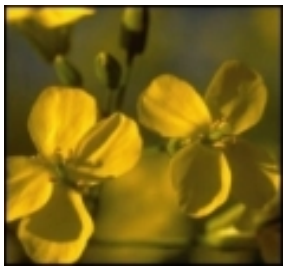
National Sclerotinia Initiative

USDA-ARS

National Sclerotinia Initiative 2023 Annual Meeting January 18-19, 2023






USA Dry Pea & Lentil Council

National Sunflower Association

US Dry Bean Council

Northern Canola Growers Association

United Soybean Board

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Fargo, ND & East Grand Forks, MN

Helping farmers produce a safe, nutritious and sustainable food supply

2023 National Sclerotinia Initiative Meeting

January 18 - 19, 2023

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2023 National Sclerotinia Initiative Annual Meeting
(ALL TIMES CENTRAL TIME)

January 18, 2023

7:00 – 8:30 am Breakfast (City A)

8:30 am Welcome & Introductions **– Mike Grusak, USDA-ARS, Fargo, ND**

8:45 am Welcome & Update from Plains Area **–Joe Rich, USDA-ARS, Fort Collins, CO**

8:55 am Welcome & Update from Office of National Programs **– Roy Scott, USDA-ARS, Beltsville, MD**

Sclerotinia Research Progress – Biocontrol/Disease Management

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

9:05 am Exploring RNA-based management strategies to confer plant resistance to white mold infection **– Gayathri Beligala, Shin-Yi Marzano, USDA-ARS, Toledo, OH**

9:25 am Small cationic cysteine-rich peptide with potent antifungal activity controls white mold in soybean **– Arnaud Thierry Djami-Tchatchou, Meenakshi Tetorya, Dilip M. Shah, Donald Danforth Plant Science Center, St. Louis, MO**

9:45 am Induction of resistance to Sclerotinia Stem Rot (SSR) in canola plants by application of exogenous RNA **– Abdolbaset Azizi, Luis del Rio Mendoza, North Dakota State University, Fargo, ND**

Sclerotinia Research Progress – Breeding

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

10:05 am Developing soybean varieties with resistance to Sclerotinia Stem Rot **– Feng Lin, Raju Thada Magar, Drew Mitchell, Randy Laurenz, Martin Chilvers, and Dechun Wang, Michigan State University, East Lansing, MI**

10:25 – 11 am Break (City A)

11:00 am Screening and introgression of Sclerotinia Stem Rot (SSR) disease resistant gene(s) into elite cultivar of canola **– Md Zahangir Alam, Luis DelRio Mendoza, Mukhlesur Rahman, North Dakota State University, Fargo, ND**

11:20 pm Detection of quantitative trait loci associated with reaction of a doubled haploid *Brassica napus* mapping population to Sclerotinia Stem Rot **– Bita Babakhani, Susan Ruud, Dua'a Al Salman, Dante Marino, Luis del Rio Mendoza, North Dakota State University, Fargo, ND**

11:40 am Improved white mold resistance in dry and snap beans through multi-site screening throughout major production areas **– Rebecca Higgins, Evan Wright, Leonardo Volpato, and Francisco E. Gomez, Michigan State University, East Lansing, MI**

12:00 noon Lunch Break (City A)

Sclerotinia Research Progress – Breeding (continued)

Moderator ***Dechun Wang, Michigan State University***

1:30 pm Evaluation and optimization of genomic selection for durable white mold resistance in dry bean **– Molly Irvin, Qijian Song, and Francisco E. Gomez, Michigan State University, East Lansing, MI**

1:50 pm A QTL approach toward understanding and improving genetic resistance to white mold in common bean **– Phil Miklas, Jim Myers, Ahmet Agir, Jayanta Roy, Phil McClean, Jose C. Figueroa-Cerna, Juan M. Osorno, USDA-ARS Prosser, WA; Oregon State University, Corvallis, OR; North Dakota State University, Fargo, ND**

2:50 – 3:30 pm Break (City A)

3:30 – 5:00 pm Poster Session (City A)

5:00 – 6:00 pm Free Time

6:00 – 7:30 pm Group Dinner (City A)

January 19, 2023

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

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

Sclerotinia Research Progress – Pathogen Biology

Moderator ***Francisco Gomez, Michigan State University***

8:30 am Characterization of a methionine biosynthesis gene (SsMet1) in *Sclerotinia sclerotiorum* **– Nickisha Pierre-Pierre, Wei Wei, George Vandemark, Weidong Chen, USDA-ARS, Pullman, WA**

8:50 am Root endophytic colonization of *Sclerotinia sclerotiorum* disarmed by a mycovirus infection induced disease resistance and photosynthesis activity **– Connor Pedersen, Gayathri Beligala, Shin-Yi Marzano, USDA-ARS, Toledo, OH**

9:10 am Fungicide sensitivity and genetic characterization of *Sclerotinia sclerotiorum* from USA soybean and dry bean, compared to different regions and climates using AmpSeq **– Edgar Nieto-Lopez, Rachel A. Koch Bach, Srikanth Kodati and Sydney E. Everhart, University of Connecticut, Storrs, CT**

9:30 am Evaluating *Sclerotinia sclerotiorum* detoxification genes as drivers of pathogenicity and as potential targets for disease control **– Mehdi Kabbage, Nathaniel Westrick, Dandan Shao, Damon Smith, University of Wisconsin, Madison, WI**

9:50 am Break (City A)

Sclerotinia Research Progress – Host Resistance

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

10:30 am Characterization of oxalic acid tolerance in sunflower resistance to Sclerotinia basal stalk rot **– William Underwood, USDA-ARS, Fargo, ND**

10:50 am Two newly described polygalacturonase-inhibiting proteins (PGIPs) in chickpea inhibit *Sclerotinia sclerotiorum* polygalacturonases (SsPG1 and SsPG5) **– Vishnutej Ellur, Wei Wei, Rishikesh Ghogare, Shyam Solanki, George Vandemark, Robert Brueggeman, Weidong Chen, USDA-ARS, Pullman, WA; University of Washington, Pullman, WA**

11:10 am Enhancing the resistance to Sclerotinia by co-expressing the AAE3 and OCD1 genes **– Chenggang Wang, Zhonglin Mou, Jeffrey A. Rollins, University of Florida, Gainesville, FL**

