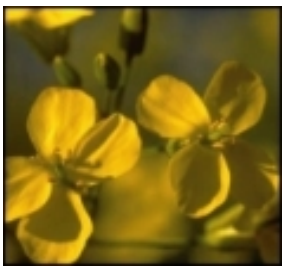
National Sclerotinia Initiative

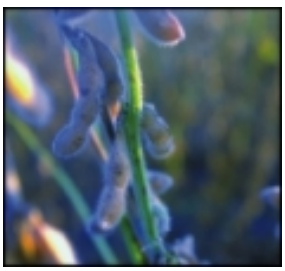
USDA-ARS

National Sclerotinia Initiative 2020 Annual Meeting

Fargo, ND

January 22-23, 2020



2020 National Sclerotinia Initiative Meeting

January 22-23, 2020

Radisson Hotel Fargo
201 North 5th Street, Fargo, ND

AGENDA

2020 National Sclerotinia Initiative Meeting
January 22-23, 2020

Wednesday – January 22, 2020

11 am - 1 pm	Registration & Poster Set-Up (lunch on your own) *(posters are displayed for the entire meeting)*	**City A**
1:00 pm	Welcome & Introductions – **Mike Grusak, USDA-ARS, Fargo, ND**	**City B**
1:10 pm	Welcome & Update from the Plains Area – **Bryan Kaphammer, USDA-ARS, Fort Collins, CO**	
1:20 pm	Welcome & Update from Office of National Programs – **Roy Scott, USDA-ARS, Beltsville, MD**	
	Sclerotinia Research Activities – Session 1 **Moderator – William Underwood, USDA-ARS, Fargo ND**	**City B**
1:30 pm	Characterizing pathogenicity effectors of *Sclerotinia sclerotiorum* preferentially expressed under acidic conditions and during plant infection (Abstract p. 13, Poster #6) – **Wei Wei, USDA-ARS, Pullman, WA**	
1:50 pm	Targeting essential genes in *Sclerotinia sclerotiorum* to achieve Sclerotinia stem rot resistance in soybean (Abstract p. 9) – **Mehdi Kabbage, University of Wisconsin, Madison, WI**	
2:10 pm	Developing environment friendly fungicides for managing white mold (Abstract p. 11; Poster #5) – **Shin-Yi Marzano, South Dakota State University, Brookings, SD**	
2:30 pm	Break	**City A**
3:00 pm	Biological control of white mold using the Mycovirus SsHADV-1 infected hypovirulent strain DT-8 of *Sclerotinia sclerotiorum* (Abstract p. 15; Poster #2) – **Weidong Chen, USDA-ARS, Pullman, WA**	
3:20 pm	Developing gemycircularvirus-based pesticide for the control of *Sclerotinia sclerotiorum* (Abstract p. 10; Poster #1) – **Shin-Yi Marzano, South Dakota State University, Brookings, SD**	
3:40 pm	Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas (Abstract p. 22; Poster #12) – **Sydney Everhart, University of Nebraska, Lincoln, NE**	

4:00-5:30 pm	Poster Session	**City A**
5:30-7:30 pm	Dinner & Posters	**City A**

Thursday – January 23, 2020

7:00-8:00 am	Steering Committee Breakfast Meeting	**Prairie Rose B**
7:00 am	Continental Breakfast	**City A**

***Sclerotinia* Research Activities – Session 2** — **City B**
Moderator – Weidong Chen, USDA-ARS, Pullman, WA

8:30 am	Role of WRKY transcription factors in quantitative resistance to *Sclerotinia sclerotiorum* (Abstract p. 25; Poster #3) – **William Underwood, USDA-ARS, Fargo, ND**
8:50 am	Enhancing basal resistance to *Sclerotinia sclerotiorum* in Brassica (Abstract p. 24; Poster #13) – **Jeffrey Rollins, University of Florida, Gainesville, FL**
9:10 am	QTL mapping of Sclerotinia basal stalk rot resistance derived from sunflower wild species (Abstract p. 20; Poster #10) – **Lili Qi, USDA-ARS, Fargo, ND**
9:30 am	Improving resistance to *Sclerotinia sclerotiorum* in spring canola (Abstract p. 14, 17; Poster #8, 9) – **Jayanta Roy and Fereshteh Shahoveisi, North Dakota State University, Fargo, ND**
9:50 am	Refining genomic tools for *Sclerotinia* resistance and agronomic breeding of sunflower towards dissection of resistance phenotype (Abstract p. 19) – **Nolan Kane, University of Colorado, Boulder, CO**
10:10 am	Understanding how sunflower soil microbiome impacts resistance to Sclerotinia stalk rot (Abstract p. 18) – **Alisha Quandt, University of Colorado, Boulder, CO**
10:30 am	Break

***Sclerotinia* Research Activities / Group Discussion – Session 3** — **City B**
Moderators – Mike Grusak, USDA-ARS, Fargo, ND; Roy Scott, USDA-ARS, Beltsville, MD

11:00 am	Discussion Topic: *Updating the National Sclerotinia Initiative Strategic 5-Year Plan*
Noon	Lunch and Poster Session — **City A**

Sclerotinia Research Activities – Session 4 **City B**
Moderator – Shin-Yi Marzano, South Dakota State University, Brookings, SD

1:20 pm White mold resistance-QTL: identification, interactions, and fine mapping in common bean (Abstract p. 23) – **Phil Miklas, USDA-ARS, Prosser, WA; Jim Myers, Oregon State University, Corvallis, OR; Phil McClean, North Dakota State University, Fargo, ND**

2:20 pm Enhancing soybean for resistance to Sclerotinia stem rot (Abstract p. 12; Poster #7) – **Zixiang Wen, Michigan State University, East Lansing, MI**

2:40 pm Break

3:20 pm Validation and characterization of cultivated sunflower lines with resistance to Sclerotinia basal stalk rot (Abstract p. 16) – **William Underwood, USDA-ARS, Fargo, ND**

3:40 pm Screening for resistance sources to Sclerotinia white mold in recently acquired germplasm of cool season grain legumes (Abstract p. 21, Poster #11) – **Weidong Chen, USDA-ARS, Pullman, WA**

4:00 pm Meeting Wrap-Up & Future Plans **– Mike Grusak, USDA-ARS, Fargo, ND**

4:30 pm Safe Travels Home or Networking at Local Restaurants

National Sclerotinia Initiative Poster Session

City A
Radisson Hotel Fargo

Epidemiology & Disease Management		
Poster No.	Title	Author(s)
1	Developing germycircularvirus-based control strategies of *Sclerotinia spp*.	C. Feng, C. Pedersen, Z. Wang, S-Y. Marzano
2	Horizontal transmission of the mycorvirus SsHADV-1 from strain DT-8 to US isolates of Sclerotinia sclerotiorum	M. Fu, Z. Qu, D. Jiang, P. Miklas, L. Porter, G. Vandemark, W. Chen

Genomics		
Poster No.	Title	Author(s)
3	Investigating quantitative resistance of Arabidopsis to Sclerotinia using comparative transcriptomics and genome-wide association study	R. Sharma Poudel, W. Underwood

Pathogen Biology & Development		
Poster No.	Title	Author(s)
4	Characterization of Sclerotinia sclerotiorum isocitrate lyase gene in pathogenicity/virulence on canola	K. Chittem, S. Upadhaya, L. del Rio Mendoza
5	Dissecting RNA silencing pathways in Sclerotinia sclerotiorum	A. Neupane, C. Feng, J. Bermudez, S-Y. Marzano
6	Functional analyses of Sclerotinia sclerotiorum extracellular effector (SsE1) in transgenic plants	W. Wei, L. Xu, K. Tanaka, G. Vandemark, W. Chen

Variety Development/Germplasm Enhancement		
Poster No.	Title	Author(s)
7	Enhancing soybean for resistance to Sclerotinia stem rot	Z. Wen, F. Lin, P. Collins, W. Li, C. Gu, M. Chilvers, D. Wang
8	Genome-wide association study for Sclerotinia stem rot resistance in a world collection of Brassica napus L.	J. Roy, L. del Rio Mendoza, M. Rahman
9	Identification of genomic regions associated with resistance to Sclerotinia stem rot in a Brassica napus double haploid population	F. Shahoveisi, A. Oladzad, L. del Rio Mendoza, K. Chittem, S. Ruud, S. Hosseinirad, B. Rissato
10	Screening of Sclerotinia basal stalk rot (BSR) and head rot populations and QTL mapping of BSR resistance derived from the wild annual sunflower species Helianthus argophyllus	Z. Talukder, W. Underwood, C. Misar, G. Seiler, X. Cai, L. Qi
11	Screening pea germplasm collection for resistance to Sclerotinia white mold	Y. Chen, C. Coyne, R. McGee, G. Vandemark, W. Chen
12	Sources of white mold resistance derived from wide crosses in common bean and fungicide sensitivity of Sclerotinia sclerotiorum from multi-site locations	R. Higgins, C. Wulkop, E.H. Nieto-Lopez, S.E. Everhart
13	Understanding and improving basal resistance to Sclerotinia sclerotiorum	J. Rollins, C. Wang, Z. Mou

National Sclerotinia Initiative Abstracts

Characterization of *Sclerotinia sclerotiorum* isocitrate lyase gene in pathogenicity/virulence on canola

Kishore Chittem, Sudha G.C Upadhaya and Luis del Río Mendoza
Department of Plant Pathology North Dakota State University, Fargo, ND 58102.

