National Sclerotinia Initiative

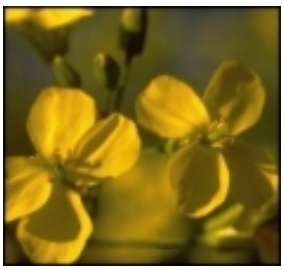
USDA-ARS

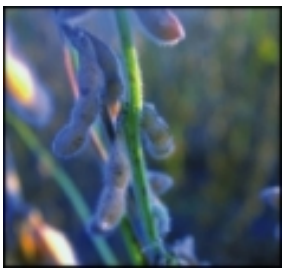
National Sclerotinia Initiative

2016 Annual Meeting

Bloomington, MN

January 20-22, 2016



14th Annual National Sclerotinia Initiative Meeting

January 20-22, 2016

Crowne Plaza Hotel & Suites
Three Appletree Square, Bloomington, MN

AGENDA

2016 Sclerotinia Initiative Annual Meeting
January 20-22, 2016

Wednesday – January 20, 2016

6-8 pm	Poster Session/Reception *(posters are displayed for the entire meeting)*	**Cortland Room**

Thursday – January 21, 2016

7:15 am	Registration/Continental Breakfast	**Cortland Room**
8:15 am	Welcome, Introductions & Meeting Charge – **Bill Kemp, USDA-ARS, Fargo, ND**	
8:25 am	Welcome & Update from the Plains Area – **John McMurtry, USDA-ARS, Fort Collins, CO**	
8:35 am	ARS Office of National Programs Update – **Roy Scott, USDA-ARS, Beltsville, MD**	
8:45 am	Introduction of ***Featured Speaker*** **– Mike Foley, USDA-ARS, Fargo, ND**	
	Developing mechanistic perspectives on *Sclerotinia* virulence and host defense to inform rational strategies for improving crop resistance – **William Underwood, USDA-ARS, Fargo, ND**	
10:00 am	Discussion Break	**Ballroom Foyer**

Sclerotinia Research Activities – Session 1 **Fireside Room**
Moderator – Martin Chilvers, Michigan State University, East Lansing, MI

10:30 am	Synergistic enhancement of resistance to *Sclerotinia sclerotiorum* (Abstract p.18; Poster #22) – **Jeffrey Rollins, University of Florida, Gainesville, FL**
10:45 am	Strategies to identify functionally significant defense genes against *Sclerotinia sclerotiorum* (Abstract p. 33; Poster #5) – **Daina Simmonds, Agriculture and Agri-Food Canada, Ottawa, ONT**
11:00 am	Identifying and verifying genes for defense to *Sclerotinia* (Abstract p. 20; Poster #4) – **Wei Wei, University of Illinois & Steven Clough, USDA-ARS, Urbana, IL**
11:15 am	The potential of genomic selection to improve white mold resistance in soybean (Abstract p. 34; Poster #24) – **Dechun Wang, Michigan State University, East Lansing, MI**

11:30 am Changes to ARS cooperative agreements process – **Marcie Currie-Gross, USDA-ARS, Fort Collins, CO**

Noon Working Lunch **Cortland Room**

Sclerotinia Research Activities – Session 2 **Fireside Room**
Moderator – Gerald Seiler, USDA-ARS, Fargo, ND

1:15 pm Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial *Glycine latifolia* (Abstract p. 16; Poster #3) – **Leslie Domier, USDA-ARS, Urbana, IL**

1:30 pm Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas (Abstract p. 32; Poster #21) – **James Steadman, University of Nebraska, Lincoln, NE**

1:45 pm Breeding Common Bean for Resistance to White Mold – **Shree Singh, University of Idaho, Kimberly, ID**

2:00 pm Quantitative trait loci analysis of white mold resistance in dry bean (Abstract p. 26; Poster #19) – **James Kelly, Michigan State University, East Lansing, MI**

