hypothesize that these tRFs do not play a role in endogenous gene regulation in *S. sclerotiorum*, but instead are involved in pathogenesis by silencing specific plant gene expressions in a cross-kingdom manner. The objective is to determine the roles of these major tRFs in white mold virulence towards plants. The invertase gene in *Nicotiana benthamiana* is one of the predicted target genes of tRF2. Our results showed that transient overexpression of the invertase gene in *N. benthamiana* improves its tolerance to *S. sclerotiorum*. Additionally, the tRFs' function in a trans-kingdom manner is investigated by immunoprecipitation of argonaute protein for small RNA-Seq. Short tandem target mimics are also being expressed *in planta* or produced *in vitro* to block tRF functions. The study will identify a novel class of effectors used by white mold to attack plants. Furthermore, building on our published findings, we will update on our dsRNA pesticide targeting the fungal ago-2 transcript with some preliminary testing of including Mg-Fe layered double hydroxide nanosheets as an external spray.

Contact information - Shin-Yi Marzano, PhD, Research Molecular Biologist, Application Technology Research Unit, USDA-ARS, 3101 W Towerview Blvd, 4271A Wolfe Hall, Toledo, OH 43606; Tel: 419-530-5053; shinyi.marzano@usda.gov

Hypovirulent mycovirus-infected strains of *Sclerotinia sclerotiorum* grow endophytically and induce host plant resistance to white mold

Min Fu, Daohong Jiang, Phil Miklas, Lyndon Porter, George Vandemark and Weidong Chen

Washington State University, Pullman, WA 99164,
Huazhong Agricultural University, Wuhan, China,
USDA ARS, Grain Legume Genetics and Physiology Research Unit, Pullman, WA 99164

Research Project: *"Biological control of white mold using the mycovirus SsHADV-1 infected hypovirulent strain DT-8 of Sclerotinia sclerotiorum"*

ABSTRACT:

Strains of *Sclerotinia sclerotiorum* harboring the mycovirus Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) are hypovirulent and unable to cause significant disease on normally susceptible plants. The mycovirus SsHADV-1 is readily transmitted horizontally among strains of *S. sclerotiorum*. The horizontal transmission was confirmed using nuclear DNA markers. All the strains harboring the mycovirus SsHADV-1 were hypovirulent. Furthermore, the hypovirulent strains can be used as a biocontrol agent in reducing white mold disease. The hypovirulent strains were used to treat bean seeds. The plants from the treated seeds showed enhanced resistance to white mold caused by *S. sclerotiorum* and, also to gray mold caused by *Botrytis cinerea*. To explore the underlying mechanisms further, it was found that the hypovirulent strain grew endophytically in bean plants growing in the growth chamber and both the fungus *S. sclerotiorum* and the mycovirus SsHADV-1 were detected using specific PCR in different parts (e. g. young leaves, old leaves, apical meristems, and stems) of four-week-old bean plants. This phenomenon was also tested in aseptic conditions. Hypovirulent strain-treated bean seeds were germinated in a sterile agar medium and grown for two weeks in the laboratory under aseptic conditions. The SsHADV-1 virus was detected in the leaves, stems and roots of two-week old plants from the treated seeds but not in the plants from control seeds. Expression of five disease resistance genes (pathogenesis-related gene 1/PR1, pathogenesis-related gene 2/PR2, phenylalanine ammonia lyase/PAL, peroxidase 1/POD1, and polygalacturonase inhibiting protein-1/PGIP1) in bean plants were monitored. In unchallenged plants (not inoculated with virulent *S. sclerotiorum)*, the expression levels of *PR1*, *PR2* and *POD1* were more than twofold higher in treated bean plants than that of untreated plants, implying that endophytic growth of hypovirulent strain in the plant may have activated the salicylic acid signalling pathway and reactive oxygen species production. When challenged with virulent *S. sclerotiorum*, the expression level of *PR1, PR2, PAL, POD1* and *PGIP1* on treated plant were significantly higher than those of the untreated plant at 6 hpi, indicating that the treated plants may respond to *S. sclerotiorum* infection earlier than the untreated plants.

Contact information: Dr. Weidong Chen, USDA ARS, Grain Legume Genetics and Physiology Research Unit, Washington State University, Pullman, WA 99164. 509-335-9178; Weidong.chen@usda.gov

Hypovirulence-determinant of a genomovirus infecting plant pathogen white mold fungus, *Sclerotinia sclerotiorum* identified

Connor Pedersen, Shin-Yi Marzano

USDA-ARS, ATRU, Wooster, OH (Satellite group at Toledo, OH)

Research Project: Developing gemycircularvirus-based pesticide for the control of *Sclerotinium sclerotiorum*.