Funded Plan of Work: Characterizing resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum* (FY 2018-2019).

ABSTRACT:
The objective of this project has been to characterize putative resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum.* From differential gene expression data, genes involved in peroxisome associated pathways like fatty acid ß-oxidation and glyoxylate cycle were identified as novel potential pathogenicity candidates. The role of these pathways/genes has been shown to be essential for pathogenicity in several plant pathogens, including *Leptosphaeria maculans*, another important pathogen of canola. Based on these findings, isocitrate lyase (SS1G_04975 – *SsICL*), a key glyoxylate pathway gene was selected as a candidate pathogenicity/virulence gene for functional characterization. Gene disruption mutants for *SsICL* were developed by targeted gene replacement following split marker approach. PCR assays confirmed correct replacement of the gene. The deletion of *SsICL* did not result in significant reduction in mycelial growth on PDA or production of oxalic acid. However, *SsICL* deletion mutant significantly affected the ability of *S. sclerotiorum* to utilize oleic acid as sole source of carbon on minimal media. Pathogenicity assays through leaf inoculations indicated that *SsICL* gene is required for full virulence on canola. Ectopic complementation strain (*SsICL-com*) of the *SsICL* gene restored to the wild-type in their ability to utilize oleic acid as sole carbon source.

Contact Information – Dr. Luis del Río Mendoza, 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

Controlling *Sclerotinia sclerotiorum* in *Glycine max* by Targeting Oxalic Acid Production Using Host-Induced Gene Silencing

Megan McCaghey, Dandan Shao, Damon Smith, Mehdi Kabbage, University of Wisconsin-Madison, Madison, WI

Funded Plan of Work: Targeting essential genes in *Sclerotinia sclerotiorum* to achieve sclerotinia stem rot resistance in soybean

ABSTRACT:
Sclerotinia sclerotiorum, the causal agent of Sclerotinia stem rot (SSR), is a yield-limiting, fungal pathogen of *Glycine max*. The pathogenic success of *S. sclerotiorum* requires the secretion of oxalic acid (OA), a virulence factor for this pathogen. Virus-induced gene silencing (VIGS) using *Bean pod mottle virus* (BPMV) was used to target OA biogenesis in *S. sclerotiorum*. A sequence of 366 bp, corresponding to the fungal oxaloacetate acetylhydrolase (*Ssoah1*), a key enzyme in OA biogenesis, was cloned into a BPMV vector in an antisense orientation. BPMV constructs were introduced into *G. max* using particle bombardment, and viral symptoms paired with RT-PCR were used to confirm viral replication prior to inoculation. Accumulation of target sRNAs was also verified using RNA sequencing. Disease progress and target mRNA levels were monitored over a five-day period. Remarkably, plants containing BPMV vectors targeting Ss*oah*1 showed enhanced resistance to *S. sclerotiorum* compared to empty-vector control plants, in three replicated experiments. This phenotype was coupled by decreased expression of the target gene as determined by RT-PCR. These results provide evidence supporting host-induced gene silencing targeting virulence factors as a viable strategy to control SSR. We are also evaluating the targeting of other potential virulence factors. This study evaluated the uptake of dsRNA and sRNA by *S. sclerotiorum*. Using fluorescence microscopy, we clearly show the uptake of labeled RNAs by the fungus, suggesting that the exogenous application of these molecules can also be used to target gene expression. Genetic resistance to SSR is inadequate in commercial soybean varieties, and fungicidal control can be inconsistent and expensive. Herein, we propose that RNAi strategies will provide new tools to control *S. sclerotiorum* in *G. max*.

Contact Information – Mehdi Kabbage, Dept. of Plant Pathology, Univ. of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706; 608-262-0506; kabbage@wisc.edu

Developing Gemycircularvirus-based Control Strategies of *Sclerotinia spp.*

Chenchen Feng, Connor Pedersen, Ziyi Wang, Shin-Yi L. Marzano
South Dakota State University, Brookings, SD

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

ABSTRACT:
Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1(SsHADV-1) is a gemycircularvirus that infects and causes hypovirulence in *Sclerotinia spp*. SsHADV-1 codes for a replication initiation protein (Rep) and a coat protein (CP). To dissect the effects of viral CP and Rep proteins on fungi and identify the hypovirulence-associated genes, CP and Rep genes were expressed separately in *S. sclerotiorum* and *Botrytis cinerea*. We successfully obtained the site-specific integration transformants of CP and Rep genes expressed in *B. cinerea* (BCP-SS and BRep-SS) and multiple transformants of ectopic expression in *S. sclerotiorum* (SCP-SS and SRep-SS). On potato dextrose agar, BRep-SS grew much slower than BCP-SS ($P<0.05$), indicating Rep is a greater factor for hypovirulence than CP. For the *S. sclerotiorum* transformants, SRep-SS grew significantly slower than SCP-SS ($P<0.05$). Consistently, the pathogenicity tests of the transformants on tomato fruits and spinach leaves showed that BRep-SS had significantly reduced virulence for tomato and spinach compared to BCP-SS ($p<0.05$) by the measuring of lesion size. Therefore, the viral Rep protein exhibited stronger anti-fungal activity than the CP protein. Our result demonstrates that Rep protein alone is enough to provide the hypovirulence-inducing activity of gemycircularvirus SsHADV-1, which suggests a transgenic approach expressing the viral Rep protein in plants may enhance the disease resistance. We have also obtained a USDA-APHIS permit with the field trials of SsHADV-1 in Lincoln, Brookings, or Clark Counties of South Dakota that are under planning for the next growing season.

Contact Information – Shin-Yi Lee Marzano, 2140 North Campus Drive, SNP 252 Box 2140D, Brookings, SD 57007-2142; shinyi.marzano@sdstate.edu

Dissecting RNA silencing Pathways in *Sclerotinia sclerotiorum*

Achal Neupane, Chenchen Feng, Johan Manuel Murcia Bermudez, Shin-Yi Lee Marzano
South Dakota State University, Brookings, SD

Funded Plan of Work: Developing Environmental Friendly Fungicides for Managing White Mold

ABSTRACT:
RNA silencing or RNA interference (RNAi) is an essential mechanism in animals, fungi, and plants that functions in gene regulation and defense against foreign nucleic acids. In fungi, RNA silencing has been shown to function primarily in defense against invasive nucleic acids. We previously determined that mycoviruses are triggers and targets of RNA silencing in Sclerotinia sclerotiorum. However, recent progresses in RNAi or dsRNA-based pest control requires more detailed characterization of the RNA silencing pathways in S. sclerotiorum to investigate the utility of dsRNA-based strategy for white mold control. This study elucidates the roles of argonaute enzymes, *agl*-2 and *agl*-4, in small RNA metabolism in S. sclerotiorum. Gene disruption mutants of *agl*-2 and *agl*-4 were compared for changes in phenotype, virulence, viral susceptibility, and small RNA profiles. The *agl*-2 mutant but not the *agl*-4 mutant had significantly slower growth and virulence prior to virus infection. Similarly, the *agl*-2 mutant but not the *agl*-4 mutant, showed greater debilitation under virus infection compared to uninfected strains. The responses were confirmed in complementation studies and revealed the antiviral role of *agl*-2. Gene disruption mutants of *agl*-2, *agl*-4, Dicer-like (*dcl*)-1, and *dcl*-2 did not change the stability of the most abundant endogenous small RNAs, which suggests the existence of alternative enzymes/pathways for small RNA biogenesis in *S. sclerotiorum*. Furthermore, *in vitro* synthesized dsRNA targeting *agl*-2 showed a significantly reduced average lesion diameter ($P < 0.05$) on canola leaves with *agl*-2 down-regulated compared to controls. In summary, we demonstrated the effectiveness of RNA pesticides targeting *S. sclerotiorum* RNA silencing pathway for the control of the economically important pathogen. Host-induced gene silencing targeting *agl*-2 and the evaluations of the RNA-dependent RNA polymerases as targets of RNA pesticides are ongoing.