2:15 pm Identification of major QTL conditioning partial resistance to white in dry bean (Abstract p. 13, 19, 24, 35; Poster #13, #17, #25) – **Phil Miklas, USDA-ARS, Prosser, WA; James Myers, Oregon State University, Corvallis, OR; & Phil McClean, North Dakota State University, Fargo, ND**

3:00 pm Break & Poster Session **Cortland Room**

Sclerotinia Research Activities – Session 3 **Fireside Room**
Moderator – Phil Miklas, USDA-ARS, Prosser, WA

3:15 pm Discovery and use of novel sources of head and stalk rot resistance in sunflower and studies of *Asteracea* genera stimulating myceliogenic germination (Abstract p. 15; Poster #23) – **Gerald Seiler, USDA-ARS, Fargo, ND**

3:30 pm Transferring Sclerotinia resistance genes from wild *Helianthus* species into cultivated sunflower (Abstract p. 25; Poster #18) – **Chao-Chien Jan, USDA-ARS, Fargo, ND**

3:45 pm Using genomic selection to optimize prediction of Sclerotinia and agronomic phenotypes for more efficient breeding (Abstract p. 36; Poster #15) – **Brent Hulke, USDA-ARS, Fargo, ND**

4:00 pm Sclerotinia stalk rot resistance in sunflower: QTL mapping and gene introgression from wild *Helianthus* species (Abstract p. 29; Poster #20) – **Lili Qi, USDA-ARS, Fargo, ND**

4:15 pm Wrap-up & Adjourn (Dinner on your own)

Friday – January 22, 2016

7:00 am Steering Committee Breakfast Meeting **Executive Conference Room**

7:15 am Continental Breakfast **Cortland Room**

Sclerotinia Research Activities – Session 4 **Fireside Room**
Moderator – Lili Qi, USDA-ARS, Fargo, ND

8:15 am *Agrobacterium*-mediated transformation of sunflower (Abstract p. 11) – **John Finer, The Ohio State University, Wooster, OH**

8:30 am Improved head rot resistance screening in sunflowers and impacts & implications of Sclerotinia infection timing in dry bean, soybean, and sunflower (Abstract p. 22; Poster #1, #16) – **Michael Wunsch, North Dakota State University, Carrington, ND**

8:45 am Characterization and validation of two distinct mechanisms for resistance to *Sclerotinia sclerotiorum* in *Pisum sativum* (Abstract p. 12; Poster #12) – **Kevin McPhee, North Dakota State University, Fargo, ND & Lyndon Porter USDA-ARS, Prosser, WA**

9:00 am Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics assisted breeding (Abstract p. 23; Poster #9) – **Martin Chilvers, Michigan State University, East Lansing, MI**

9:15 am Sclerotial development and pathogenicity of *Sclerotinia sclerotiorum* depend on low pH, independent of oxalic acid (Abstract p. 30; Poster #10) – **Weidong Chen, USDA-ARS, Pullman, WA**

9:30 am Discussion Break **Ballroom Foyer**

10:00 am Characterizing resistance and pathogenicity genes associated with infection of *Brassica napus* by *Sclerotinia sclerotiorum* (Abstract p. 14; Poster #14) – **Luis del Rio, North Dakota State University, Fargo, ND**

10:15 am Identifying candidate genes for aggressiveness via association analysis of a diverse population of *Sclerotinia sclerotiorum* (Abstract p. 21; Poster #7) – **Robert Brueggeman & Berlin Nelson, North Dakota State University, Fargo, ND**

Sclerotinia Initiative Research: The next steps **Fireside Room**
Moderator – Bill Kemp, USDA-ARS, Fargo, ND

10:30 am ***Guest Speaker***
Strategic Planning & Reporting Progress – **Rich Wilson, USDA-ARS, Office of National Programs–Retired, Raleigh, NC**

11:15 am Strategic Plan Discussion – Writing Team Input/Revisions
Annual Reports

11:45 am Working Lunch **Cortland Room**

1:00 pm Assignment of Additional Tasks & Wrap-up of Initiative Business

2:00 pm Adjourn (Travel Safely!)