ABSTRACT:

Uncharacterized viral genomes that encode circular replication-associated proteins of single-stranded DNA viruses have been discovered by metagenomics/metatranscriptomics approaches. Some of these novel viruses are classified in the newly formed family Genomoviridae. Here, we determined the host range of a novel genomovirus, SlaGemV-1, through the transfection of Sclerotinia sclerotiorum with infectious clones. Inoculating with the rescued virions, we further transfected Botrytis cinerea and Monilinia fructicola, two economically important members of the family Sclerotiniaceae, and Fusarium oxysporum. SlaGemV-1 causes hypovirulence in S. sclerotiorum, B. cinerea, and M. fructicola. SlaGemV-1 also replicates in Spodoptera frugiperda insect cells but not in Caenorhabditis elegans or plants. Moreover, by expressing viral genes separately through site-specific integration, the replication initiator protein (REP) alone was sufficient to cause debilitation. REP is currently hypothesized to bind to fungal retinoblastoma (Rb) protein, Whi5, to alter S. sclerotiorum transcriptional control, which induces the synthesis of the plant DNA replication machinery. In other eukaryotes, Rb is a regulator of the E2F family of transcription factors. Our study is the first to demonstrate the reconstruction of a metagenomically discovered genomovirus without known hosts with the potential of inducing hypovirulence, and the infectious clone allows for studying molecular mechanisms of genomovirus-host interactions that are conserved across genera to design new disease control strategies.

Contact information – Shin-Yi Marzano, PhD, Research Molecular Biologist, Application Technology Research Unit, USDA-ARS, 3101 W Towerview Blvd, 4271A Wolfe Hall, Toledo, OH 43606; Tel: 419-530-5053; shinyi.marzano@usda.gov

Development of RNA fungicides for management of *Sclerotinia sclerotiorum* on canola

L. del Río Mendoza[1], A. Bezbaruah[2]

[1]Dept. Plant Pathology, North Dakota State University, Fargo, ND.
[2]Department of Civil and Industrial Engineering, North Dakota State University, Fargo, ND

Research Project: Development of RNA fungicides for management of *Sclerotinia sclerotiorum* on canola

ABSTRACT:

Sclerotinia stem rot continues to be a threat to the canola industry in North Dakota. For every percentage incidence of disease, yields can be reduced on average by 0.5%, although in certain areas more severe losses have been observed. The lack of adequate levels of genetic resistance among commercial cultivars and the limited benefit of crop rotations have forced farmers to heavily rely on fungicide use to protect their crops. Most fungicides currently registered for use against Sclerotinia have single mode of action and a history of development of fungicide resistance. Finding alternative, environmentally friendly options is critical to keep canola production profitable. Recent developments in RNAi-mediated gene silencing have shown that exogenous spray applications of dsRNA also known as SIGS have the potential to control diseases caused by fungal pathogens. We have identified several *S. sclerotiorum* genes that were essential for virulence of the fungus on canola from a previously supported Sclerotinia Initiative project. For this project we selected genes SS1G_05491 (Cytochrome P450 monooxygenase) and SS1G_04975 (isocitrate lyase) as candidates for fungicide formulation. Primers have been designed and will be formulated with multiple nanomaterials as carriers.

Contact information – Dr. Luis del Rio, Dept. of Plant Pathology, North Dakota State University, Dept. 7660 P.O. Box 6050, Fargo ND 58108-6050; (701) 231-7073; luis.delriomendoza@ndsu.edu

Small cationic cysteine-rich peptide with potent antifungal activity controls white mold in soybean

Arnaud Thierry Djami-Tchatchou, Meenakshi Tetorya and Dilip M. Shah

Donald Danforth Plant Science Center, St Louis, MO 63132

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

ABSTRACT:

White mold (WM) caused by a necrotrophic pathogen *Sclerotinia sclerotiorum* results in serious economic losses of soybean yield in the US. For lack of effective genetic resistance to this disease in soybean germplasm and rapid evolution of pathogen resistance to fungicides, WM is difficult to manage. We are exploring the potential of small cysteine-rich *Ssc*-inhibitory peptides for development as multi-target environmentally friendly biofungicides. PD30.6 is a small cationic cysteine-rich antifungal peptide derived from a plant defensin MtDef4 of *Medicago truncatula*. Antifungal activity of PD30.6 was determined against an aggressive strain *S. sclerotiorum* 555 in *in vitro*, semi-*in planta* and *in planta* antifungal assays. PD30.6 exhibited potent fungicidal activity against *S. sclerotiorum* in *vitro* with an EC_{50} value of 14 µM and the minimum inhibitory concentration (MIC) of 24 µM. We found that external application of PD30.6 to detached Williams 82 soybean leaves, stems and pods at concentrations between 24 and 96 µM significantly reduced lesion sizes or completely inhibited pathogen growth. In preliminary experiments, employing spray-application of PD30.6 on leaves of a soybean plant, we observed much reduced lesion sizes at inoculation points with complete inhibition observed at 96 µM. In addition, we also assessed the antifungal activity of a combination of PD30.6 and tebuconazole fungicide at concentrations below their MIC values against *S. sclerotiorum in vitro* and *in planta.* Our results revealed a synergistic increase in antifungal activity against *S. sclerotiorum*. The dual application of PD30.6 and tebuconazole led to complete inhibition of fungal growth *in vitro* and lesion development on soybean leaves, stems and pods. The synergistic peptide/tebuconazole combination reduced the amount of fungicide required for complete prevention of disease symptoms almost tenfold. Our results demonstrate that PD30.6 has significant potential as a peptide-based bio-fungicide for WM management in soybean.

Contact information - Dr. Dilip M. Shah, Donald Danforth Plant Science Center; 975 North Warson Road St Louis, MO 63132; (636)346-9846, dshah@danforthcenter.org

Identification and Characterization of a *Sclerotinia sclerotiorum* Effector (SsE3) that targets *Arabidopsis thaliana* fatty acid hydroxylase 2 (AtFAH2)

Wei Wei, Liangsheng Xu, Kiwamu Tanaka, George Vandemark and Weidong Chen

Washington State University, Pullman, WA; Northwestern A&F University, Yangling, Shaanxi, China; and USDA ARS, Washington State University, Pullman, WA

Research Project: Characterizing pathogenicity effectors of *Sclerotinia sclerotiorum* preferentially expressed under acidic conditions and during plant infection

ABSTRACT:

Sclerotinia sclerotiorum is a necrotrophic pathogen causing the white mold disease on many economically important crops such as common bean, canola, soybean, sunflower and pea, chickpea and lentil. *S. sclerotiorum* secrets numerous polypeptides, some of which play important roles in pathogenesis. Previously we characterized an extracellular effector that targets and nullifies plant cell wall-bound polygalacturonase-inhibiting proteins (PGIPs), compromising plant defense and enhancing pathogen virulence. This study is focused on a cytoplasmic effector (SsE3). *SsE3* is a single copy gene in the *S. sclerotiorum* genome and its encoded protein has a signal peptide, suggesting SsE3 is a secreted protein. Expression of *SsE3* is significantly induced at early stages of infection (in the initial 24 hours after inoculation). Deletion of *SsE3* did not affect the growth and life cycle of *S. sclerotiorum*, but significantly reduced its virulence on bean and pea. Complementation of *SsE3* restored the virulence. Subcellular localization using transient expression of GFP-labelled SsE3 in tobacco leaves showed that SsE3 is localized in the plant cytoplasm. Yeast 2-hybrid assays showed that SsE3 interacts with *Arabidopsis thaliana* fatty acid hydroxylase 2 (AtFAH2). AtFAH2 is required for the Bax inhibitor-1 (AtBI-1)-mediated suppression of programmed cell death. We hypothesize that the interaction with AtFAH2 will disrupt the process of suppressing programmed cell death, thus will actually enhance programmed cell death. Enhanced programmd cell death will increase susceptibility to necrotrophic pathogens including *S. sclerotiorum*. Further research effort is directed to analyzing the effect of SsE3 on plants by expressing *SsE3* in transgenic *Arabidopsis thaliana* plants.

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

Evaluating *Sclerotinia sclerotiorum* detoxification genes as drivers of pathogenicity and as potential targets for disease control

Nathaniel Westrick[1], Dandan Shao[1], Damon Smith[1], and Mehdi Kabbage[1]

[1]Department of Plant Pathology, University of Wisconsin – Madison, Madison, WI

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

ABSTRACT:

Sclerotinia sclerotiorum, the causative agent of Sclerotinia Stem Rot (SSR), is a major yield limiting pathogen of soybean. Control of this disease is complicated by the lack of robust commercially available resistance in soybeans, thus novel disease control approaches are needed. We showed that SSR resistance found in our soybean germplasm material rely on the accumulation of antifungal compounds and SSR development is likely dependent on the pathogen's ability to detoxify or control the accumulation of these compounds. We identified two genes, *Sslac2* and *SsAOX* as likely drivers of this detoxification activity. CRISPR-Cas9 Knockout mutants of *Sslac2* (*ΔSslac2)* were found to be non-pathogenic on soybean and were significantly more susceptible to several antifungal compounds produced by resistant soybean lines. Knockout mutants of SsAOX (*ΔSsAOX*) are markedly less virulent on soybeans, likely due to their inability to properly colonize soybean stem tissue. Chemical genomics suggest that SsAOX may function as an aromatic alcohol oxidase, required for the utilization and detoxification of stem extracts. Profiling of known plant metabolites point towards the monolignol coniferyl alcohol (CA) as a likely substrate for SsAOX and oxidation of CA by SsAOX appears to facilitate both the detoxification and metabolism of this compound. Previously our lab has shown that partial SSR resistance can be achieved by silencing the expression of the fungal enzyme oxaloacetate acetylhydrolase (*Ssoah1*) through host-induced gene silencing (HIGS) and it is believed that a similar approach targeting *Sslac2* and *SsAOX* would help to facilitate the plants own immune system to successfully subvert SSR infection. Additional virulence targets, including the effector *Sscm1* will also be considered. *Sscm1*, which modulates the host's secondary metabolites, including antifungals, in order to facilitate infection, may also serve as a valuable target for HIGS. In the future we will focus on a stacked approach to silence multiple virulence factors which have demonstrated importance in SSR infection in order to develop soybean lines with robust resistance to this devastating disease.

Contact information - Dr. Mehdi Kabbage, Department of Plant Pathology, UW-Madison, 1630 Linden Dr., Madison, WI USA 53706; 608-262-0506, kabbage@wisc.edu

Multi-site evaluation of white mold resistance derived from wide crosses in common bean and AmpSeq genotyping for population variation and fungicide resistance in *Sclerotinia sclerotiorum*

R. Higgins[1], E. Nieto-Lopez[1], E. Wright[2], F. Gomez[2], and S.E. Everhart[1,3].
[1]Department of Plant Pathology, University of Nebraska
[2]Department of Plant, Soil, and Microbial Genomics, Michigan State University
[3]Department of Plant Sciences and Landscape Architecture, University of Connecticut

Collaborators: F. Gomez (MI), M. Wunsch (ND), J. Myers (OR), P. Miklas (WA), C. Urrea (NE), V. Hoyos-Villegas (CAN), M. Munoz-Amatriain (CO)

Research Project: Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas

ABSTRACT: Our research goal is to identify putative sources of resistance of dry bean lines to white mold in adapted backgrounds at multiple sites located in major bean-production areas of the United States. We employ two types of evaluations, greenhouse-based straw test and field trials carried out within white mold nurseries in several locations throughout the northern U.S. and Canada. In 2021, trials were conducted in NE, MI, OR, ND, CO, WA and CAN with a total of 30 lines that included Black, Pink, Navy, Great Northern, Pinto, Red, Tan, Small Red, and Dark Red Kidney bean types. In our preliminary analysis of greenhouse data from three sites, there were seven lines that out-performed the known resistant line (G122), which included: Z0726-9-50-1, ND122454, M25-13-1, USPT-WM-12-1, P3-047-1, USGN-WM-3-1, and P3-032-2. Analysis of the results of field data and a retrospective G x E analysis are currently underway, which will provide greater insight into potential cultivars with both genetic and physiological resistance to white mold. Complementary investigation of the pathogen population used a hierarchical sampling of 178 *S. sclerotiorum* isolates from dry bean and soybean along with development of an AmpSeq technique that enables next-generation sequencing of multiple target loci. An AmpSeq primer array was developed to amplify and sequence variants in SSRs, genes conferring fungicide resistance (β-tubulin, Sdh complex, and cytochrome b gene), SNPs, and putative virulence loci. Raw demultiplexed reads were received and subjected to an AmpSeq custom pipeline that used the *S. sclerotiorum* reference genome to identify variants and produce a variant call format (VCF) file for downstream analysis. Preliminary analysis of fungicide resistance loci showed 16 samples out of 192, along with the positive control, had a mutation for fungicide resistance in the SdhB gene which represented 24–33% of all samples with sequence data at those loci. No sequence data for other resistance loci was found, possibly due to low sequence depth, quality control filters, or poor primer amplification. With an ability to evaluate large numbers of samples and loci, this technique represents a promising new approach for broad scale characterization of *S. sclerotiorum*.