Contact Information – Shin-Yi Lee Marzano, 2140 North Campus Drive, SNP 252 Box 2140D, Brookings, SD 57007-2142; shinyi.marzano@sdstate.edu

Enhancing soybean for resistance to Sclerotinia stem rot

Zixiang Wen, Feng Lin, Paul J. Collins, Wenlong Li, Cuihua Gu, Martin Chilvers & Dechun Wang, Department of Plant, Soil and Microbial Sciences, Michigan State University

Funded Plan of Work: Enhancing soybean for resistance to Sclerotinia stem rot

ABSTRACT:
Sclerotinia stem rot of soybean is caused by *Sclerotinia sclerotiorum*, which is capable of infecting a wide range of plants. To continue the breeding pipeline for enhancing resistance to this disease in soybean, 69 $F_{3:5}$ lines derived from 518 $F_{3:4}$ marker-assisted selected lines were advanced to 1st year yield trials (preliminary yield test). Of these 69 lines, 9 were advanced based on yield performance and will be entered in 2020 advance yield test. Moreover, 15 new cross combinations were made focusing on yield, protein, and the disease package. Sixty-one hybrid pods were harvested from these combinations. To facilitate varieties releasing and parental lines' selection, 138 advanced breeding lines were evaluated for white mold resistance in disease nursery at Montcalm. Of these tested lines, 8 lines showed resistance (disease severity index < 15) to white mold. We also tried to optimize genomic selection model through implementing non-parametric methods (random forest and neural networks). Non-parametric methods did not outperform the linear model by a sizable margin. More research is needed to adapt non-linear models to build genomic selection model for white mold resistance in soybean.

Contact Information – Dr. Dechun Wang, 1066 Bogue St., Rm. A384E, East Lansing, MI 48824-1325; 1-517-355-0271 Ext. 1188; wangdech@msu.edu

Functional Analyses of *Sclerotinia sclerotiorum* Extracellular Effector (SsE1) in Transgenic Plants

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

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

ABSTRACT:
Sclerotinia sclerotiorum causes the necrotrophic disease white mold on many economically important crops such as common bean, canola, soybean, sunflower and pea, chickpea and lentil, with tissue maceration as the most prominent symptom, due to degradation of plant cell wall. *S. sclerotiorum* produces a number of polygalacturonases (SsPGs) that degrade the major plant cell wall component pectin. As a countermeasure, plants have evolved to produce polygalacturonase-inhibiting proteins (PGIPs) that bind to pathogen's PGs and inhibit PG activity in protecting plant cell wall from fungal PG degradation. In this research project, we previously showed that *S. sclerotiorum* secretes a small effector protein (SsE1) that specifically interacts with plant AtPGIP1, based a variety of techniques such as yeast two-hybrid, co-immunoprecipitation and bimolecular fluorescence complementation assays. In this reporting period, we specifically tested the biological functions of *SsE1 in vivo* by heterologously expressing *SsE1* in plant. Transgenic lines of *Arabidopsis thaliana* (Col-0 background) overexpressing *SsE1* or *AtPGIP1* were generated. Their reactions to infection by three *S. sclerotiorum* genotypes (wildtype strain, *SsE1*- and *SsPG1*-deletion mutants were compared to those of the wildtype plant. Results showed expression of *SsE1* in plant increased susceptibility to infection by all three pathogen genotypes. The reactions of AtPGIP1-overexpressing line to infection by the wildtype strain and *SsE1*-deletion mutant are very telling. Overexpressing *AtPGIP1* significantly limited infection by the *SsE1*-deletion mutant, but had minimum effect on infection by the wildtype strain because the wildtype strain possesses SsE1 that can nullify the effect of AtPGIP1, demonstrating the important role of *SsE1* in virulence of *S. sclerotiorum.*

Contact Information – Dr. Weidong Chen, USDA- ARS, and WSU, Pullman, WA 99164; 509-335-9178; weidong.chen@usda.gov

Genome-Wide Association Study for Sclerotinia Stem Rot Resistance in a World Collection of *Brassica napus* L.

Jayanta Roy[1], Luis E. del Río Mendoza[2], and Mukhlesur Rahman[1]
[1]Department of Plant Sciences, North Dakota State University, Fargo, ND
[2]Department of Plant Pathology, North Dakota State University, Fargo, ND

Funded Plan of Work: Improving resistance to *Sclerotinia sclerotiorum* in spring canola.

ABSTRACT:
We proposed to introduce NEP63-derived resistance into elite NDSU canola breeding lines (WC1417 and WC1421) using a backcross program. The F_1 (NEP63 X 17B01) were backcrossed with elite breeding line 17B01 and BC_1 seeds were harvested. BC_1 seeds will be planted soon in the greenhouse. The backcrossing program is in progress.

Sclerotinia stem rot (SSR) is one of the most destructive fungal diseases of canola and is caused by *Sclerotinia sclerotiorum* (Lib) de Bary. The disease can significantly reduce seed yield as well as oil content and quality. In North Dakota, average yield losses have been estimated at 13-50%, with annual economic losses estimated at US$ 20.8 million. Since there are no completely resistant varieties available, identification of resistant genotypes and genes in diverged germplasm accessions is one of the best options to develop cultivars with durable resistance against this disease. In the present study, a panel of 296 germplasm accessions consisting of spring, winter and semi-winter types, originated from 29 countries were evaluated for their reaction to *S. sclerotiorum* in a controlled environment using RCBD with three replications and five plants per replication. The germplasm was evaluated using isolate WM#031 by mycelial stem inoculation at flowering stage. For each accession, lesion length (cm), stem girdling (%), and plant mortality were recorded between the third and fifteenth days after inoculation at two-day intervals. Relative area under disease progress curve (rAUDPC) was calculated for lesion length, stem girdling and plant mortality to evaluate each accession. For both phenotypic evaluation and association mapping, spring type accessions were evaluated as one group and semi-winter and winter types as another group. The germplasm accessions have been genotyped using next generation sequencing at next generation sequencing at University of Texas Southwestern Medical Center at Dallas, TX and 19,836 high quality single nucleotide polymorphisms have been identified. Finally, genome-wide association is being conducted to identify the genomic region containing SSR resistant genes in *B. napus.*

Contact Information – Dr. Mukhlesur Rahman (corresponding author), Department of Plant Sciences, North Dakota State University, Loftsgard Hall 470G, Fargo, ND-58102; 701-793-1408; md.m.rahman@ndsu.edu

Horizontal transmission of the mycovirus SsHADV-1 from strain DT-8 to US isolates of *Sclerotinia sclerotiorum*

Min Fu, Zheng Qu, Daohong Jiang, Phil Miklas, Lyndon Porter, George Vandemark and Weidong Chen, Washington State University, Pullman, WA 99164, Huazhong Agricultural University, Wuhan, China, and USDA ARS, Grain Legume Genetics and Physiology Research Unit, Pullman, WA 99164

Funded Plan of Work: Biological control of white mold using the mycovirus SsHADV-1 infected hypovirulent strain DT-8 of Sclerotinia sclerotiorum

ABSTRACT:
The mycovirus Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) was discovered in the strain DT-8 of *Sclerotinia sclerotiourm* in China. The strain DT-8 is hypovirulent and can be used as an agent in biological control of Sclerotinia white mold. In order to explore the potential of using the mycovirus SsHADV-1 in the United States, attempts were made to transfer the mycovirus from DT-8 to US strains. Strain DT-8 was used as the donor and co-cultured on a 150-mm PDA plate for two weeks with the US strain WMA1 (ATCC MYA-4521) originally isolated from a pea plant in the Columbia Basin of US Pacific Northwest. The dramatic differences in sclerotial size served as a convenient morphological marker for separating the two isolates. Agar plugs were taken from near the inoculation plug of the WMA1 colony and transferred to new PDA plates and were further purified through hyphal transfers in order to generate WMA1-derived strains carrying the mycovirus SsHADV-1. Initially the WMA1-derived strains were examined for colony morphology and scleotial formation in comparison with the progenitor and donor strains on PDA. Slower growth rates and serrated colony edges suggested presence of the mycovirus, but the obviously large sclerotial size suggested the strains were derived from WMA1. Presence of the mycovirus was detected in the WMA1-derived strains using PCR with the mycovirus-specific PCR primers (CP-FP/CP-RP and REP-FP/REP-RP). Nuclear DNA markers are being developed for further confirmation of the origin of the derived strains. In pathogenicity tests, the derived strains became hypovirulent, and functioned as a biocontrol agent in reducing white mold disease on dry bean plant, features of strains carrying the SsHADV-1 mycovirus. The derived strain WMA1-V3-1 is being used as a donor in transmitting the mycovirus to other US isolates of *S. sclerotiorum*.