11:30 am General Discussion – **Mike Grusak, USDA-ARS, Fargo, ND**

12:00 noon Lunch Break (City A)

Sclerotinia Research Progress – Host Resistance (continued)

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

1:30 pm Exploring mechanisms of effector-triggered susceptibility in the soybean-Sclerotinia pathosystem **– Mitchell G. Roth, Tiffanna J. Ross, The Ohio State University, Wooster, OH**

1:50 pm Identification and characterization of soybean phenylalanine ammonia-lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) homologs genes' role in white mold disease control **– Nick Talmo, Ji Hyun Kim, Robert Stupar, Ashish Ranjan, University of Minnesota, St. Paul, MN**

2:10 pm A *Sclerotinia sclerotiorum* effector protein interacts with plant fatty acid hydroxylases (FAHs) that mediate cell death control **– Wei Wei, Liangsheng Xu, George Vandemark, Weidong Chen, USDA-ARS, Pullman, WA**

2:30 pm Break (City A)

3:00 pm General Discussion/Wrap Up – **Mike Grusak, USDA-ARS, Fargo, ND**

4:00 pm Departure

Oral Abstracts

Exploring RNA-based management strategies to confer plant resistance to white mold infection

Gayathri Beligala and Shin-Yi Marzano

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

ABSTRACT:

Growers lack effective genetic tools to manage losses caused by *Sclerotinia sclerotiorum* because no complete disease resistance exists in the crop germplasms. This necessitates the identification of alternative sources of resistance for the disease. Previously, we have identified strong candidate genes in the *Sclerotinia sclerotiorum* RNA silencing pathway as the targets for the development of an RNAi-based pesticide for spray-induced gene silencing (SIGS). This year, we compared dsRNA segments generated from different regions of the Argonaute 2 (Ago2) transcript and discovered that the dsRNA segment "fragment 5", named as FF#5, is most effective in suppressing white mold infection, achieving a near complete suppression. The FF#5 segment targets the PIWI/RNaseH domain of Ago2 functioning in the dsRNA guided hydrolysis of ssRNA which makes Argonaute a slicer that cleaves mRNA in a RISC complex. We have cloned all the segments for RNA expression in *Escherichia coli* HT115 and are in the process of optimizing crude preparations of *E. coli* culture for pathogenicity assays. Next, we will investigate whether differences in the secondary structure of these corresponding ssRNA segments also explain for the accessibility of dsRNA binding to ssRNA by SHAPE analysis. Development of RNAi-based pesticide for SIGS will allow for biologically friendly and pathogen-specific control of white mold disease.

Contact information - **Shin-Yi Marzano**, University of Toledo, 3050 W Towerview Blvd rm 4271, Toledo, OH 43606; (330) 621-2678; shinyi.marzano@usda.gov

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

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

Donald Danforth Plant Science Center, St Louis, MO 63132

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

ABSTRACT:

White mold (WM) caused by a necrotrophic pathogen *Sclerotinia sclerotiorum (Ssc)* results in serious economic losses of soybean yield in the US. For lack of effective genetic resistance to this disease in soybean germplasm and rapid evolution of pathogen resistance to fungicides, SSR is difficult to manage. We are exploring the potential of small cysteine-rich *Ssc*-inhibitory peptides for development as multi-target environmentally-friendly biofungicides. PD30.6 is a small cationic cysteine-rich antifungal peptide derived from a plant defensin MtDef4 of *Medicago truncatula*. Antifungal activity of PD30.6 was tested against an aggressive strain of *Ssc* (*Ssc* 555) using *in vitro*, semi-*in planta* and *in planta* antifungal assays. PD30.6 exhibited potent fungicidal activity against *Ssc 555* in *vitro* with the EC_{50} value of 14 µM and the minimum inhibitory concentration (MIC) of 24 µM. We found that external application of PD30.6 to detached Williams82 soybean leaves, stems and pods significantly decreased lesion sizes with complete inhibition of growth or decay at concentrations between 24 and 96 µM. In preliminary experiments, spray application of PD30.6 on leaves of a soybean plant, we observed much reduced lesion sizes at inoculation points with complete inhibition at 48 µM. We also observed that PD30.6 reduces the sclerotia production by *Ssc* 555. In contrast, *Ssc* 555 mycelium challenged with a PD30.6 variant having markedly reduced antifungal activity *in vitro* enhances the sclerotia production as compared with *Ssc* 555 mycelium sprayed with water alone. We assessed the dual treatment of PD30.6 and tebuconazole fungicide at concentrations below their MIC values against *Ssc in vitro* and *in planta.* Our results showed a synergistic increase in antifungal activity against *Ssc* 555. The dual treatment of PD30.6 and tebuconazole led to complete inhibition of *fungal* growth *in vitro* and lesion development on soybean leaves, stems and pods . In addition, synergy between PD30.6 and tebuconazole reduced the amount of fungicide required almost 10-fold to achieve complete prevention of disease symptoms. Our results demonstrate that a small antifungal peptide has significant potential for development as a biofungicide and for reducing the use of chemical fungicides to manage WM in soybean.

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

Induction of resistance to Sclerotinia stem rot (SSR) in canola plants by application of exogenous RNA

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

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

ABSTRACT:

Sclerotinia sclerotiorum is the causal agent of Sclerotinia stem rot (SSR) of canola. Every percentage unit of incidence of this disease reduces canola yields by approximately 13 kg/ha, making it one of the most important production problems for this crop in North Dakota. Currently, SSR disease is managed mainly using fungicides and crop rotations; however, concerns about the side effect of their chemical residues on biodiversity and human health, have warranted the search for safer alternatives. One such alternative is the silencing of RNA. Work supported by the National Sclerotinia Initiative has identified five *S. sclerotiorum* genes, Cytochrome P450 (P450), Chitin binding domain (CBD), Glycosyltransferase, SsBGT1 (GSF), Serine/Threonine kinase (STK), and Oxaloacetate acetylhydrolase (OA), that are highly upregulated during infection. In this project, invitro hairpin RNA (hpRNA) for from these genes were prepared using MEGAscript™ T7 Transcription Kit. The effect of these hpRNAs on the expression of target genes was investigated by adding them (100 ng/µl) to a fungal liquid culture. Confocal microscopy showed RNA uptake by mycelia for all hpRNAs. Analysis of gene expression showed 7, 4, 4, and 1/2-fold suppression for CBD, STK, OA, and P450 genes respectively, while no suppression for GSF. Bioassay using detached leaf assay and spreading application of hpRNAs (100 ng/µl) on the stem of canola plants for all genes were studied. Fungal inoculation of samples was conducted 24 h after RNA application. Leaf bioassay results showed that all genes reduced lesion sizes with different degrees. A significant ($P = 0.05$) reduction in lesion size and plant mortality was observed when hpRNAs were applied to canola stems. Investigation about the effect of the combination of different hpRNAs for all genes and finding the best combination is ongoing. The research will also continue to develop RNA nanoparticles to increase the hpRNAs steady on the plant surfaces.