National Sclerotinia Initiative Poster Session

January 20-22, 2016
Cortland Room
Crowne Plaza Hotel & Suites

Epidemiology & Disease Management		
Poster No.	Title	Author(s)
1	Impact of timing of Sclerotinia disease development on seed yield and quality in dry beans, soybeans and sunflowers	M.J. Wunsch, L. Besemann, M. Schaefer, S. Kallis, B. Kraft
2	Relationship between environmental conditions and Sclerotinia sclerotiorum apothecia production, ascospore release and primary plant infection of soybean	M.L. Fall, M. Chilvers, A.M. Byrne, J. Willbur, D. Smith

Genomics		
Poster No.	Title	Author(s)
3	Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial Glycine latifolia	Q. Liu, G.L. Hartman, L.L. Domier
4	RNA-Seq data analysis of soybean leaves infected with Sclerotinia	W. Wei, X. Wu, M. Belaffif, M. Hudson,S.J. Clough, L. Blahut-Beatty, L. Koziol, D. Simmonds
5	Strategies to identify functionally significant defense genes against Sclerotinia sclerotiorum	A. Itaya, L. Blahut-Beatty, Y. Zhang, S. Zheng, L. Koziol, S. Clough, D. Simmonds

Pathogen Biology & Development		
Poster No.	Title	Author(s)
6	A reevaluation of myceliogenic germination of sclerotia for Sclerotinia sclerotiorum strain Sun-87	M.E. Foley, M. Dogramaci, W. Underwood
7	Identifying candidate genes for aggressiveness via association analysis of a diverse population of Sclerotinia sclerotiorum	R. Brueggeman, S. Jain, J. Richards, C. Qiu, S. Solanki, L. Aldrich-Wolfe, J. LeBoldus, W. Underwood, B.D. Nelson, Jr.
8	Genotypic diversity of Sclerotinia sclerotiorum populations from New York	A. Dunn, S. Pethybridge, J. Kikkert

Pathogen Biology & Development (cont.)		
Poster No.	Title	Author(s)
9	Molecular characterization of partial resistance in pea (Pisum sativum) to Sclerotinia sclerotiorum	J. Trinh, P. Santos, D. Kosma, X. Zhuang, T. Coram, K. McPhee, M. Chilvers
10	Sclerotia development and pathogenicity of Sclerotinia sclerotiorum depend on low pH, independent of oxalic acid	L. Xu. M. Xiang, D. White, W. Chen
11	Small RNA processing in Sclerotinia sclerotiorum	S.L. Marzano, L. Domier

Variety Development/Germplasm Enhancement		
Poster No.	Title	Author(s)
12	Characterization and validation of two distinct mechanisms for resistance to Sclerotinia sclerotiorum in Pisum sativum	R. AshtariMahini, K. McPhee
13	Characterization of two new recombinant inbred populations in common bean for Sclerotinia resistance	J.R. Myers, H. Arwazee
14	Characterizations of candidate genes within QTL associated with resistance to stem rot in a double haploid breeding population of canola	J.V. Anderson, K. Chittem, L. del Rio Mendoza
15	Developing genomic selection models for sunflower	B.S. Hulke, Q. Gao, N.C. Kane
16	Impact of differences in susceptibility to shattering of diseased tissue on the impact of Sclerotinia head rot on seed yield and quality in sunflowers	M.J. Wunsch, L. Besemann, M. Schaefer, S. Kallis, B. Kraft
17	Physical mapping QTL for resistance to white mold from multiple common bean sources	R. Vasconcellos, B. Oraguzie, A. Soler, P. McClean, S. Moghaddam, P. Miklas
18	Progress on transferring Sclerotinia resistance genes from wild Helianthus species into cultivated sunflower	Z. Liu, H. Want, X. Cai, G.J. Seiler, C. Jan
19	Quantitative trait loci analysis of white mold avoidance in pinto bean	V. Hoyos-Villegas, W. Mkwaila, E. Wright, J.D. Kelly
20	Sclerotinia stalk rot resistance in sunflower: QTL mapping and gene introgression from wild Helianthus species	Z. Talukder, G. Seiler, W. Underwood, L. Qi
21	Sources of white mold resistance derived from wide crosses in common bean and progress in characterization of relevant pathogen isolates	S. Everhart, R. Higgins, J.R. Steadman
22	Synergistic enhancement of resistance to Sclerotinia sclerotiorum	C. Wang, J. Rollins, Z. Mou
23	Update on the discovery and use of novel sources of head and stalk rot resistance in sunflower and studies of Asteraceae Genera stimulating myceliogenic germination	G.J. Seiler, W. Underwood, S. Markell, M. Gilley, M. Wunsch, K. Olander, B. Flett
24	The potential of genomic selection to improve white mold resistance in soybean	Z. Wen, S. Zhang, R. Tan, W. Du, P. Collins, C. Gu, M. Chilvers, D. Wang
25	Using common bean seedlings for white mold resistance evaluation via the straw test	H. Arkwazee, M. Myers