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@ars.usda.gov

Identification and characterization of sunflower lines with high levels of resistance to Sclerotinia basal stalk rot

Jesse Pahl[1], Julie Pasche[1], and William Underwood[2]
[1]Department of Plant Pathology, North Dakota State University, Fargo, ND
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND

Funded Plan of Work: Validation and characterization of cultivated sunflower lines with resistance to Sclerotinia basal stalk rot.

ABSTRACT:
Sclerotinia basal stalk rot (BSR) of sunflower is a root rot that begins with infection of sunflower roots by *Sclerotinia sclerotiorum* mycelium derived from myceliogenic germination of sclerotia in the soil. Due to the unusual mode of infection, evaluation of sunflower germplasm and experimental materials for BSR resistance requires inoculation of the root zone with *S. sclerotiorum* mycelium. In our previous NSI-funded project, we developed an improved, higher resolution greenhouse-based method for evaluating sunflower resistance to BSR. Prior to this advance, BSR evaluations were conducted in field nurseries either relying on natural infection or inoculated with sclerotia or *Sclerotinia*-infested millet seed deposited in furrows next to sunflower rows. These field-based trials could roughly identify genotypes with some degree of resistance but were too variable to effectively separate highly-resistant from only moderately-resistant or average genotypes. The goals of this project are: 1) to re-evaluate 60 sunflower lines for BSR resistance that had previously exhibited some degree of resistance in field trials; 2) to characterize the most resistant lines using molecular markers for BSR resistance identified in a prior QTL mapping effort; 3) to confirm broad-spectrum resistance in the most resistant lines by evaluating resistance to 6 diverse *S. sclerotiorum* isolates. We have completed initial BSR resistance evaluations for the set of 60 sunflower lines. Through these efforts, we identified three lines with significantly higher levels of resistance than our previous most resistant inbred line RHA 801. We have selected 15 lines with levels of resistance statistically similar or superior to RHA 801 for molecular marker analysis and evaluation with multiple *S. sclerotiorum* isolates. This information will allow us to prioritize lines with high levels of physiological resistance for further genetic mapping efforts and characterization of resistance mechanisms.

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

Identification of Genomic regions associated with resistance to Sclerotinia stem rot in a *Brassica napus* double haploid population

Fereshteh Shahoveisi[1], Atena Oladzad[2], Luis E. del Río Mendoza[1], Kishore Chittem[1], Susan Ruud[1], Seyedali Hosseinirad[2], Bruna Rissato[1]
[1]Department of Plant Pathology, North Dakota State University, Fargo, ND 58102
[2]Department of Plant Sciences, North Dakota State University, Fargo, ND 58102

Funded Plan of Work: Improving resistance to *Sclerotinia sclerotiorum* in spring canola

ABSTRACT:
We proposed to characterize and transfer the resistance present in the doubled-haploid line NEP63 and to characterize resistance present in plant introduction 436554. Here we report the identification of two QTL derived from NEP63. A mapping population derived from 436554 was developed.

A doubled haploid population, developed from a cross between *Brassica napus* cv. Topas (susceptible) and DH line NEP63 (resistant), was used to identify quantitative trait loci (QTL) associated with resistance to Sclerotinia stem rot caused by *Sclerotinia sclerotiorum*. For phenotyping, forty-day old plants were inoculated using the agar plug stem method. Lesion size was measured between the third- and eleventh-days post inoculation at two-day intervals. Three relative areas under the disease progress curve (rAUDPC) were calculated using different lesion size reading intervals. In addition, the original data sets for plant mortality and lesion size were used to generate binary and polynomial data sets. In all, nine phenotypic data sets were used in the analysis. DNA extracted from leaves of 20-day old plants was sent to the Genomics Center of the University of Minnesota for genotyping by sequencing. A total of 1019 single nucleotide polymorphism markers were used to construct genetic linkage maps with LOD of 10. Nineteen linkage groups were developed at LOD 4 using markers that passed the Chi-square test ($\alpha \geq 0.0001$). Two QTL were identified when using the original rAUDPC and lesion size data sets. The first was located on chromosome A02, had an LOD of 4.5 to 4.8, and explained 14 to 15.5% of the phenotypic variation, respectively. The second QTL was located on chromosome C01, had an LOD of 3.2 to 3.4, and explained 10.7 to 11.5% of the phenotypic variation, respectively. In the next step, potential candidate genes associated with these QTL will be identified.

Contact Information – Dr. Luis del Río Mendoza, 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

Isolation and characterization of bacterial communities associated with sunflower resistance to Sclerotinia stalk rot

C. Alisha Quandt[1], Meg Glaser[1], Cloe Pogoda[1], Stephan Reinert[1], Nolan Kane[1] and Brent Hulke[2]
[1] Ecology and Evolutionary Biology, University of Colorado, Boulder, CO
[2] USDA-ARS Sunflower and Plant Biology Unit, Fargo, ND

Funded Plan of Work: Understanding how sunflower soil microbiome impacts resistance to Sclerotinia stalk rot

ABSTRACT:
New research has linked the microbial community of sunflower (*Helianthus annuus*) with its resistance to Sclerotinia stalk rot caused by the fungus *Sclerotinia sclerotiorum*. The potential to identify, isolate, and use specific microbes associated with Sclerotinia resistance could have both prophylactic and therapeutic impacts. In our new study, we have begun targeted isolation of sunflower field soil bacteria previously correlated with resistance via DNA sequencing. Many of the bacteria we isolated are Actinobacteria, a group of microbes known for their prolific production of antimicrobial secondary metabolites. We will also present preliminary data on competition assays between these bacterial isolates and the pathogen *S. sclerotiorum*.

Contact Information – Dr. C. Alisha Quandt, EBIO Department, Box 334, University of Colorado, Boulder, CO, 80309-0334; 303-735-6550; alisha.quandt@colorado.edu; Dr. Brent S. Hulke, USDA-ARS Sunflower and Plant Biology Unit, Fargo, ND 58102-2765; 701-239-1321; brent.hulke@usda.gov

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

Nolan Kane, Cloe Pogoda, Stephan Reinert, Ziv Attia, Jason Corwin, and Brent Hulke

Funded Plan of Work: Refining genomic tools for Sclerotinia resistance and agronomic breeding of sunflower – towards dissection of the resistance phenotype

ABSTRACT:
Despite decades of work, gaining a full understanding of host-plant resistance to the *Sclerotinia* and *Phomopsis (Diaporthe)* pathogens remains elusive. Here we show heritable genetic plant effects on soil microbial communities, which in turn accounts 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. In addition, we sampled the plant rhizospheres of a portion of the panel (95 individuals). 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 to explain 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 all four sunflower traits. The genomics results re-affirm the results of the phenotyping experiments, which indicates association between head rot and Phomopsis stem canker, and between rhizosphere microbial composition and Sclerotinia basal stalk resistance. These findings demonstrate a previously unknown microbial component to Sclerotinia resistance in sunflower and suggest important targets for future research and breeding efforts.

Contact Information – Dr. Nolan C. Kane, EBIO Department, Box 334, University of Colorado, Boulder, CO, 80309-0334; 303-492-2736; nolan.kane@colorado.edu; Dr. Brent S. Hulke, USDA-ARS Sunflower and Plant Biology Unit, Fargo, ND 58102-2765; 701-239-1321; brent.hulke@usda.gov

Screening of Sclerotinia basal stalk rot (BSR) and head rot populations and QTL mapping of BSR resistance derived from the wild annual sunflower species *Helianthus argophyllus*

Zahirul Talukder[1], William Underwood[2], Christopher Misar[2], Gerald Seiler[2], Xiwen Cai[1] and Lili Qi[2]
[1]Department of Plant Sciences, North Dakota State University, Fargo, ND
[2]USDA-ARS, Edward T. Schafer Agricultural Research Center, Fargo, ND

Funded Plan of Work: QTL mapping of Sclerotinia basal stalk rot resistance derived from sunflower wild species