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

Developing Soybean Varieties with Resistance to Sclerotinia Stem Rot

Feng Lin, Raju Thada Magar, Drew Mitchell, Randy Laurenz,
Martin Chilvers, and Dechun Wang

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

ABSTRACT:

Sclerotinia stem rot (white mold), caused by *Sclerotinia sclerotiorum*, is one of the most destructive soybean diseases in the U.S. From 2010 to 2014, white mold has caused a total yield loss of more than 100 million bushels of soybean across 28 states of the U.S. and Ontario, Canada. To improve soybean resistance to white mold, the Michigan State University soybean breeding program has established a steady breeding pipeline for selecting and releasing elite soybean varieties with high resistance to white mold. In 2022, a total of 192 lines, including 64 elite lines and 128 advanced breeding lines, were evaluated in our white mold disease nursery at Montcalm, Michigan. The disease severity index (DSI) ranged from 0 to 100, with an average DSI of 72.3. Correlation analysis identified a significant positive correlation (0.177, $p < 0.01$) between plant density and DSI. In 2022, a variety E17069 with resistance to white mold was released for commercial production.

Contact Information – Dr. Dechun Wang, Dept. of Plant, Soil and Microbial Sciences, Michigan State University, 1066 Bogue St., East Lansing, MI 48824; 517-353-0219, wangdech@msu.edu

Screening and Introgression of Sclerotinia Stem Rot (SSR) Disease Resistant Gene(s) into Elite Cultivar of Canola

Md Zahangir Alam[1], Luis DelRio Mendoza[2], and Mukhlesur Rahman[1*]

[1]Department of Plant Sciences, NDSU, Fargo, ND 58102
[2]Department of Plant Pathology, NDSU, Fargo, ND 58102

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

ABSTRACT:

The SSR disease caused by the notorious fungus, *Sclerotinia sclerotiorum,* is the most important disease in canola. The existing management practices e.g. crop rotation with non-host crops and application of chemical pesticides are not effective, economic, and eco-friendly. However, development of disease-resistant cultivar has the potential to address these issues. To introgress the resistant gene(s) into the elite breeding line, the 4-parents (3 resistant and one elite) Multi-parent Advanced Generation Inter-Cross (MAGIC) population was crossed with the elite cultivar, NDOLA-2 to get first backcross (BC1) population. We have screened 850 BC1 seedlings with the Petiole Inoculation Technique (PIT). A total of 30 BC1 seedlings were survived and screened again with the Stem Inoculation Technique (SIT) at the flowering stage. After screening with SIT, 3 BC1 plants survived and were backcrossed to NDOLA-2 to get BC2 generation. A total of 600 BC2 seedlings were screened again with PIT and 20 BC2 plants survived. These 20 plants were screened with the SIT and 2 plants survived which were backcrossed to NDOLA-2 to get BC3 generation. This screening and backcrossing activities will be continued and the best resistant lines at BC6 will be evaluated in the field to identify and release the elite lines with improved SSR resistance. In addition, 520 F3 seedlings of 8-parents (five elite breeding lines/cultivars and three resistant accessions) MAGIC population are being advanced to the F4 population with the single seed descent (SSD) method to generate and evaluate recombinant inbred lines (RILs) in the field in order to identify and release the elite lines with improved SSR resistance. Finally, to facilitate the rapid screening and marker-assisted selection, 23 sets of primers have already been designed and nuclear DNA has been extracted from the resistant and susceptible cultivars of canola for the detection of single nucleotide polymorphism (SNP) marker associated with SSR resistance by the Kompetitive Allele Specific PCR (KASP) marker technology.

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Detection of quantitative trait loci associated with reaction of a doubled haploid *Brassica napus* mapping population to Sclerotinia stem rot

Bita Babakhani, Susan Ruud, Dua'a Al Salman, Dante Marino, and Luis del Río Mendoza, Dept. of Plant Pathology, North Dakota State University. Fargo, ND

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

Abstract:

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is an economically important disease that is endemic to canola (*Brassica napus*) production areas in North Dakota. *Brassica napus* plant introduction 649136 was identified as resistant to *S. sclerotiorum* in a project previously funded by the National Sclerotinia Initiative. The identification of molecular markers associated with reaction of this PI to infection by *S. sclerotiorum* will facilitate the transfer of its resistance into modern canola cultivars. The goals of this study are to develop a doubled haploid mapping population and to identify quantitative trait loci associated with resistant reaction to this disease. To accomplish these goals, DH line NEP32, derived from this PI was crossed with *S. sclerotiorum*-susceptible cv. Topas. Microspores produced by the F_1 of this cross were cultured in NLN medium and treated with colchicine to double their chromosome count. Treated plantlets were transferred to the greenhouse for seed production. After harvest, seeds from 296 DH lines, both parents, and breeding line NDC12121 were planted in the greenhouse as a mapping population in the spring of 2022. Each entry had four plants per replication and three replications. Plants were inoculated at flowering time using the agar plug method. Briefly, the hyphal side of an agar plug containing hyphal tips of a 48-h old *S. sclerotiorum* culture was placed in contact with the stem approximately 20-30 cm above the pot and carefully wrapped with parafilm. Reaction to inoculation was assessed by measuring lesion length one week after inoculation. The average plant mortality and lesion size were 58% and 48 mm, respectively. Lines with plant mortality < 40% and average lesion size < 25 mm were considered resistant. At the time of inoculation, plant tissues from each line were collected for DNA extraction using the MagMax Plant DNA isolation kit. Paired-end sequencing will be performed once the samples have been purified. Phenotyping of these lines will be repeated in the winter of 2023.