Contact Information: Dr. Sydney Everhart, Department of Plant Science and Landscape Architecture, University of Connecticut, Storrs, CT, 06269; everhart@uconn.edu

Searching for resistance sources to Sclerotinia white mold in wild relatives of cultivated pea (*Pisum sativum*)

Yungchun Chen, Clare Coyne, Rebecca McGee, George Vandemark and Weidong Chen

USDA ARS, Grain Legume Genetics and Physiology Research Unit,
Pullman, WA 99164

Research Project: *"Screening for resistance sources to Sclerotinia white mold in recently acquired germplasm of cool season grain legumes"*

ABSTRACT:

Sclerotinia sclerotiorum causes the economically important disease white mold in grain legumes (pea, chickpea and lentil). Management of white mold is challenging because resistance to white mold is lacking in the cultivated pea and fungicide application is not economical for grain legume crops. There is a lack of resistance sources since previous screening of the available germplasm collections did not identify any sources with sufficient levels of resistance. Additional resistance sources are needed for resistance breeding programs. This research project screened the recently acquired germplasm of pea. Under this project, among the 218 recently acquired and propagated pea germplasm lines, more than 150 accessions have been tested for resistance to white mold. We used a moderately resistant line DSP as a standard for comparison. All the tested accessions showed disease although the accessions exhibited variations in reaction to white mold inoculation. The accession W6 44561 is the most resistant line, and disease lesions on W6 44561 plants were smaller than the moderately resistant line DSP. This year we decided to focus on screening wild relatives of pea (*Pisum abyssinicum*, *P. fulvum*, and subspecies of *P. sativum*). Seed source was a limitation because the number of seeds of wild accessions are insufficient for the destructive sampling and need to be propagated. We started the screening with the accessions that have sufficient seeds. Initial test with 14 accessions including some accessions of *P. sativum* subsp. *asiaticum* and some advanced pea breeding lines showed that all the wild accessions in the batch of screening were very susceptible with disease lesions greater than the DSP. However, one advanced breeding line Ps17100078 was the best among the screened and was compatible with DSP. This shall be good news for the breeding program. We will continue to screen the remaining accessions of the wild relatives of pea in the coming months.

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Deciphering soybean phenylpropanoid pathway for resistance against *Sclerotinia sclerotiorum*

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

ABSTRACT:

Soybean is one of the most economically important crop plants impacted by *Sclerotinia sclerotiorum*, a broad host range fungal pathogen. One of the most sustainable and economical ways to control crop diseases is to generate resistant varieties of plants. Understanding molecular plant resistance mechanisms to *S. sclerotiorum* could deliver novel strategies to deploy resistant soybean varieties. Our previous research, using a combination of metabolomics and transcriptomics approaches, has identified key phenylpropanoid pathway genes and metabolites to be implicated in soybean resistance responses to *S. sclerotiorum*. The identified phenylpropanoid pathway genes include phenylalanine ammonia-lyase (PAL), ferulic acid 5-hydroxylase (F5H), caffeoyl-CoA O-methyltransferase (CcoAOMT), and cinnamyl alcohol dehydrogenase (CAD). Our non-targeted metabolomics approach also suggested significant up-regulation of some important antimicrobial metabolites resulting from phenylpropanoid pathway such as cinnamic acid, benzoic acid, and ferulic acid in the resistance response. Our proposed study aims to characterize these crucial phenylpropanoid pathways genes in soybean resistance/susceptibility to *S. sclerotiorum*. In our first year of study, we provide direct evidence of some of the metabolite's antifungal activity against *S. sclerotiorum*. Using protein sequence similarity searches, we have identified 8 soybean PALs (GmPALs), 11 F5H (GmPALs), 8 CcoAOMT (Gm CcoAOMTs), and 14 CAD (GmCADs). We have quantified the transcript expression of these genes in soybean following *S. sclerotiorum* challenge. For high throughput screening of the function of these genes in soybean, we are constructing virus-induced gene silencing (VIGS) of specific genes using *Bean pod mottle virus* (BPMV) derived vectors. We will complement the knockdown studies with overexpression of the candidate genes either transiently in *N. benthamiana* or by making stable *A. thaliana* transgenic plants.

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Physiological and molecular basis of oxalic acid tolerance in sunflower

William Underwood[1]

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Research Project: Characterization of oxalic acid tolerance in sunflower basal stalk rot resistance.

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. Consequently, the overall goal of this project is to examine the physiological and molecular nature of sunflower tolerance to OA. Specific objectives of the project are to: 1) Determine if sunflower tolerance to OA is associated with reduced OA accumulation in aerial tissues; 2) Determine if OA tolerance is conferred by the sunflower rootstock or shoot by performing grafting experiments; 3) Define transcriptional changes associated with OA tolerance by RNAseq comparison of tolerant and sensitive sunflower lines. Initial results of grafting experiments indicate that, somewhat surprisingly, sunflower OA tolerance appears to be conferred by the shoot tissues rather than roots (which are directly exposed to OA). The results of this project will dovetail with ongoing and future efforts to map sunflower genes contributing to OA tolerance and basal stalk rot resistance.