ABSTRACT:
In 2019, we completed the third year of Sclerotinia basal stalk rot (BSR) field screening trials for two mapping populations derived from crosses of two wild annual sunflower species, *H. petiolaris* (Pop1, 174 BC_2F_4 families) and *H. argophyllus* (Pop3, 134 BC_1F_4 families) with cultivated sunflower line HA 89. Field screening trials were conducted at Carrington, ND using a randomized complete block (RCB) design with three replications. Highly significant (p<0.001) genetic variations were observed for BSR disease incidence (DI) with no significant genotype × environment (G×E) interactions for the trait in both populations. Spearman rank correlations among DI scores of the field trials conducted during 2017-2019 were highly significant (p<0.001) with ρ ranging from 0.24 to 0.35, and 0.43 to 0.50, respectively for Pop1 and Pop3, confirming a high degree of repeatability of the screening trials. The BSR DI data for Pop1 followed a normal distribution in all three seasons with mean DI of 51.9, 47.5, and 47.3%, and DI ranged from 4.8-88.6%, 2.2-80.8%, and 5.6-85.2%, respectively. The distribution of BSR DI data of Pop3 was normal for the 2018 and 2019 seasons, but not for 2017. The mean DI of Pop3 was 20.3%, 32.5%, and 38.5%, and ranged from 0-72.3%, 0-78.5%, and 0-100%, respectively in the three seasons. Pop1 and Pop3 were also screened in the greenhouse with three replications under high disease pressure and evaluated for disease rating (DR) and area under the disease progress curve (AUDPC). A continuous variation of the traits was observed in both populations with a mean DR and AUDPC of 11.8 and 1674, respectively for Pop1, and 13.4 and 1506, respectively for Pop3. BSR resistance QTL mapping was performed for Pop3 using a linkage map spanning 2045.14 cM developed from 3,112 GBS-derived SNP markers on 17 sunflower chromosomes. A total of 21 QTL associated with BSR resistance were detected on 11 chromosomes, each explaining a proportion of the phenotypic variation ranging from 4.5% to 22.6%. Of the 21 QTL, eight QTL were detected for BSR DI measured in the field, seven were detected for traits measured in the greenhouse, and six were detected from both field and greenhouse tests. BSR resistance QTL mapping for Pop1 is in progress.

In 2019, we also screened a F_3 population consisting of 188 families segregating for head rot (HR) resistance developed from the cross of HR21 × HA 234 in two field nurseries at Carrington, ND and Staples, MN. Inoculated field screening trials were conducted using RCB design with three replications. A clear separation of parents was observed for the HR DI and disease severity (DS) at both locations with mean DI of 36.37 and 89.37%, and DS of 1.22 and 4.25 (0-5 scale) for HR 21 and HA 234, respectively. A continuous variation of DI and DS were observed for the population with mean DI of 73.0 and 68.5%, and DS of 2.97 and 2.85 for the Carrington and Staples locations, respectively, confirming a polygenic inheritance of the traits. Preliminary analysis revealed highly significant (p<0.001) genetic variations for the traits measured. The moderately high broad-sense heritability (H^2) estimates of the HR DI and DS (0.74 and 0.72, respectively) suggest potential for genetic improvement of HR resistance through breeding.

Contact Information – Dr. Lili Qi, Sunflower and Plant Biology Research Unit, USDA-ARS Edward T. Schafer Agricultural Research Center, 1616 Albrecht Blvd., Fargo, ND USA 58102-2765; (701) 239-1351; lili.qi@usda.gov

Screening pea germplasm collection for resistance to Sclerotinia white mold

Yungchun Chen, Clare Coyne, Rebecca McGee, George Vandemark and Weidong Chen
USDA ARS, Grain Legume Genetics and Physiology Research Unit, Pullman, WA 99164

Funded Plan of Work: Screening for resistance sources to Sclerotinia white mold in recently acquired germplasm of cool season grain legumes

ABSTRACT:
Sclerotinia white mold is an economically important disease of grain legumes (pea, chickpea and lentil). Management options for white mold are mainly limited to cultural practices because resistance to white mold is lacking in the cultivated pea and fungicide application is not economical for grain legume crops. Previous screening for resistance in then 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 was initiated to identify new resistance sources in the recently acquired germplasm of pea. Under this project, the newly acquired germplasm lines of cool season grain legumes were propagated for the screening in pathogenicity assays. Among the 218 recently acquired and propagated pea germplasm lines, more than 120 accessions have been tested for resistance to white mold along with a susceptible cultivar Shawne and a moderately resistant line DSP for comparison. Due to limitations of available space in the greenhouse, the germplasm accessions were tested in batches to fit the limited space and during time periods when greenhouse temperature can be managed. We completed tested screening of three batches in the greenhouse. Each time the germplasm lines that showed moderate resistance in previous tests were included in the subsequent tested to confirm their reaction to white mold. All the tested accessions showed disease although the accessions exhibited variations in reaction to white mold inoculation. No germplasm accessions were found to be more resistant than the moderately resistant accessions identified last year. The three accessions that showed slower disease onset or disease progression in 2018 remained to be the most prominent lines for resistant sources. The accession W6 44561 is the most resistant line so far, and disease lesions on W6 44561 plants were smaller than the moderately resistant line DSP. Allelic testing would be required to see if this line possesses additional resistance genes or quantitative trait loci to white mold.

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

Sources of white mold resistance derived from wide crosses in common bean and fungicide sensitivity of *Sclerotinia sclerotiorum* from multi-site locations

R. Higgins, C. Wulkop, E.H. Nieto-Lopez and S.E. Everhart
University of Nebraska–Lincoln

Collaborators: J. Kelly (MI), H. M. Wunsch (ND), J. Myers (OR), P. Miklas (WA), C. Urrea (NE), and E. Berghauer (WI)

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

ABSTRACT:
Our over-arching goal is to improve white mold (WM) disease management by identifying WM resistant bean lines and characterization of the pathogen genetic and phenotypic variation. Greenhouse and field evaluations were used to screen lines for putative sources of WM resistance in adapted backgrounds at multiple sites located in six states in the major bean-production area of the U.S. Multi-site testing is essential for robust evaluation under different environmental conditions and with different pathogen populations that our previous results have shown are significantly different in both genetic variation and aggressiveness. A straw test that consistently identifies sources of resistance in adapted and unadapted bean germplasm was used for greenhouse tests, which requires only a small number of seeds to confirm resistance. In 2019, trials were conducted in WI, NE, MI, OR, ND and WA, with eight lines that included Black, Great Northern, Pinto, Red, and Small Red bean types. Two of these lines were evaluated previously in these trials and six were new this year. In the greenhouse, two lines performed significantly better than the moderate control, Bunsi, whereas in the field trials, four lines outperformed the moderate control, indicating either resistance or escape mechanisms in the field. In both greenhouse and field trials, no lines were scored as more resistant than the resistant control G122. Complementary research to characterize phenotypic variation in the pathogen is also underway. Fungicide sensitivity of 113 *Sclerotinia sclerotiorum* isolates to four fungicides with different modes of action is in progress. Isolates were selected from multi-site testing locations in 15 counties in five states (CO, MI, ND, NE, and WA). Results will be used to determine if there is resistance or reduced sensitivity present and used to inform disease management recommendations.

Contact Information – Dr. Sydney Everhart, Department of Plant Pathology, University of Nebraska-Lincoln, 406 Plant Science Hall, Lincoln, NE 68583-0722; everhart@unl.edu

Towards QTL Analyses in Two Pinto Bean RIL Populations and Release of SR9-5 Small Red White Mold Resistant Germplasm Line

Phillip Miklas, Fernanda Souza Lopes, Alvaro Soler Garzon, Jim Myers, Phil McClean, and Juan Osorno
USDA-ARS, Prosser, WA; Oregon State University; North Dakota State University

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

ABSTRACT:
Work continues toward identifying and validating major effect QTL for resistance to white mold in pinto bean. The RIL pinto population PT9-5-6/USPT-WM-12 (abbreviated as P2) with 199 F5:6 lines was screened in the GH straw test (four replications to date). The susceptible (7.0) and resistant (4.3) parents and checks (not shown) scored as expected. The P2 population (mean = 6.0) had a normal distribution slightly skewed toward susceptibility. The P2 population was recently genotyped with the new 12K SNP chip (USDA-ARS-Beltsville). Linkage map construction and QTL analyses will be conducted in time for the meeting. The 199 P2 RILs will be planted in Puerto Rico (USDA-ARS-Mayaguez) this January to obtain enough seed of each line for a replicated white mold field trial this summer - 2020. The RIL pinto bean population PT12-37/VCP-13 (abbreviated as P3) has 164 F5:6 lines. The susceptible (6.5) and resistant (3.5) parents for P3 scored as expected in a recent straw test. The P3 RILs were recently planted in the GH (5 reps) but the straw test results will likely not be available in time for the meeing. DNA extraction and SNP genotyping for the P3 population are pending. The 164 P3 RILs will be increased this summer (2020) in Othello, WA, to generate enough seed for a field trial in 2021. From these two RIL populations we also hope to obtain one or two pinto bean lines with a combination of improved agronomic performance and resistance to white mold for germplasm/cultivar release by 2024.
The small red advanced line SR9-5, a parent of Cayenne cultivar, and many other advanced small red breeding lines exhibiting good agronomic performance and field resistance to white mold in the MI and WA breeding programs, is being considered for germplasm release. SR9-5 performed well in the 2019 Bean White Mold Nusery (BWMN) across both field and greenhouse trials. The straw test resistance in the GH was unexpected. To purify SR9-5 we will increase seed from 50 inoculated plants that score well in the straw test this winter and subsequently under white mold pressure in the field in 2020. SR9-5 will be included in the 2020 BWMN and if all goes as planned, will be released in 2021.