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

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

R. Higgins[1], E. Wright[2], L. Volpato[2], and F. E. Gomez[2].

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[2]Department of Plant, Soil and Microbial Sciences, Michigan State University

Collaborators: M. Wunsch (ND), J. Myers (OR), P. Miklas (WA), C. Urrea (NE), V. Hoyos-Villegas (CAN)

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

ABSTRACT:

Our research goal is to identify putative sources of resistance of dry bean lines to white mold in adapted backgrounds at multiple sites located in major bean-production areas of the United States. We employ two types of evaluations, greenhouse-based straw test and field trials carried out within white mold nurseries in several locations throughout the northern U.S. and Canada. In 2022, trials were conducted in NE, MI, OR, ND, WA and CAN with a total of 18 lines that included Black, Pink, Navy, Great Northern, Pinto, Red, Small Red, and Dark Red Kidney bean types. Our preliminary analysis of greenhouse and field data show moderate levels of resistance. Greenhouse data from one trial showed lines performed better or equal to the known resistant line (G122). Further greenhouse and field data as well as a retrospective G x E analysis are currently underway, which will provide greater insight into the potential cultivars with both genetic and physiological resistance to white mold and evaluate and understand the rate of genetic progress in white mold resistance.

Contact Information - Dr. Francisco Gomez, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, 48824; gomezfr1@msu.ed

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

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

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

ABSTRACT:

Dry bean (*Phaseolus vulgaris* L.) production in the U.S., and specifically in Michigan the second largest dry bean producer in the U.S., suffer severely from white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary) infection. In 2014, yield losses exceeded 20% in Michigan and overall economic losses can approach ~$5M annually in terms of lost production and fungicide costs. Dry bean cultivars lack high levels of resistance and progress to breed new cultivars with durable levels of resistance to white mold has been slow due to the quantitative inheritance of this trait and screening dependence on the presence of the pathogen under suitable environmental conditions. Marker-assisted selection (MAS) approaches would be useful to breed for white mold resistance, however, given the multigenic nature of white mold hinders the use of MAS efforts that aim of pyramid only a few target genes. An alternative is genomic prediction and selection, which utilizes genome-wide marker coverage to predict genotypic values for quantitative traits. Thus, the overall goal of this project is to evaluate and optimize genomic prediction in a dry bean breeding program. A total of 320 lines have been evaluated from 2021-2022 including previously evaluated lines from the multi-state project. All lines were genotyped using the BARCBean12K BeadChip. Preliminary results for the first year identified population structure among the breeding lines and a moderate genomic prediction accuracy for white mold given the limited training population size. Analyses are underway to evaluate all 320 lines using different cross validation approaches and genomic prediction models including known QTL for white mold resistance.

Contact Information - Dr. Francisco Gomez, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, 48824; gomezfr1@msu.ed

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

Phil Miklas[1], Jim Myers[2], Ahmet Agir[2], Jayanta Roy[3], Phil McClean[3], Jose C. Figueroa-Cerna[3], and Juan M. Osorno[3]

[1]USDA-ARS, Prosser, WA; [2]Department of Horticulture, Oregon State University, Corvallis, OR; [3]Department of Plant Sciences, North Dakota State University, Fargo, ND

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

ABSTRACT: Multiple genetic and breeding populations were generated, phenotyped and genotyped in support of detecting new and existing QTL conditioning improved resistance to white mold in snap and pinto beans with acceptable agronomic traits. A four-population snap bean NAM population of 247 lines was phenotyped in field trials for agronomic traits and disease reaction. Twenty-two QTL on 9 chromosomes were revealed in the field disease screens. The data from disease screens and agronomic trials is undergoing final analysis, synthesis and write up as part of a M.S. thesis. A new snap-dry bean MAGIC population initiated with 685 first generation plants from 8-way crosses is undergoing single seed descent and is currently in the F4 generation. Some attrition caused by dwarf lethal incompatibility has been observed, reducing population size to 622 lines. Remnant seed of the F2 population was screened using the seedling straw test with 284 genotypes (46%) exhibiting partial resistance, with 3% of genotypes with disease severity scores of 1.5 or less (compared to 2.6 and 2.9 for NY6020-4 and G122, respectively). The most resistant genotypes from this test are being advanced to homozygosity in parallel to the main population to determine whether early selection in a heterozygous population can enhance the number of lines with high levels of resistance. When grown in the field in 2022, the 622 lines were classified as either dry or snap bean phenotype. In the fall of 2022, 1,050 seeds were advanced to the next generation in the greenhouse. An existing pinto bean MAGIC population is being used to examine six genomic prediction models: the 1st set of 500 lines is being used to predict performance on the 2nd set of 500 lines using available SNPs and phenotypic data from the straw test. Model validation will also be made using available phenotypic data for the 2nd set of lines. A set of ~28 MAGIC lines were tested in the field for the 3rd year. In addition, the best predictive model will be tested in our pinto bean breeding programs. A new RIL population (Ruby/SR9-5, 172 F5:6 RILs) was developed to examine white mold reaction in the small red dry bean market class. Phenotyping in the straw test is ongoing and the genetic linkage map for this RIL population covers 605 cM (~50%) of the genome with 383 SNP markers. The low coverage sequencing Khufu technology available from Hudson Alpha Institute for Biotechnology was used to map and validate new and previously identified QTL for white mold resistance across four dry bean RIL populations (540 lines). Overall continued progress is being made toward identifying, developing, and generating QTL linked markers for use in breeding snap and dry beans with improved resistance to white mold.