A Reevaluation of Myceliogenic Germination of Sclerotia for *Sclerotinia sclerotiorum* Strain Sun-87

Michael E. Foley, Münevver Doğramacı, William R. Underwood, USDA-ARS, Northern Crop Science Laboratory, Fargo, ND

Funded Plan of Work: Not applicable

Abstract:
Basal stalk rot of sunflower is an economically important, and rather unique disease, among crops that are susceptible to *Sclerotinia sclerotiorum*. This disease is the result of myceliogenic germination of sclerotia whereby the vegetative hyphae infect the sunflower below the soil level. In contrast, sunflower head rot and similar diseases of susceptible crops result from carpogenic germination to produce airborne ascospores that infect above ground senescent or wounded tissues. We initiated research on several factors reported to affect sclerotia germination as a prelude to comparing transcriptomes associated with myceliogenic and carpogenic germination. Specifically, we reevaluated the effects of inoculum development temperature, sclerotia development temperature, conditioning temperature, conditioning of hydrated and desiccated sclerotia, and the duration of sclerotia desiccation on germination of Sun-87 sclerotia, largely as outlined by Huang (1991), Huang and Kozub (1993), and Huang et al. (1998). We were not able to use conditioning temperature to clearly differentiate myceliogenic and carpogenic germination (-20 vs. $\geq 0.5^0$C), as reported by Huang (1991), using either hydrated or desiccated Sun-87 sclerotia. Additionally, we were not able to verify that a low inoculum production temperature was the main factor affecting carpogenic germination of Sun-87. Rather, a low temperature during inoculum and/or sclerotia production enhanced germination. Finally, we were not able to verify that myceliogenic germination of Sun-87 occurred most readily when sclerotia formed at 20-25^{0}C were desiccated prior to germination. Desiccation almost always resulted in carpogenic germination, albeit at a low level relative to germination of hydrated sclerotia. Additional experiments are in progress to discover a reliable and non-confounded method that clearly differentiate myceliogenic and carpogenic germination.

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Agrobacterium-mediated transformation of sunflower

Zhifen Zhang and John J. Finer
Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH

Funded Plan of Work: Use of a transformation system in sunflower for Sclerotinia resistance studies