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Plant genetics, and soil bacteria and fungi, are associated with resistance to *Sclerotinia*

Nolan Kane[1], Lara Vimarcati[1], and Brent Hulke[2]

[1]University of Colorado, Boulder,
[2]USDA-ARS Sunflower and Plant Biology Unit

Research Project: Developing knowledge and tools to optimize sunflower breeding for Sclerotinia resistance and improved microbiome-related traits.

ABSTRACT:

Analysis of plant-associated microbes have yielded new insights into the genetic basis of host-plant resistance to the *Sclerotinia* pathogen in sunflower. We have found strong, heritable genetic plant effects on soil microbial communities, accounting for much of the heritable variation in resistance to Sclerotinia stalk rot. We conducted multi-environment, multi-pathogen assays of an open pollinated variety sunflower (*Helianthus annuus*) diversity panel (260 individuals), for which we have genome resequencing data and called variants with high confidence in quality. In addition, we sampled the plant rhizospheres of a portion of the panel (95 individuals at 1 environment, as well as 10 divergent individuals replicated twice at 16 environments across the sunflower growing region). All traits are of moderate to high heritability. Multiple bacterial and fungal taxa are strongly associated with the Sclerotinia basal stalk rot resistance phenotype, providing a unique mechanism for stalk rot resistance, which explains the neutral to slightly negative correlations previously observed between head rot and stalk rot resistance. Genome-wide association analysis resulted in the discovery of multiple quantitative trait loci for plant associations with many microbial taxa, some of which are strongly correlated with Sclerotinia resistance, others of which may be important for other plant traits. This work to improve our understanding the diverse microbial associations of sunflower is starting to shed light on how heritable plant traits may have an important environment-specific microbial component. Additionally, the fact that domesticated sunflower, especially recently developed inbred lines, vary heritably in these important microbial associations makes it an attractive system to better understand the genetics of plant-microbe interactions more generally.

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Genomic prediction for dry bean resistance against *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of white mold

Molly Irvin[1], Evan Wright[1], Qijian Song[2], and Francisco E. Gomez[1]

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[2]Beltsville Agricultural Research Center, USDA-ARS, Beltsville, MD 20705, USA

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

ABSTRACT:

Dry bean (*Phaseolus vulgaris* L.) production in the U.S., and specifically in Michigan the second largest dry bean producer in the U.S., suffer severely from white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary) infection. In 2014, yield losses exceeded 20% in Michigan and overall economic losses can approach ~$5M annually in terms of lost production and fungicide costs. Dry bean cultivars lack high levels of resistance and progress to breed new cultivars with durable levels of resistance to white mold has been slow due to the quantitative inheritance of this trait and screening dependence on the presence of the pathogen under suitable environmental conditions. Marker-assisted selection (MAS) approaches would be useful to breed for white mold resistance, however, given the multigenic nature of white mold hinders the use of MAS efforts that aim of pyramid only a few target genes. An alternative is genomic prediction and selection, which utilizes genome-wide marker coverage to predict genotypic values for quantitative traits. Thus, the overall goal of this project is to evaluate and optimize genomic prediction in a dry bean breeding program. In this first year of the project, we evaluated 176 advanced breeding lines for white mold resistance in a disease nursery at Montcalm. Significant differences between genotypes were identified and disease ratings ranged from 30 to 100% infection rate. Lines were genotypes using the BARCBean12K BeadChip and preliminary genomic analysis identified population structure among the breeding lines. Genomic prediction is being conducted to estimate prediction accuracies using different cross validation methods for white mold ratings collected in the 2021 field season.

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A QTL approach toward understanding and improving genetic resistance to white mold in common bean

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

ABSTRACT:

Genetic and breeding populations were generated, phenotyped and genotyped in support of detecting new and existing QTL conditioning improved resistance to white mold in snap and pinto beans with acceptable agronomic traits. A new snap-dry bean MAGIC population initiated with 685 first generation plants from 8-way crosses awaits multiple selfing generations to obtain homozygous inbred lines. An existing pinto bean MAGIC population is being used to examine genomic predictions: the 1st set of 500 lines is being used to predict performance on the 2nd set of 500 lines using available SNPs and phenotypic data from the straw test. A new RIL population with resistance derived from breeding line SR9-5 is in development (currently F4 generation) to examine white mold reaction in the small red dry bean market class. A second year of agronomic data in the field was successfully obtained in 2021 for a snap-dry bean NAM population (368 plots) and first year for disease reaction in a dry bean RIL population (486 plots). The beneficial effect of WM5.4 QTL was validated in a few pinto bean F2 breeding populations using new SNP markers that were converted to Tm-shift and KASP assays. New SNP markers, developed for WM3.1 and WM7.4 QTL, await validation in breeding populations. The low coverage sequencing Khufu technology available from HudsonAlpha Institute for Biotechnology will be performed on four existing RIL populations (540 lines) to further fine map QTL WM1.2, WM3.1, WM5.4, WM7.4, and WM7.5 segregating in one or more of the four populations using QTL-seq conducted by our partners at HudsonAlpha. Another fine-mapping QTL approach is using 4.4 M SNPs for downstream population genomics analyses that were generated by deep-sequencing (40x) 21 genotypes representing parental lines from the pinto MAGIC and RIL populations used to detect nine meta-QTL. For example, population statistics such as FST and π will be calculated between those genotypes with and without a specific QTL. Peak regions for these statistics will define intervals associated with the QTL. Overall steady progress is being made toward identifying, developing, and generating QTL linked markers for use in breeding snap and dry beans with improved resistance to white mold.