Contact information – Phillip N. Miklas, USDA-ARS, 24106 N. Bunn Road, Prosser, WA, 99350; 509-786-8492, phil.miklas@usda.gov

Characterization of a methionine biosynthesis gene (*SsMet1*) in *Sclerotinia sclerotiorum*

Nickisha Pierre-Pierre[1,2], Wei Wei[1], George Vandemark[1,2] and Weidong Chen[1,2]

[1]Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA[2] USDA Agricultural Research Service, Pullman, WA 99164, USA

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

ABSTRACT:

Methionine (Met), a key sulfur-containing amino acid, is involved in various functions in cellular metabolism. Genes that encode enzymes to catalyze steps of the Met biosynthesis pathway are essential for survival of pathogenic fungi in the host. The *SsMet1* gene in *Sclerotinia sclerotiorum* is an orthologue of *BcStr2*, a gene characterized in *Botrytis cinerea* that plays a key role in methionine biosynthesis. In this study, we characterized *SsMet1* in *S. sclerotiorum* by creating two *SsMet1*-deletion mutants, *Met11-2* and *Met11-4*. The *SsMet1* mutants were unable to grow on minimal medium (MM) and did not produce sclerotia. Supplementation with methionine and homocysteine rescued the defects in mycelial growth, but cysteine and glutathione did not. In addition, there was no sclerotia development of the *SsMet1*-deletion mutants. These results indicate that *Met11-2* and *Met11-4* are methionine auxotrophic mutants. In addition, the *SsMet1* mutants exhibited increased sensitivity to osmotic stress (Sodium chloride [1 M] and Potassium chloride [1 M]), oxidative stress (Sorbitol [1 M], Hydrogen peroxide [10 mM] and Calcofluor White [0.5 g/liter]), cell wall-damaging agent (Congo Red [0.5 mg/mL]), and thermal stress (15 ℃, 20 ℃, 25 ℃ and 28 ℃). Statistical tests were performed with R studio. In comparisons of the two mutants with the wild type (WMA1), the significance of differences was analyzed by a linear model and subsequently analyzed by one-way ANOVA. The *SsMet1*-deletion mutants, *Met11-2* and *Met11-4* lost pathogenicity on the tested plants. All the defects were restored by genetic complementation of the mutant with wild type *SsMet1* allele. The results of this study indicate that *SsMet1* plays a critical role in the regulation of various cellular processes in *S. sclerotiorum*. The deletion of *SsMet1* demonstrated the role of *SsMet1* in growth, virulence, sclerotia development and the response to environmental stresses. *SsMet1* is a potential antifungal drug target in *S. sclerotiorum*.

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

Root endophytic colonization of Sclerotinia sclerotiorum disarmed by a mycovirus infection induced disease resistance and photosynthesis activity

Connor Pedersen, USDA-ARS ATRU, Toledo, OH, Gayathri Beligala, University of Toledo, Toledo, OH, & Shin-Yi Marzano, USDA-ARS ATRU, Toledo, OH

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

ABSTRACT:
SlaGemV-1 is a hypovirulence-inducing CRESS-DNA mycovirus discovered through a meta-transcriptomic analysis of soybean leaves. Hypovirulence-inducing mycoviruses are a promising alternative to chemical pesticides for disease control, but the potential of the hypovirulent fungus to provide benefits beyond just disease resistance is not well understood. We've previously shown that SlaGemV-1 directly inhibits the growth of 3 species of Sclerotiniaceae through infection. Here we show endophytic effects that SlaGemV-1-infected *Sclerotinia sclerotiorum* can produce in *Glycine max* and *Phaseolus vulgaris* var. pinto through root colonization methods in greenhouse and field utilizing RNA-Seq analysis and leaf fluorometer readings. SlaGemV-1 induced hypovirulence in *S. sclerotiorum* which is then able to induce a defense response and reduce the lesion growth of pathogenic *S. sclerotiorum*. Further, RNA-Seq analysis reveals 1,500 differentially expressed genes and suggests an increase in gene ontology groups relating to photosynthetic function which is reflected in an increase in photosystem II photochemical efficiency Fv/Fm values in field-planted pinto bean plots. RNA-Seq also reveals differential expression of salicylic acid and jasmonic acid pathways, defense response-related metabolites. SlaGemV-1, a hypovirulence-inducing mycovirus, can modulate the pathogenic fungus *S. sclerotiorum* to become a potentially beneficial endophyte. Along with induction of plant resistances, colonization may improve photosynthetic capacity.

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Fungicide sensitivity and genetic characterization of *Sclerotinia sclerotiorum* from USA soybean and dry bean, compared to different regions and climates using AmpSeq

E. Nieto-Lopez[1,2], R. A. Koch Bach[1,3], S. Kodati[1,4] and S.E. Everhart[1,4].

[1]Department of Plant Pathology, University of Nebraska
[2]Department of Plant Pathology and Microbiology, Iowa State University
[3]United States Department of Agriculture, Frederic, MD
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Research Project: Genetic variability associated with the traits of fungicide resistance and pathogenicity in *Sclerotinia sclerotiorum*

ABSTRACT: Application of fungicides is a common method to reduce the yield loss due to *Sclerotinia sclerotiorum* on dry bean and soybean. Increase in the usage may lead to development of resistance or reduce the sensitivity to the most commonly used fungicides. We conducted a study to identify the difference in fungicide sensitivity between isolates from dry bean and soybean and isolates from fields with different levels of fungicides treatments. Fungicide sensitivity of 512 *S. sclerotiorum* isolates from the USA (N=443), Brazil (N=36), and Mexico (N=33) was determined using a discriminatory concentration (DC) of tetraconazole (2.0 ppm; FRAC group 3), boscalid (0.2 ppm; group 7), picoxystrobin (0.01 ppm; group 11), and thiophanate methyl (TM; group 1), which had a qualitative DC of 10 ppm. Sequence analysis of the 10 least sensitive isolates to boscalid and picoxystrobin, two isolates from Mexico presented mutations known to confer resistance in the *SdhB* (qualitative) and the other two Mexican isolates each had a total of 12 mutations in *SdhC* (qunatitative) genes, however no strong resistance was found. To investigate further, we used a total of 178 *S. sclerotiorum* isolates along with development of an AmpSeq technique that enables next-generation sequencing of multiple target loci. An AmpSeq primer array was developed to amplify and sequence variants in SSRs, genes conferring fungicide resistance (β-tubulin, Sdh complex, and cytochrome b gene), SNPs, and putative virulence loci. Raw demultiplexed reads were received and subjected to an AmpSeq custom pipeline that used the *S. sclerotiorum* reference genome to identify variants and produce a variant call format (VCF) file for downstream analysis. Preliminary analysis of fungicide resistance loci showed 16 samples out of 192, along with the positive control, had a mutation for fungicide resistance in the *SdhB* gene which represented 24–33% of all samples with sequence data at those loci. No sequence data for other resistance loci was found, possibly due to low sequence depth, quality control filters, or poor primer amplification. With an ability to evaluate large numbers of samples and loci, this technique represents a promising new approach for broad scale characterization of *S. sclerotiorum*.