Contact Information – Dr. Phil Miklas, USDA-ARS, Prosser, WA 99350; 509-786-8492; phil.miklas@usda.gov

Understanding and Improving Basal Resistance to *Sclerotinia sclerotiorum*

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

Funded Plan of Work: Enhancing basal resistance to *Sclerotinia sclerotiorum* in Brassica

ABSTRACT:
This project is focused on using the *Arabidopsis thaliana* gene hypersusceptible to *S. sclerotiorum* (*HSS1*) for engineering high levels of basal disease resistance in canola. Through map-based cloning, we have determined that *HSS1* encodes the Mediator complex subunit MED16, indicating that MED16 is a key component regulating basal resistance to *S. sclerotiorum*. Although loss of function mutants are hypersusceptible to *S.* sclerotiorum, overexpression of the *Arabidopsis HSS1* gene did not provide an increase in resistance to *S. sclerotiorum*. To determine if other forms of MED16 could provide an increase in resistance, we have screened orthologous genes from fungi and non-host plants for their ability to confer resistance to *S. sclerotiorum*. We found that the rice *HSS1* (*OsHSS1*) homolog complemented the *A. thaliana hss1* mutant and the *S. sclerotiorum HSS1* homolog did not. Over-expression of *OsHSS1* in the *A. thaliana hss1* mutant background increased resistance to *S. sclerotiorum* above the wild-type level Results indicate that the OsMED16 protein much like the AtMed16 protein is sensitive to proteolysis resulting form plant infection with *S. sclerotiorum*. This indicates that the mechanism of enhanced resistance is likely not based on resistance to proteolysis. We are currently focusing our efforts on engineering other components of the MED16 signaling cascade utilizing a rice ortholog of WRKY33 to determine if a further increase in OsMed16-mediated resistance is possible. Expression of interacting nonhost components of basal resistance pathways is expected to increase the functionality of the individual components and enhance the resistance response.

Contact Information – Dr. Jeffrey Rollins, Department of Plant Pathology, University of Florida, Gainesville, FL 32611; 352-273-4620; rollinsj@ufl.edu

WRKY transcription factors influencing levels of resistance to Sclerotinia in *Arabidopsis thaliana*

William Underwood[1], Megan Overlander[1], and Luis del Rio[2]
[1]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND
[2]Department of Plant Pathology, North Dakota State University, Fargo, ND

Funded Plan of Work: Role of WRKY transcription factors in quantitative resistance to *Sclerotinia sclerotiorum.*

ABSTRACT:
Resistance to *Sclerotinia sclerotiorum* is quantitative and genetically complex. While many efforts have been made to map loci contributing to Sclerotinia resistance in numerous agriculturally important crop hosts, the identities and functions of genes governing resistance to this pathogen are still mostly unknown. We conducted a genome-wide association mapping effort using the model dicot Sclerotinia host *Arabidopsis thaliana* as a step toward identifying and characterizing genes controlling quantitative resistance. We evaluated an Arabidopsis diversity panel of 325 accessions for resistance at two time-points after leaf inoculation with the sequenced reference isolate, 1980, as well as a second, less aggressive isolate, BN325. As anticipated for the quantitative nature of resistance typical for this pathogen, we observed a continuous distribution of resistance/susceptibility. In total, across both time-points and isolates, we identified over 30 loci significantly associated with resistance. Five transcription factors of the WRKY family, known to play a role in disease resistance, were found among plausible candidate genes within mapped regions. The goal of this project is to evaluate the potential role of these Arabidopsis WRKY family transcription factors, and their canola and sunflower orthologues, in resistance to Sclerotinia. To date, we have acquired T-DNA insertion knockout mutants disrupted in *AtWRKY3*, *AtWRKY4*, *AtWRKY19*, *AtWRKY27*, and *AtWRKY61*. Disease evaluations indicated that *wrky4* mutants are significantly more susceptible to *S. sclerotiorum* than the Col-0 parent ecotype while, in contrast, *wrky27* mutants are significantly more resistant. *WRKY4* transcript levels, as determined by qPCR, increased 2-3-fold in resistant Arabidopsis accessions at 24h after Sclerotinia inoculation, but were unchanged or decreased in susceptible accessions, consistent with a positive role for *WRKY4* in regulating resistance. In contrast, *WRKY27* expression was strongly down-regulated after Sclerotinia inoculation in both resistant and susceptible accessions and this transcription factor appears to negatively impact resistance. These results provide initial insights into the identities of genes controlling quantitative resistance to this disease. Future efforts to characterize resistance mechanisms will be discussed.

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

National Sclerotinia Initiative Annual Report – FY19

Project Title: Sclerotinia Initiative

Project No: 3060-21220-031-00D Accession No: 0432211
SY(s): GRUSAK, MICHAEL
UNDERWOOD, WILLIAM

Location: FARGO, NORTH DAKOTA
EDWARD T. SCHAFER AGRICULTURAL RESEARCH CENTER
SUNFLOWER AND PLANT BIOLOGY RESEARCH

Start Date: 02/01/2017 Termination Date: 01/31/2022

Final Report: No NPL: ROY A SCOTT

Objectives (from AD-416):
Coordinate the development of a Sclerotinia initiative for expanded research to control this devastating disease which affects canola, sunflowers, soybeans, edible dry beans, lentils, peas and other crops. Research should be coordinated with interested ARS, state, and industry cooperators and administered through specific cooperative agreements. Planning workshops and annual meetings involving interested parties will be organized throughout the funding period.

Approach (from AD-416):
Exotic and emerging plant diseases pose severe problems throughout the United States. Their increasing importance may be attributed to the introduction of pathogens into new geographic regions; modification of the environment that favor diseases; change in crop management practices; genetic shifts in the pathogen population; and other processes that may give them a competitive advantage.

Milestones for FY2019:
1. Develop and implement integrated management strategies for Sclerotinia.
Milestone Substantially Met

2. Identify germplasm and/or wild species with resistance to Sclerotinia.
Milestone Fully Met

3. Identify and/or evaluate chemical or biological fungicides and application technologies for use in Sclerotinia management.
Milestone Fully Met

4. Characterize genetic and biological variation among different Sclerotinia populations.
Milestone Fully Met

5. Study host-parasite interactions to determine disease vulnerability during crop development.
Milestone Fully Met

6. Develop additional information on effect of crop rotations/sequences and related crop production techniques on Sclerotinia severity and management.
Milestone Not Met – Insufficient resources (lack of operational funds)

7. Conduct research on host/pathogen genomics including genome-wide gene expression, gene profiling of susceptible and resistant hosts, and marker development.
Milestone Fully Met

8. Develop and/or refine Sclerotinia forecasting models/risk maps for integration into IPM programs.
Milestone Not Met – Insufficient resources (lack of operational funds)

Progress Report:
This report documents progress for cooperative research performed as part of the National Sclerotinia Initiative and involves researchers at several U.S. universities and USDA-ARS locations, in cooperation with USDA-ARS in Fargo, North Dakota.

Dry bean research: To identify, transfer, and validate white mold (WM) resistance factors from Phaseolus coccineus and Phaseolus vulgaris in common bean breeding lines, a nested association mapping (NAM) population was evaluated for WM in the field for a 2nd year; genotyping is under way. Studies with the Unidor/5630 population (differential WM resistance) identified one quantitative trait locus (QTL) that was mapped to bean chromosome Pv02. Efforts continued to define meta-QTL for resistance to white mold in common bean and to verify their phenotypic effect. Three Andean lines ADP-0014, -0436, and -0734 were studied and continue to perform well in the straw test. Physical regions of the common bean genome associated with major white mold resistance QTL were defined to investigate candidate genes. Work continued toward fine-mapping the WM2.2 QTL.

To identify and/or verify resistance to white mold in wide dry bean crosses and adapted dry and snap bean lines, greenhouse and field trials (multiple locations) were conducted using pinto, navy, black, and small red bean seed classes. In FY19, 11 seed lines were received (9 for field and 11 for greenhouse screening) from four breeders. To identify pathogen phenotypic variation that may guide new bean line breeding, data from 366 Sclerotinia sclerotiorum isolates were genotyped and analyzed.

Soybean research: A long-term goal of this research is to develop soybean varieties or germplasm with a high level of resistance to soybean white mold (SWM). Marker-assisted

selections (MAS) were carried out for screening F2:3 lines, which were developed from 8 cross combinations between new resistance sources and elite cultivars. New advanced breeding lines (132) were evaluated for SWM in a naturally infected field. Among those lines, 16 lines showed high level of resistance with disease severity index (DSI) less than 10. For germplasm release, line E12076T with enhanced resistance to SWM was increased by a seed company to enable release of seeds for commercial production. The reliability of a genomic selection (GS) model was maximized by optimizing statistical methods and the choice of reference individuals in a training set. Eighty advanced breeding lines were evaluated for white mold resistance in a disease nursery and genotyped with SoySNP6K BeadChip.