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Improving resistance of spring canola to Sclerotinia stem rot

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[2]Dept. Plant Sciences, North Dakota State University, Fargo, ND

Research Project: Improving resistance of spring canola to Sclerotinia stem rot

ABSTRACT:

Sclerotinia stem rot (SSR) is a major disease affecting canola (*Brassica napus*) production in the North central region of the US. Several *B. napus* plant introduction materials have been identified as promising sources of resistance against this disease in previous years by projects supported by the Sclerotinia Initiative but the resistance carried by very few materials has been characterized. The objectives of this project are to characterize the resistance present in plant introduction accession 649136. characterize the reaction of ten elite *Brassica napus* plant introductions to multiple isolates of *Sclerotinia sclerotiorum*, and to transfer resistance present in PI 436554 and NEP63 to modern canola breeding line. For the first objective, approximately 200 doubled haploid lines from the cross between 649136 and cv. Topas have been produced. These materials will be planted for phenotypic characterization in January 2022. The second objective will be addressed in the winter months of 2022. For the third objective, crosses were made with NDOLA-01 and the resulting F_1 seeds have been planted in the greenhouse. These plants will be backcrossed to NDOLA-01 to produce the BC_2F_1 generation.

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Introgression and Pyramiding of Sclerotinia Stem Rot Disease Resistant Gene(s) into Canola Cultivars.

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Research Project: Introgression and pyramid the resistant gene(s) from the resistant/tolerant accessions into elite breeding line, and develop breeding lines with enhanced SSR disease tolerance to release as cultivar.

ABSTRACT:

The sclerotinia stem rot disease (SSR) disease caused by *Sclerotinia sclerotiorum* is considered by growers and scientists the most important disease in canola. Non-host crop rotation and fungicides application are commonly used to manage the disease. However, breeding for disease resistant cultivars would be an efficient, economically feasible, and environmentally friendly option. We have screened 350 globally distributed rapeseed/canola germplasm accessions against the SSR disease from 2016 to 2020 under greenhouse and field conditions, and identified three accessions (g151, g127, g339) with high levels of resistance/tolerance to the disease. For gene(s) introgression, we made crosses between g151 x NDC-E16198 (an elite breeding line), and g127 x g339 in the greenhouse during summer 2021. We also made 4-way crosses were made between F1s (g151 x NDC-E16198) and F1s (g127 x g339) in fall 2021. A total of 400 four-way F1s were harvested from the greenhouse. The new 4-way F1s [(g151 x NDC-E16198) x (g127 x g339)] will planted in the greenhouse in spring 2022 and will be screened against SSR disease using petiole inoculation technique (PIT) at seedling stage followed by stem inoculation technique (SIT) at flowering stage. The resistant/tolerant F1s will be backcrossed to NDC-E16198 to introgress the resistant gene(s) into the breeding line. Finally, the best resistant lines will be evaluated in the field to identify elite lines to release as cultivar. For cultivar development, five elite breeding lines/cultivars and three resistant accessions were used for Multi-parent Advanced Generation Inter-Cross *(*MAGIC) population development. The 1st crosses were made between P_1xP_2, P_3xP_4, P_5xP_6, P_7xP_8 in spring 2021. The 2nd crosses were also made between each of six F_1s such as [$F_{1(P1xP2)}$ x $F_{1(P3xP4)}$], and [$F_{1(P5xP6)}$ x $F_{1(P7xP8)}$] in summer 2021. The 3rd crosses were made between each of six F_1s $_{(P1xP2)x(P3xP4)]}$ x $F_{1[(P5xP6)x(P7xP8)]}$ in fall 2021. A total of 890 MAGIC F_1 seeds have been harvested and will be planted in spring 2022. Single seed descent method will be applied to generate recombinant inbred lines (RILs). Again, the best resistant lines will be evaluated in the field to identify elite lines to release as cultivar.