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

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

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

ABSTRACT:

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

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Characterization of oxalic acid tolerance in sunflower resistance to Sclerotinia basal stalk rot

William Underwood[1]

[1]USDA-ARS, Sunflower & Plant Biology Research Unit, Fargo, ND

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

ABSTRACT:

Oxalic acid (OA) is an important virulence factor for *Sclerotinia sclerotiorum*, causing plant cell death and contributing to disease development. Treatment of sunflower roots with OA via soil drench or hydroponic application mimics the symptoms of basal stalk rot disease caused by *S. sclerotiorum*, including wilting, leaf necrosis, stem streaking, and development of basal stem lesions. In addition to recapitulation of disease symptoms upon OA treatment, some sunflower lines with moderate to high levels of resistance to basal stalk rot exhibit tolerance to OA, suggesting that OA tolerance may contribute to stalk rot disease resistance. Consequently, the overall goal of this project is to examine the physiological and molecular nature of sunflower tolerance to OA. Specific objectives of the project are to: 1) Determine if sunflower tolerance to OA is associated with reduced OA accumulation in aerial tissues; 2) Determine if OA tolerance is conferred by the sunflower rootstock or shoot by performing grafting experiments; 3) Define transcriptional changes associated with OA tolerance by RNAseq comparison of tolerant and sensitive sunflower lines. Grafting experiments in which scion (aerial) tissues of OA tolerant sunflower genotypes are grafted onto rootstocks of a sensitive genotype, or vice versa, have revealed that sunflower OA tolerance appears to be conferred by the aerial tissues rather than roots (which are directly exposed to OA). Experiments to compare OA accumulation in aerial tissues of tolerant and sensitive genotypes are currently being completed and results will be presented. Finally, collection of root tissues from tolerant and sensitive genotypes over a time-course after hydroponic treatment with OA has been completed and RNA samples will be isolated and submitted to an external provider for RNA sequencing in the final year of the project.

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Two newly described polygalacturonase-inhibiting proteins (PGIPs) in chickpea inhibit *Sclerotinia sclerotiorum* polygalacturonases (SsPG1 and SsPG5)

Vishnutej Ellur[1], Wei Wei[2], Rishikesh Ghogare[3], Shyam Solanki[4], George Vandemark[5], Robert Brueggeman[6], Weidong Chen[5]

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

Abstract:

Polygalacturonase inhibiting proteins (PGIPs) are cell wall-anchored proteins that selectively inhibit pathogen polygalacturonases (PGs). Previous study identified two PGIPs (*Capgip1* and *Capgip2*) in chickpea (*Cicer arietinum*). In this study, we identified and characterized two novel *Capgips* (*Capgip3* and *Capgip4*). Our analysis showed that CaPGIP1, CaPGIP3, and CaPGIP4 proteins contain N-terminal signal peptides, ten leucine rich repeats (LRR)s like other legume PGIPs. However, CaPGIP2 cannot be classified as a true PGIP since it lacked a signal peptide, more than half of the LRRs, and other characteristics of a typical PGIP. Localization experiments of *Capgips* in *Nicotiana benthamiana* leaves showed that *Capgip1*, *Capgip3*, and *Capgip4* are located to the cell wall or membrane, whereas *Capgip2* is found in the endoplasmic reticulum. Phylogenetic analysis revealed that the *Capgip* amino acid sequences are similar to the other reported PGIPs of legumes. The chickpea PGIPs were cloned from cultivar Dwelley and their recombinant proteins were expressed in yeast *Pichia pastoris* to investigate the efficacy of their inhibitory activity. The recombinant proteins of all five *S. sclerotiorum* polygalacturonases (SsPG1 to 5) were also expressed in *Pichia pastoris*. Enzyme assays revealed that only CaPGIP3 and CaPGIP4 were capable of inhibiting SsPG1 and SsPG5, with CaPGIP4 showing the strongest inhibition activity. CaPGIP overexpression transgenic *Nicotiana tabacum* lines were generated and purified, and their effects on Sclerotinia infection are being assessed. These findings will allow us to design experiment to investigate the interaction of CaPGIP with Sclerotinia sclerotiorum PGIP INactivating Effector (SsPINE1).

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

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

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

ABSTRACT:

The goal of this project is to develop effective and durable disease resistance for Sclerotinia in *Brassica napus* through manipulating endogenous host pathways. The oxalic acid secreted by *Sclerotinia sclerotiorum* is essential to colonize and produce disease symptoms in *Brassica napus* and other Brassicaceae species. A two-step oxalate metabolic pathway is present in Brassicaceae species. First, oxalyl-CoA synthetase (*AAE3*) ligates oxalate and CoA to form oxalyl-CoA, and then Oxalyl-CoA Decarboxylase1(*OCD1*) catalyzes oxalyl-CoA into formyl-CoA and CO2. Interestingly, *AAE3* and *OCD1* genes are induced by oxalate and Sclerotinia infection in Arabidopsis, and *aae3* mutants in Arabidopsis show increased susceptibility to Sclerotinia. We have cloned the *BnAAE3* and *BnOCD1* genes from *Brassica napus* and created transgenic lines overexpressing *BnAAE3* and *BnOCD1*, respectively. We are finishing steps to obtain homozygous lines and test lines for level of gene overexpression and enhanced resistance. Subsequently, we will prepare transgenic lines simultaneously overexpressing *BnAAE3* and *BnOCD1*genes. Following these proof of concept experiments we will plan to enhance durable disease resistance in *B. napus* by CRISPR mutagenesis of *BnAAE3* and *BnOCD1* gene promoters to increase gene expression, the level of endogenous oxalate metabolism and disease resistance.