ABSTRACT:
An efficient and reliable sunflower transformation system will be useful for basic research and characterization of mechanisms of resistance of sunflower to Sclerotinia. In spite of the multiple sunflower transformation reports in the literature, the production of transgenic sunflower continues to be quite challenging. *Agrobacterium*-mediated transformation of sunflower cells is not difficult in itself, but the targeting of those specific cells that give rise to shoots or germ line tissue remains inefficient. Previously, we have reported an increased frequency of production of shoot primordia using the RHA280 line with plant regeneration from both cotyledonary and leaf tissues. Regeneration of plants was improved when the tissues were exposed to a cytokinin pulse treatment and the induced shoots were micrografted onto scions from seedlings. Unfortunately, using standard *Agrobacterium*-mediated transformation protocols, almost all sunflower plants that were recovered were either not transformed or chimeric, and transgenic progeny were not recovered. Therefore, targeting of the regenerable sunflower cells remains limiting. Using an alternate *Agrobacterium* inoculation approach, the production of transgenic shoot primordia was increased multiple-fold, with some explants producing large numbers of transgenic shoots on each piece of tissue. We hypothesize that this inoculation method reduces the hypersensitive response of sunflower tissue to *Agrobacterium* and makes tissues more receptive to this bacterium for transformation. But, due to the nature of the new inoculation approach, the variability in transformation efficiency remains high and large numbers of chimeric shoots are produced. However, our new inoculation approach shows tremendous promise for transformation of sunflower as well as other crops.

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Characterization and Validation of Two Distinct Mechanisms for Resistance to *Sclerotinia sclerotiorum* in *Pisum sativum*

Kevin McPhee and Rahil Ashtari Mahini, North Dakota State University, Fargo, ND
Funded Plan of Work: Characterization and validation of two distinct mechanisms for partial resistance to *Sclerotinia sclerotiorum* in pea

ABSTRACT:

White mold caused by *sclerotinia sclerotiorum* (Lib.) de Bary is one of the most important fungal diseases of pea, can causes severe economic losses. Pea genotypes with partial resistance to *sclerotinia* have been previously identified. Resistance to *sclerotinia* is partial and quantitative in nature and expressed in two forms, lesion expansion inhibition (LEI) and nodal transmission inhibition (NTI), in order to use these accession in breeding program, genetic study of *sclerotinia* resistance in pea should be more validated. Recombinant inbred line (RIL) populations from crosses between PI 169603/Medora (365 individuals) and Lifter/PI 240515 (190 individuals) have been established by single seed decent and used for phenotyping and genotyping. Individual RILs will be screened in the greenhouse for LEI and NTI and for further investigation a detached stem assay method will be developed to quantify the time required for *S. sclerotiorum* to pass through the node and isolate the nodal transmission inhibition resistance phenotype. Genotype by sequencing methodology will be employed to identify single nucleotide polymorphism markers in each population and will be combined with phenotypic data to identify QTLs responsible for LEI and NTI. The *Pisum* genetic map will be enhanced and greater understanding of the interaction between pea and *Sclerotinia sclerotiorum* will be developed.

Characterization of two new recombinant inbred populations in common bean for Sclerotinia resistance

James R. Myers and Haidar Arkwazee, Oregon State University, Corvallis, OR

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

ABSTRACT:
Two recombinant inbred populations (G122/WMG904-20-3 and OSU6137/A195) have been developed and characterized for field for resistance to white mold. Both were in the F_6 generation and consisted of 80 and 114 individuals, respectively. G122 is a well characterized source of partial resistance from common bean with a major QTL on PV07. WMG904-20-3 was derived from a backcross-inbred population to introgress white mold resistance from *P. coccineus* accession PI255956 and was previously shown to have a QTL for resistance on PV03. The OSU6137/A195 population was developed from the cross of a highly susceptible blue lake green bean line to A195, a partially resistant dry bean that has not been subjected to genetic analysis. A195 is of particular interest because it expresses high levels of resistance relative to other resistant accessions such as G122 and NY6020. For the G122/WMG904-20-3 population, both parents were not significantly different from one another and showed partial resistance. The distribution of the progeny three clusters consisting of highly susceptible individuals, intermediate genotypes and a third of resistant types. The most resistant lines not significantly different from either parent but one line showed no infection at all in the trial. The distribution of resistance suggests that different QTL for resistance exist in the two parents and can be combined to achieve enhanced levels of resistance. The complexity of the distribution with three clusters may be indicative of fairly large QTL conditioning resistance. In the OSU6137/A195 population, a normal distribution was apparent for disease index. None of the RI lines showed as great a resistance to white mold as A195 although some were not significantly different. The more normal distribution of this population is indicative of several QTL with small individual effect and/or strongly influenced by environment. DNA was isolated from lines of both populations and was subjected to genotype by sequencing (GBS) in the OSU CGRB service lab. Results from the Tassel pipeline will be reported.