Efforts were carried out to target essential genes in S. sclerotiorum to achieve sclerotinia stem rot (SSR) resistance in soybean. Host Induced Gene Silencing (HIGS) was used to target an important virulence factor, oxalic acid (OA), to debilitate S. sclerotiorum growth and pathogenic development. The RNA silencing machinery of the soybean plant was recruited to target an essential fungal gene for OA biosynthesis, Ssoah1 (oxaloacetate acetyl hydrolase). Targeting this gene in S. sclerotiorum leads to limited lesions on soybean plants, suggesting that HIGS can be a viable strategy to control SSR.

Chickpea, lentil, and pea research: Recently acquired germplasm accessions for chickpea, lentil and pea are being screened for new resistance sources to Sclerotinia white mold. In FY19, recently acquired pea germplasm accessions from China were screened for resistance to white mold in the greenhouse, with plant reactions to Sclerotinia inoculation monitored by measuring the lesion length. Ten accessions showed disease severity lower than the tolerant cultivar DPS used as a check. The most tolerant lines were W6 44561 and W6 44593 originally from Inner Mongolia and the Qinghai provinces of China, respectively.

Sunflower research: A small graphical user interface (GUI) software package that integrates the best genomic selection models for sunflower is being developed. Many of the R packages typically used for GS are not flexible enough to deal with a hybrid crop where genotypic effects are subdivided into “female” and “male” line effects (GCA and SCA) for single cross hybrid data; However, we found new packages, including ‘BGLR’ and ‘sommer’ that are more flexible in the number of random effects that can be handled. An updated (v.2) GWAS model to infer candidate genes and develop hypotheses on necrotrophic disease resistance architecture was used to find 7 major stalk rot resistance loci, 4 major head rot loci, 7 major Phomopsis stalk canker resistance loci (another important necrotrophic disease) and a sizeable number of low to moderate effect loci.

QTL mapping of Sclerotinia basal stalk rot (BSR) resistance derived from sunflower wild species was continued with three advanced backcross (AB) populations. Field screening trials were conducted at Carrington, North Dakota, using randomized incomplete block design with three replications. Highly significant ($p<0.001$) genetic variations were observed for BSR disease incidence in all three populations in both individual and combined analysis. A genetic linkage map was developed to identify QTL on twelve linkage groups (LGs) associated with BSR resistance for the three data sets.

Canola research: To develop effective and durable disease resistance for Sclerotinia stem rot in canola, overexpression of MED16, a subunit of the Mediator protein complex that functions in transcriptional regulation of defense genes, is being used. To determine the molecular basis of OsMED16 resistance, an assay was developed to determine proteolytic sensitivity of MED16 orthologs, because MED16 is shown to be degraded during infection. The MED16 signaling cascade was also being engineered to test for a synergetic increase in resistance. Previous research had identified AtWRKY33 as an interactor of MED16 and thus efforts are underway to identify a WRKY33 ortholog from rice that could be used in combination with the resistance-conferring MED16, also from rice.

A mapping population derived from the cross of canola cultivars NEP63 (resistant) x Topas was developed using doubled haploid technique. This population will be evaluated in 2019. A second population derived from NEP32 x Topas is being produced and we expect to have it ready for evaluation late in 2019. These populations will be used for further validating Cleaved Amplified Polymorphic Sequence (CAPS) markers for resistance to Sclerotinia.

Sclerotinia pathogen biology research: A previously identified S. sclerotiorum effector protein (SsE1) that interacts with the plant defense protein polygalacturonase-inhibiting protein (PGIP) was studied to verify the specific interaction and to demonstrate its function. SsE1 has the same expression pattern as S. sclerotiorum polygalacturonase 1 (SsPG1) and the importance of SsPG1 in virulence was shown through deletion analyses. This research provides the first example that S. sclerotiorum secretes a small effector protein (SsE1) that specifically interacts with and mitigates the inhibitory effects of plant PGIPs.

Disease management research: Studies with mutants of S. sclerotiorum genes encoding disrupted homologues of Dicer-like (DCL), Argonaute (AGO) and RNA-dependent RNA polymerase (RdRp) proteins were conducted to assess alterations in growth, sclerotial development, pathogenicity on plants and sensitivity to infection by mycoviruses. Wild-type and mutant strains were transfected with a single-stranded RNA virus, SsHV2-L, and copies of a single-stranded DNA mycovirus, SsHADV-1, as a synthetic virus constructed in this study. Disruption of dcl-1 or dcl-2 resulted in no changes in phenotype compared to wild-type S. sclerotiorum; however, the double dicer mutant strain exhibited significantly slower growth. Furthermore, the delta dcl-1/dcl-2 double mutant, which was slow growing without virus infection, exhibited much more severe debilitation following virus infections. Deletion of ago-2 gene but not ago-4 resulted in compromised growth and virulence prior to virus infection suggesting the contributions made by ago-2 to physiological and developmental processes.

The feasibility of using the hypovirulent strain DT-8 of S. sclerotiorum as an option in managing Sclerotinia white mold is being tested. Several experiments were carried out by spraying the DT-8 mycelium homogenates onto detached leaves before inoculating with a virulent strain, anticipating that the virus particles will enter the virulent strain and render it less virulent. Some trials showed promise, but difficulties with virus titer lead to inconsistencies in the

results. New procedures will be developed to improve our culturing of the DT-8 mycovirus before further plant tests are executed.

Accomplishments:
1. Soybean varieties developed with resistance to white mold disease. Soybean white mold (SWM) is caused by Sclerotinia sclerotiorum, a fungus capable of infecting a wide range of plants. Resistance mechanisms within a crop plant, like soybean, can play a key role in effective management of SWM. Modern breeding technologies, including the use of molecular markers, have been used to incorporate white mold resistance traits with other agronomic and seed quality traits. Soybean line E14077 is in progress to be released as a new variety in 2019 and line E13268 is under development for future release, both having good resistance to SWM. Another newly developed soybean line, E12076T, exhibiting enhanced resistance to SWM was released to a seed company for commercial production in 2019, such that seeds would be available to farmers in 2020.

303 3 A 2017

Publication(s):
Westrick, N.M., Ranjan, A., Jain, S., Grau, C.R., Smith, D.L., Kabbage, M. 2019. Gene regulation of Sclerotinia sclerotiorum during infection of Glycine max: on the road to pathogenesis. BMC Genomics. 20:157. https://doi.org/10.1186/s12864-019-5517-4.

Mochama, P., Jadhav, P., Neupane, A., Marzano, S.-Y.L. 2018. Mycoviruses as triggers and targets of RNA silencing in white mold fungus Sclerotinia sclerotiorum. Viruses. 10:214. https://doi.org/10.3390/v10040214.

Kandel, R., Chen, C.Y., Grau, C.R., Dorrance, A.E., Liu, J.Q., Wang, Y., Wang, D. 2018. Soybean resistance to white mold: evaluation of soybean germplasm under different conditions and validation of QTL. Frontiers in Plant Science. 9:505. https://doi.org/10.3389/fpls.2018.00505.

Wang, C., Zhang, X., Li, J., Zhang, Y., Mou, Z. 2016. The Elongator complex-associated protein DRL1 plays a positive role in immune responses against necrotrophic fungal pathogens in Arabidopsis. Molecular Plant Pathology. 19(2):286-299. https://doi.org/10.1111/mpp.12516.

Liang, X., Rollins, J.A. 2018. Mechanisms of broad host range necrotrophic pathogenesis in Sclerontinia sclerotiorum. Phytopathology. 108:1128-1140. https://doi.org/10.1094/PHYTO-06-18-0197-RVW.

Neupane, A., Feng, C., Feng, J., Kafle, A., Bucking, H., Marzano, S.-Y.L. 2018. Metatranscriptomic analysis and in silico approach identified mycoviruses in the arbuscular mycorrhizal fungus Rhizophagus spp. Viruses. 10:707. https://doi.org/10.3390/v10120707.

Wen, Z., Tan, R., Zhang, S., Collins, P.J., Yuan, J., Du, W., Gu, C., Ou, S., Song, Q., An, Y., Boyse, J.F., Chilvers, M.I., Wang, D. 2018. Integrating GWAS and gene expression data for functional

characterization of resistance to white mold in soya bean. Plant Biotechnology. https://doi.org/10.1111/pbi.12918.