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Exploring mechanisms of effector-triggered susceptibility in the soybean-*Sclerotinia* pathosystem

Mitchell G. Roth, and Tiffanna J. Ross, The Ohio State University, Wooster, OH

Funded Plan of Work: "Less is More: Removing R-gene Mediated Susceptibility to *Sclerotinia sclerotiorum* in Soybean"

ABSTRACT:
To date, strong genetic resistance to white mold is lacking in commercial soybean lines, partially due to the lack of R-genes effective against *S. sclerotiorum*. Utilizing a unique set of criteria, an analysis of a time-course RNA-seq data set in a pair of recombinant inbred lines (RILs) has revealed strong evidence that *S. sclerotiorum* might be manipulating R-gene mediated defense mechanisms, triggering uncontrolled programmed cell death (PCD), and increasing disease susceptibility. In the partially resistant RIL, this R-gene mediated susceptibility is lacking although the R-gene appears to be present and expressed. Candidate effectors used by *S. sclerotiorum* to trigger the R-gene mediated susceptibility have already been identified through homology searches to known elicitors in other fungi. We have begun whole-genome sequencing of the two RILs with long read (Nanopore) technologies and will be performing de novo assemblies to examine structural variation between the RILs, particularly in the region containing the R-gene. We have identified and cloned two candidate *S. sclerotiorum* effectors and the corresponding soybean R-gene into *Agrobacterium* expression vectors and yeast 2-hybrid bait and prey vectors to examine the effects of transient expression of the *Sclerotinia* effectors *in planta* and for screening of direct interactions respectively. Lastly, two unique silencing constructs were generated using the cowpea severe mosaic virus system to perform virus induced gene silencing (VIGS) experiments. These VIGS constructs will be used to silence this R gene in soybean and perform inoculations with *Sclerotinia* and examine if silencing the R-gene confers enhanced resistance, as expected. Overall, this work aims to identify and validate that less is more; removing an R-gene could lead to enhanced resistance to SSR in soybean.

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Identification and characterization of soybean phenylalanine ammonia-lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) homologs genes' role in white mold disease control

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

ABSTRACT:

Sclerotinia stem rot (SSR; white mold) caused by *Sclerotinia sclerotiorum*, a broad host range fungal pathogen, was reported as the second most destructive soybean disease in the United States in the year 2021, leading to an estimated loss of nearly 25 million bushels of yield. Previous studies have indicated the role of the phenylpropanoid biosynthesis pathway and accumulation of metabolites, including cinnamic acid, in response to SSR, indicating this pathway's importance in the defense response to SSR. Identification and characterization of specific enzymatic genes in this pathway may improve SSR resistance in soybean. Phenylammonia layse (PAL) and cinnamyl alcohol dehydrogenase (CAD) are key genes of the phenylpropanoid biosynthesis pathway that catalyzes the conversion of precursor phenylalanine to cinnamic acid and aldehyde moiety such as coniferaldehyde and sinpaldehyde to their alcohol derivatives, respectively. We have shown that purified cinnamic acid, coniferaldehyde, and sinpaldehyde inhibit *S. sclerotiorum* growth. The role of *GmPALs* and *GmCADs* genes in disease response in soybean is currently unknown. Using sequence similarity searches, we have identified eight putative GmPALs and thirteen GmCADs genes in the soybean genome. To characterize the role of *GmPALs* and *GmCADs* involved in *S. sclerotiorum* infection, we have conducted time-course (6hpi, 12hpi, 24hpi, 48hpi, and 72hpi) experiments in the growth chambers using Soybean var. Williams 82, challenged with *S. sclerotiorum*. The plant samples were destructively collected and processed for RNA isolation. qRT-PCR was performed on the eight *GmPALs* and thirteen *GmCADs* using gene-specific primers to assess expression changes over time compared to mock-inoculated plants. The expression study indicated that eight *GmPALs* and eleven *GmCADs* were differentially regulated in response to *S. sclerotiorum* infection, but two homologs of *GmCADs* did not express. We are currently performing overexpression and silencing studies of the candidate genes by transiently overexpressing them in *Nicotiana benthamiana* and by constructing high throughput virus-induced gene silencing (VIGS) of specific genes using *Bean pod mottle virus* (BPMV) derived vectors in soybean, respectively. The study will provide a potential target for developing white mold-resistant soybean lines.

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A *Sclerotinia sclerotiorum* effector protein interacts with plant fatty acid hydroxylases (FAHs) that mediate cell death control

Wei Wei, Liangsheng Xu, 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: *Sclerotinia sclerotiorum* hijacks host cell death control in infecting plant

ABSTRACT:

Sclerotinia sclerotiorum is a typical broad-host-range necrotrophic pathogen causing white mold disease on many dicotyledonous crops. Plant pathogens often work through protein effectors that are delivered into the host plant cells to disrupt critical cellular processes. We previously reported that a novel secretory protein SsE3 is critical for virulence of *S. sclerotiorum*. Deletion of *SsE3* significantly reduced virulence. Using three independent techniques - yeast two-hybrid (Y2H), coimmunoprecipitation (co-IP), and bimolecular fluorescence complementation (BiFC) assays, we demonstrated that SsE3 without signal peptide ($SsE3^{\Delta SP}$) interacts with both *Arabidopsis* fatty acid hydroxylases AtFAH1 and AtFAH2. AtFAH1 and AtFAH2 are functionally different fatty acid 2-hydroxylases that mediate cell death suppression. A previously published paper showed that AtFAH1/AtFAH2 interact with AtB1-1 via AtCb5 suppressing programmed cell death. Immunoprecipitation assays showed that SsE3 has a higher binding affinity with AtFAH2 than with AtCb5. Over-expression of *SsE3* in *Arabidopsis* reduced expression levels of defense-related genes, such as the pattern recognition receptor (PRR) brassinosteroid insensitive 1 associated receptor kinase1, NADPH oxidase, respiratory burst oxidase homolog D (RBOHD), and heterotrimeric G. In addition, Salicylic acid (SA) level was increased in the *SsE3*-overexpressing lines compared to the wildtype plant. We are currently analyzing the expression levels of AtFAH1 and AtFAH2 in the *SsE3*-overexpressing lines. We hypothesize that SsE3 binding with AtFAH1/AtFAH2 inhibits fatty acid 2-hydroxylases activities, which disrupts the process of suppressing programmed cell death, thus promoting cell death. These results will show that SsE3 is critical for *S. clerotiorum* necrotrophy in host plant.

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Poster Abstracts

Genomic predictions for resistance to White Mold in Dry Beans using a MAGIC population

Jose C. Figueroa Cerna, Juan M. Osorno, Kristin Simons & Phillip McClean, North Dakota State University, Fargo, ND, & Phil N Miklas, USDA-ARS, Prosser, WA.