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Characterizing resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum*

Kishore Chittem, and Luis del Río Mendoza, North Dakota State University, Fargo, ND

Funded Plan of Work: Characterizing resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum*

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 a previously funded project by the Initiative, an RNASeq experiment was conducted to study canola – *S. sclerotiorum* interaction using two doubled haploid lines NEP32 (susceptible) and NEP63 (resistant), derived from a *Brassica napus* PI accession. From this analysis, we have identified several *B. napus* genes that were differentially expressed when inoculated with *S. sclerotiorum* compared to non-inoculated conditions. Functional categorization and gene enrichment analysis of these differentially expressed transcripts showed that genes belonging to several categories of biological processes including those involved in response to biotic and biotic stimulus, pathogenesis related proteins were significantly over represented in the upregulated gene set. Further analyses by combining RNASeq analysis and QTL data obtained from another project funded by the Initiative, we have identified potential candidate resistance genes within the five QTL regions, including a novel gene, putatively identified as ethylene responsive transcription factor (ER-TF), which was exclusively expressed in resistant parent. qRT-PCR assays did not show a close relationship between expression profiles of the putative resistance genes identified within these five QTLs and the F_2 phenotypes scored as resistant or susceptible to Sclerotinia stem rot. However, 4 out of the 5 most resistant or susceptible F_2 phenotypes had increased transcript abundance of *AOX* and the novel transcript identified in this study, *ER-TF* in response to inoculation with NE152 at either 16 or 48 HAI. Based on these results ER-TF was selected for further functional characterization. Functional characterization of the identified candidate resistance genes is being conducted.

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Discovery and Use of Novel Sources of Head and Stalk Rot Resistance in Sunflower and Studies of Asteraceae Genera Stimulating Myceliogenic Germination

Gerald J. Seiler, William Underwood, USDA-ARS, Sunflower and Plant Biology Research Unit, Fargo ND, Sam Markell and Michelle Gilley, NDSU, Department of Plant Pathology, Fargo, ND, Michael Wunsch, NDSU, Carrington Research Extension Center, Carrington, ND, and Keith Olander, Central Lakes College, Staples, MN, and Bradley Flett, Agricultural Research Council, Grain Crops Institute, Potchefstroom, South Africa

Funded Plan of Work: Discovery of novel sources of resistance to head and stalk rot in cultivated sunflower and studies of Asteraceae genera stimulating myceliogenic germination