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QTL mapping of Sclerotinia head rot resistance introgressed from the wild perennial *Helianthus maximiliani* species into cultivated sunflower

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[3]USDA-ARS, Wheat, Sorghum and Forage Research Unit, Lincoln, NE

Research Project: QTL mapping of Sclerotinia head rot resistance and pyramiding of basal stalk rot QTL in sunflower

ABSTRACT:

Sclerotinia head rot (HR), caused by the fungal pathogen *Sclerotinia sclerotiorum*, is one of the most devastating diseases of sunflower worldwide. The use of host resistance is the most economical and effective method of controlling the disease. The objective of this study was to dissect the quantitative trait loci (QTL) associated with Sclerotinia HR resistance introgressed from a wild perennial sunflower species, *Helianthus maximiliani*, into cultivated sunflower. A population of 188 $F_{3:4}$ progeny lines was developed from a cross of HR 21, a HR resistant line with HA 234, a HR susceptible line. The population was evaluated for HR resistance in inoculated field trials at two locations with three replications during 2019-2020 seasons. A clear separation of the parents HR 21 and HA 234 for the HR disease incidence (DI) and disease severity (DS) was observed in all environments with a mean DI of 32.3% and 94.7%, and mean DS of 1.12 and 4.38, respectively. The distribution of HR DI and DS in the population was continuous, confirming a polygenic inheritance of the trait. Transgressive segregation was observed for both the traits in the population where some progenies showed more extreme phenotypes than either of the parents. Analysis of variance of the DI and DS data revealed highly significant variation (p <0.001) for genotype (G), genotype × year (G×Y), and genotype × year × location (G×Y×L) interactions effect in the population. However, Spearman's rank correlations for HR DI and DS measured across years and locations were highly significant (p <0.001), suggesting a high degree of collinearity of the field trials. The moderately high broad sense heritability (H^2) estimates (~0.70) across environments for both the traits indicates that there is ample opportunity for improving HR resistance through breeding. QTL analyses were performed for HR DI and DS using both individual and combined environments data. Preliminary analyses identified 19 QTL on ten sunflower chromosomes associated with Sclerotinia HR resistance in this population. Fourteen of the QTL had HR resistance alleles contributed by the resistant HR 21 parent, while the remaining five QTL had resistance alleles derived from the susceptible parent HA 234.

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Enhancing soybean for resistance to Sclerotinia stem rot

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Research Project: Enhancing soybean for resistance to Sclerotinia stem rot

ABSTRACT:

Soybean sclerotinia stem rot, caused by *Sclerotinia sclerotiorum* (Lib.) deBary, is a major soybean (*Glycine max* L. Merr.) disease in the North Central region of the United States. It causes yield losses through the reduction of seed number and weight as well as seed quality. In 1994, 2004 and 2009, sclerotinia stem rot ranked the second to soybean cyst nematode on total yield lost in the US soybean production. Host plant resistance plays a key role in effective disease management, and progress has been made in developing soybean varieties with partial resistance. In 2021, we released two soybean varieties with sclerotinia stem rot resistance, and both are in progress of being licensed for commercial production. In addition, 11 new crosses were made with sclerotinia resistant parents. Sixteen F2 populations derived from crosses with sclerotinia resistant parents are current grown in the greenhouse. We also evaluated 199 advanced breeding lines for sclerotinia stem rot resistance in Montcalm disease nursery in Michigan. In the next funding year, we propose to 1) continue the breeding pipeline for enhancing resistance to this disease in soybean, 2) incorporate molecular data-driven decisions towards improvement for sclerotinia stem rot resistance, and 3) continue evaluation of advanced breeding lines towards releasing high yield varieties with excellent sclerotinia stem rot resistance.

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Strategic Plan

United States Department of Agriculture

Research, Education & Economics

Agricultural Research Service

Northern Plains Area

January 2016

Version 1.0

National Strategic Plan for the Sclerotinia Research Initiative

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

2017 to 2021

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Executive Summary

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

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

NSI Program Performance 2013-2017

Sclerotinia Initiative Research Progress Evaluation

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

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

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

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

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

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

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

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

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

Background

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

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

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

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

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

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

National Sclerotinia Research Initiative Strategic Plan (2017 to 2021)

Crop Germplasm Resources & Translational Genomics

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

- Use of allele specific markers and high-throughput phenotyping methods to facilitate pyramiding genes that mediate resistance to Sclerotinia diseases.
- Canola, chickpea, lentil, and pea lines with resistance to Sclerotinia and a broad

portfolio of desirable agronomic traits developed and released.

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

Pathogen Biology & Mechanisms of Resistance

Goal 2: Understand *Sclerotinia sclerotiorum* biology and development

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

Disease Management & Crop Production

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

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

Milestones & Deliverables:

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

Appendix

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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