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

ABSTRACT:
Combined with other techniques, genomic predictions can improve the selection efficiency of the genotypes in a plant breeding program, especially for quantitative traits such as white mold (*Sclerotinia sclerotia* Lib. de Bary) resistance in dry beans (*Phaseolus vulgaris* L.). Screening new genotypes for this disease under field conditions is complex. The presence of the pathogen in the soil, climate conditions, plant density, and avoidance and physiological mechanisms, interact together, hindering the selection process of resistant genotypes. In this study, accuracy of six genomic prediction models (RR-BLUP, Bayes A, Bayes B, Bayes Cπ, BLASSO, and BRR) will be measured using a Multiparent Advance Generation Inter-Cross (MAGIC) population. Genotypic data was obtained via GBS from a subset of 500 Recombinant Inbred Lines (RILs) from the MAGIC population. Phenotypic data was obtained using the seedling straw method in the greenhouse. The total amount of genotypic data used in the models differ depending on the reference genome used. For the G19833 v2.1 genome reference (Andean), a total of 52,201 SNPs were identified, while a total of 76,286 SNPs were obtained using the UI111 v1.1 reference genome (Middle American). To identify the best predictive model, the predictive ability was calculated as the Pearson correlation between the average of the predicted values and the observed phenotypes. The model with the highest predictive ability will be validated as a genomic selection tool for a second subset of the MAGIC population as well as for the advanced breeding lines in the dry bean breeding program at North Dakota State University.

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Nested Association Mapping (NAM) of Resistance to White Mold and Agronomic Performance in Four Common Bean Populations

Ahmet R. Agir, Haidar Arkwazee, Joel Davis and James R. Myers
Department of Horticulture, Oregon State University, Corvallis OR

Funded Plan of Work: A QTL Approach Toward Understanding and Improving Genetic Resistance to White Mold in Common Bean

ABSTRACT:

The objective of this project was to use single nucleotide polymorphism (SNP) markers to identify quantitative trait loci (QTL) associated with white mold resistance and agronomic performance traits in common bean. Four biparental populations were developed with the common parent, WMG904-20-03 (snap bean derived from a backcross-inbred interspecific population introgressing resistance from runner bean (*Phaseolus coccineus*) crossed to donor parents Cornell 501 and NY6020-4 (snap beans), and M0070 and A195 (bot dry beans). Lines of the four recombinant inbred (RI) populations were scanned with the BARCBEAN12K bead chip at the F_5. Field data measuring disease incidence and severity was recorded for 2018 and 2019. In the summers of 2020 and 2021, the NAM population was evaluated for agronomic traits, including 100-seed weight, whole-plot weight, seed emergence, vigor, leaf heliotropism ratio, number of germinated plants, maturity, leaf color, flower color, pod ground color, secondary pod color, degree of pod curvature, shape of pod curvature, pod shape in cross-section, pod stringiness, and the leaf crumple (LCR) trait. SNPs from the NAM population was used to construct linkage maps and find QTLs for the traits listed above. Linkage maps were generated using JoinMap 5.0 and QTL analysis was performed using the Interval Mapping and MQM functions of MapQTL 6.0. Potential QTLs ($P \leq 0.05$, $LOD \geq 2.0$) were identified in all four populations for both years. For white mold resistance, we detected 22 QTLs on nine chromosomes. Multiple QTLs for incidence and severity were found on Pv02, Pv03, Pv04, Pv06 and Pv08. For agronomic traits, 54 QTLs were present on 10 chromosomes. The most abundant QTLs were on chromosome Pv07 (12), related to 100-seed weight, whole-plot weight, and number of plants in a plot. The second-highest number of QTLs (11) related to 100-seed weight was on chromosome Pv03. Six QTLs for bean leaf color were identified on Pv01, Pv06 Pv07 and Pv08. Most QTL were present in both years with congruency between individual and the combined NAM populations.

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Multiple QTL Conferring Genetic and Physiological Resistance to White Mold Identified in Two Common Bean Bi-Parental Mapping Populations

Jayanta Roy[1], Phillip N. Miklas[2], Alvaro Soler-Garzón[3], Josh Clevenger[4], Rian Lee[1] and Phillip E. McClean[1]

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

ABSTRACT: White mold (WM), caused by the ubiquitous fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a devastating disease that limits production and quality of common bean globally. The complex host-pathogen interaction and lack of complete host resistance coupled with low to medium heritability often limits breeding efforts to develop improved WM resistant common bean cultivars. In the present study, linkage maps of the two recombinant inbred line (RIL) populations 'Montrose'/I9365-25 (M25) and 'Raven'/I9365-31 (R31) were constructed consisting of 9575 and 4175 SNPs, respectively, which covered a total length of 342 cM and 288 cM for M25 and R31, respectively. The RILs were phenotyped for WM reactions under greenhouse (straw test) and field environments to identify QTL that contribute to WM resistance using QTL sequencing (QTL-Seq) and classic QTL mapping analyses. Major QTL WM2.2 were delimited within the physical interval of WM2.2a (4.38-4.60 Mb) detected in R31 and WM2.2b (27.7-29.6 Mb) in M25 corresponding to 26.7% and 10.9% of phenotypic variance (PV), respectively. Another major QTL WM5.4 conferring physiological and field resistance was identified in the heterochromatic genomic region in M25 (25.9-31.4 Mb; LOD 13.2; PV 25.3%) and in R31 (24.8-29.8 Mb; LOD 12.6; PV 15.6%). On chromosome Pv07, major QTL WM7.5 (4.27-4.85 Mb; PV 39.7%) and another fine mapped QTL WM7.4 (22.3-23.6 Mb; PV 18.4%) were identified for physiological resistance in R31 population. Gene models encoding TIR-NBS-LRR domain, NB-ARC domain, pentatricopeptide repeat, legume lectin family, and gibberellin 2-oxidase proteins, found within the major QTL confidence intervals, represent potential candidate genes associated with WM resistance. Acquired knowledge of the narrowed major QTL intervals, flanking markers, and candidate genes provides promising opportunities to develop functional molecular markers to implement marker-assisted selection for WM resistant common bean cultivars.

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Strategic Plan

United States Department of Agriculture

Research, Education & Economics

Agricultural Research Service

Northern Plains Area

January 2016

Version 1.0

National Strategic Plan for the Sclerotinia Research Initiative

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

2017 to 2021

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

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

Universities/Institutions

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

Commodity Organizations

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