ABSTRACT:
In 2015, we had two successful inoculated stalk rot (SR) nurseries (Carrington, ND and Grandin, ND) totaling 3,200 rows, and two successful inoculated, misted head rot (HR) nurseries (Carrington, ND and Staples, MN) totaling 500 rows evaluating germplasm in various stages of breeding for four USDA scientists' Sclerotinia projects developing SR and HR resistant germplasm. In addition to testing of the USDA materials, germplasm from the Grain Crops Institute, Potchefstroom, South Africa was evaluated for SR resistance. Sclerotinia SR resistance was successfully transferred from three wild annual *Helianthus* species into cultivated sunflower with two *H. petiolaris*, six *H. argophyllus*, and five *H. praecox* introgression lines selected as potential candidates for germplasm release based on their higher levels of SR resistance in multiple environments during 2012-2015. The advance backcross recombinant inbred (AB-RIL) population of 150 lines derived from HA 89/*H. argophyllus* (PI 494573) was evaluated for SR resistance in the field screening trials at Carrington, ND and Grandin, ND in 2015. Highly significant variation (p <0.001) for SR disease incidence was observed in both the environments. The other AB-RIL population derived from HA 89/*H. praecox* was advanced to the BC_2F_5 generation for future evaluation. Additionally, 411 families from amphiploid, hexaploid, and diploid perennial crosses were tested for SR at Carrington, ND and Grandin, ND, and 336 families for HR at Carrington, ND and Staples, MN. Families with better resistance than the recurrent parents identified in the different trials will be retested or released as a germplasm. More than 150 new early generation families of perennial *H. hirsutus*, *H. salicifolius*, and *H. occidentalis* tested in replicated SR field trials in 2015 suggested excellent SR resistance, further confirming successful gene introgression. Two interspecific HR and six SR germplasms are scheduled for release in 2016. Sunflower is unique among crops affected by *Sclerotinia sclerotiorum* in that it is susceptible to mycelial invasion of the fungus through the root system. This observation may conceivably be explained by differential susceptibility of roots of different plant species to invasion by mycelia or, alternatively, by differential stimulation or inhibition of myceliogenic germination of sclerotia. To determine if other members of the Asteraceae genera are susceptible to root invasion by mycelia and/or stimulate germination of sclerotia, we are currently evaluating a cross-section of eight Asteraceae species to determine susceptibility to *S. sclerotiorum* inoculated in soil as mycelium on millet or as sclerotia. Our preliminary results indicate that many Asteraceae species are susceptible to root invasion by mycelia. In parallel, we are evaluating susceptibility of sunflower, dry bean, and canola to root invasion by mycelia. Preliminary results of this study indicate that sunflower roots are uniquely and highly susceptible, suggesting some resistance to root invasion in bean and canola and lack of resistance to this mode of infection in sunflower and other Asteraceae.

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Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial *Glycine latifolia*

Qiong Liu, Glen L. Hartman and Leslie L. Domier, USDA-ARS2, Department of Crop Sciences, University of Illinois, Urbana, IL 61801

ABSTRACT:
Glycine latifolia, a wild perennial relative of soybean (*Glycine max*), is recognized for its resistance to multiple phytopathogenic diseases and tolerance to abiotic stresses. Better understanding of the *G. latifolia* genome and its characteristics may assist in the identification of genes underlying favorable traits in wild perennial soybean relatives. For that purpose, RNASeq and small RNA libraries were prepared using RNAs purified from various *G. latifolia* tissue types and analyzed by Illumina high-throughput sequencing, yielding over 400 million and 200 million reads, respectively. Trinity transcriptome *de novo* assembly generated more than 300,000 contigs with an N50 of 1,394 nt. Among the 158,427 protein sequences predicted by TransDecoder, 70% aligned to predicted products of *G. max* nuclear, mitochondrial or chloroplast coding sequences. The transcriptome assembly also facilitated annotation by MAKER of a draft *G. latifolia* genome sequence that predicted 59,400 protein-coding loci - similar to the number (56,044) for *G. max*. The products of predicted genes included 66 pathogenesis-related proteins and 311 putative disease resistance proteins. Small RNA data were analyzed using miRPlant for expression levels, secondary structure of the primary transcripts and genome locations utilizing the draft *G. latifolia* genome sequence as reference. Subsequent manual selection based on miRPlant results yielded 231 microRNAs (miRNAs), including 45 novel miRNAs and 186 conserved miRNAs from 41 families. The most abundantly expressed miRNAs were miR166, miR396, miR482, and miR1507, which display similar expression patterns in *G. max*. Degradome sequence analysis will be required to confirm the activity of the novel miRNAs.