Liu, Q., Chang, S., Hartman, G.L., Domier, L.L. 2018. Assembly and annotation of a draft genome sequence for Glycine latifolia, a perennial wild relative of soybean. Plant Journal. 91:71-85.

Marzono, S.L., Neupane, A., Domier, L.L. 2018. Transcriptional and small RNA responses of the white mold fungus Sclerotinia sclerotiorum to infection by a virulence-attenuating hypovirus. Viruses. 10:713. https://doi.org/10.3390/v10120713.

Talukder, Z.I., Long, Y., Seiler, G.J., Underwood, W., Qi, L. 2019. Introgression and monitoring of wild Helianthus praecox alien segments associated with Sclerotinia basal stalk rot resistance in sunflower using genotyping-by sequencing. PLoS One. 14(3):e0213065. https://doi.org/10.1371/journal.pone.0213065.

Talukder, Z.I., Long, Y.M., Seiler, G.J., Underwood, W., Qi, L.L. 2019. Registration of oilseed sunflower germplasms HA-BSR6, HA-BSR7, and HA-BSR8 highly resistant to sclerotinia basal stalk rot and downy mildew. Journal of Plant Registrations. https://doi.org/10.3198/jpr2018.10.0071crg.

Money, K.L., Koehler, B.D., Misar, C.G., Grove, M., Underwood, W., Hulke, B.S. 2019. Registration of oilseed sunflower germplasms RHA 485, RHA 486, and HA 487, selected for resistance to Phomopsis stalk canker and Sclerotinia, in a high yielding and high-oil background. Journal of Plant Registrations. https://doi.org/10.3198/jpr2019.02.0008crg.

Qi, L.L., Talukder, Z.I., Long, Y.M., Seiler, G.J. 2018. Registration of oilseed sunflower germplasms HA-BSR2, HA-BSR3, HA-BSR4, and HA-BSR5 with resistance to sclerotinia basal stalk rot and downy mildew. Journal of Plant Registrations. https://doi.org/10.3198/jpr2017.11.0083crg.

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
John McMurtry
Barry Coleman
Greg Varner
William P. Kemp
John Sandbakken
Tim McGreevy
Kelly Whiting
Todd Scholz
Rich Wilson

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

2020 National Sclerotinia Initiative Meeting Participants								
Last Name	First Name	Company	Address	City	State	Zip	Phone	Email
Anderson	James	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	701-239-1263	james.anderson@ars.usda.gov
Bales	Scott	Michigan State University	9923 Kruger Rd	Frankenmuth	MI	48734	810-304-3698	balessco@msu.edu
Bateson	Zachary	National Agricultural Genotyping Center	1616 Albrecht Boulevard N.	Fargo	ND	58102	701-239-1452	Zack.bateson@genotypingcenter.com
Chen	Weidong	USDA-ARS	303 Johnson Hall, WSU	Pullman	WA	99164	509-335-9178	weidong.chen@ars.usda.gov
Chittem	Kishore	North Dakota State University	209 Walster Hall	Fargo	ND	58108	701-429-8381	kishore.chittem@ndsu.edu
Coleman	Barry	Northern Canola Growers Association	125 Slate Drive, Ste. #4	Bismarck	ND	58503	701-319-3130	coleman@ndpci.com
del Rio Mendoza	Luis	North Dakota State University	324 Walster Hall	Fargo	ND	58108	701-231-7073	luis.delriomendoza@ndsu.edu
Everhart	Sydney	University of Nebraska	406 Plant Science Hall	Lincoln	NE	68587-0722	402-472-2879	everhart@unl.edu
Fransen	Lindsey	National Agricultural Genotyping Center	1616 Albrecht Boulevard N.	Fargo	ND	58102	701-239-1478	Lindsey.fransen@genotypingcenter.com
Grusak	Mike	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	701-239-1371	mike.grusak@ars.usda.gov
Hogness	Angelia	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102	701-239-1347	angelia.hogness@usda.gov
Hulke	Brent	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	701-239-1321	brent.hulke@ars.usda.gov
Jones	Jennifer	Smith Bucklin/United Soybean Board	16305 Swingley Ridge Road #210	St. Louis	MO	63122	314-775-3154	niferj14@yahoo.com
Kabbage	Mehdi	University of Wisconsin-Madison	1630 Linden Drive	Madison	WI	53706	979-587-1148	kabbage@wisc.edu
Kane	Nolan	University of Colorado	3851 Orion Court	Boulder	CO	80304	206-200-0859	nolan.kane@colorado.edu
Kaphammer	Bryan	USDA-ARS	2150 Centre Ave., Bldg D, Suite 300	Fort Collins	CO	80526	970-492-7054	bryan.kaphammer@usda.gov
Marzano	Shin-Yi Lee	South Dakota State University	1309 College Ave	Brookings	SD	57007	605-688-5469	shinyi.marzano@sdstate.edu
McClean	Phillip	North Dakota State University	Loftsgard Hall	Fargo	ND	58108-6050	701-231-8443	phillip.mcclean@ndsu.edu
Miklas	Phillip	USDA-ARS	24106 N. Bunn Road	Prosser	WA	99350	509-786-8492	phil.miklas@ars.usda.gov
Miorini	Thomas	North Dakota State University	CREC - 663 Hwy 281 N.	Carrington	ND	58421-0219	402-314-9470	thomas.miorini@ndsu.edu
Misar	Chris	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	605-661-2530	christopher.misar@ars.usda.gov
O'Neil	Megan	National Agricultural Genotyping Center	1616 Albrecht Boulevard N.	Fargo	ND	58102	701-239-1449	megan.oneil@genotypingcenter.com
Osorno	Juan	North Dakota State University	Loftsgard Hall	Fargo	ND	58108-6050	701-235-5841	juan.osorno@ndsu.edu
Piche	Lisa	National Agricultural Genotyping Center	1616 Albrecht Boulevard N.	Fargo	ND	58102	701-239-1460	lisa.piche@genotypingcenter.com
Qi	Lili	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	701-239-1351	lili.qi@usda.gov
Quandt	C. Alisha	University of Colorado	1900 Pleasant St.	Boulder	CO	80309	303-735-6550	alisha.quandt@colorado.edu
Rahman	Mukhlesur	North Dakota State University	470G Loftsgard Hall	Fargo	ND	58108-6050	701-231-5768	md.m.rahman@ndsu.edu
Rollins	Jeffrey	University of Florida	1450 Fifield Hall	Gainesville	FL	32611	352-273-4620	rollinsj@ufl.edu
Roy	Jayanta	North Dakota State University	460C Loftsgard Hall	Fargo	ND	58108	701-793-0224	jayanta.roy@ndsu.edu
Sandbakken	John	National Sunflower Association	2401 46th Avenue SE	Mandan	ND	58554	701-328-5100	johns@sunflowernsa.com
Scholz	Todd	American Pulse Assoc./USA Dry Pea & Lentil	2780 W Pullman Road	Moscow	ID	83843	208-596-5025	tscholz@usapulses.org
Scott	Roy	USDA-ARS	5601 Sunnyside Ave Rm 4-2216	Beltsville	MD	20705	301-504-4670	roy.scott@usda.gov
Seiler	Gerald	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	701-239-1380	gerald.seiler@usda.gov
Shahoveisi	Fereshteh	North Dakota State University	318A Walster Hall	Fargo	ND	58108-6050	224-856-0353	fereshteh.shahoveisi@ndus.edu
Shamimuzzaman	MD	USDA-ARS	1307 18th St. N.	Fargo	ND	58102	701-239-1324	md.shamimuzzaman@usda.gov
Sharma Poudel	Roshan	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102	701-630-1718	roshan.sharmapoudel@ndus.edu
Souza Lopes	Fernanda	Washington State University	24106 N. Bunn Rd	Prosser	WA	99350	509-212-4670	fernanda.souzalopes@wsu.edu
Swanson	Kim	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	701-239-1370	kimberly.swanson@ars.usda.gov
Talukder	Zahirul	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	701-239-1324	zahirul.talukder@usda.gov
Underwood	William	USDA-ARS	1616 Albrecht Boulevard N.	Fargo	ND	58102-2765	701-239-1316	william.underwood@usda.gov
Varner	Greg	Michigan Dry Bean Research Bd.	8439 North Blair Road	Breckenridge	MI	48615-9725	989-751-8415	varnerbean@hotmail.com
Wang	Dechun	Michigan State University	1066 Bogue St., Rm. A384E	East Lansing	MI	48824-1325	517-614-2939	wangdech@msu.edu
Wei	Wei	Washington State University	345 Johnson Hall	Pullman	WA	99164	509-335-4008	weiwei2@wsu.edu
Wen	Zixiang	Michigan State University	1066 Bogue St., Rm A366	East Lansing	MI	48824-1325	517-575-5029	wzxsoy@msu.edu