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Genotypic diversity of *Sclerotinia sclerotiorum* populations from New York

Amara R. Dunn*, Sarah J. Pethybridge, School of Integrative Plant Science, Plant Pathology and Plant-Microbe Biology Section, Cornell University, Geneva, NY, 14456, and Julie R. Kikkert, Cornell University Cooperative Extension, Cornell Vegetable Program, Canandaigua, New York, 14424

ABSTRACT:
The genotypic diversity of *Sclerotinia sclerotiorum* isolates from snap, dry and lima bean fields in 2014 in New York was quantified. Isolates were collected from diseased plants as sclerotia which were dried and stored at room temperature, bisected and placed on potato dextrose agar or 10% clarified V8 agar. One hyphal-tipped isolate was obtained from each of 10 sclerotia within the ten fields. DNA was extracted from mycelia grown in potato dextrose broth after 3 to 7 days using the DNeasy Plant Mini Kit. The identity of each isolate was confirmed by PCR using species-specific primers (SSaspr F and SSaspr R) and ITS4/5. Alleles at eight microsatellite loci were amplified for each isolate. Capillary electrophoresis was conducted on an ABI 3730xl DNA Analyzer at the Cornell University Biotechnology Resource Center, Ithaca, NY. Resulting electropherograms were scored manually using PeakScanner software. Amplification and fragment analysis were repeated for 8% of isolates to confirm the reproducibility of results. One allele was amplified from each isolate at each of the eight microsatellite loci and observed allele sizes were reproducible for all loci. Twenty-four multilocus genotypes (MLGs) were detected with one MLG (MLG.4) present in all fields. MLG.4 was the most frequently detected MLG in eight of the ten fields. Moreover, between three and eight unique MLGs were detected within each field. Indices for genotypic richness, diversity and evenness were calculated prior to clone correction. The modified index of genotypic richness (R) was 0.12 and ranged between 0.11 and 0.37 within individual field populations. The Stoddart and Taylor's Index (G) of genotypic diversity ranged from 1.65 to 6.67 within individual fields and averaged across the entire population was 4.24. Across the population, the unbiased genotypic diversity, evenness and β parameter (slope) of the Pareto distribution were 0.77, 0.47, and 0.18, respectively. These findings suggest a predominantly clonal population with little evidence of outcrossing depicted by low genotypic richness and a high frequency of one MLG across fields sampled in 2014. This finding may assist in informing the effective deployment of chemical-based management strategies for white mold and support the selection of representative isolates for resistance screening to local populations.

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Identification and Enhancement of Basal Resistance to *Sclerotinia sclerotiorum* in Brassicaceae

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Funded Plan of Work: Synergistic enhancement of resistance to *Sclerotinia sclerotiorum*

ABSTRACT:
This project is investigating the potential to use a newly identified *Arabidopsis thaliana* gene hypersusceptible to *S. sclerotiorum* (*HSS1*) for engineering high levels of disease resistance in canola. Simultaneously and in combination, we have been investigating the efficacy of plant over-expression of an oxalate decarboxylase gene (*odc2*) isolated from the pathogen for enhancing disease resistance. Following fine mapping of the *hss1* mutation and confirming the identity of the *HSS1* gene by transforming candidate genes into the *A. thaliana hss1* mutant, we have determined that *HSS1* encodes a subunit of the Mediator complex. During the past year we have cloned the *HSS1* gene into pCAMBIA1300S and identified single-insertion, homozygous lines. Disease screening of these lines indicated no increase or decrease in resistance relative to the wild type. To better understand this result, we analyzed the fate of the HSS1 protein during infection. This analysis revealed that the HSS1 protein is specifically degraded during infection with *S. sclerotiorum*. We have since cloned an *HSS1* homolog from *Brassica napus* and are currently evaluating its conserved function via complementation of the *A. thaliana hss1* mutant and its resistance to proteolytic degradation during infection. We plan to screen other novel forms of the HSS1/MED16 protein from fungi and non-host plants for resistance to *S. sclerotiorum* proteolysis. As a second prong in our approach to enhancing disease resistance, we have transformed *A. thaliana* with the ODC2 over-expression construct and identified homozygous, single insertion lines. Preliminary screens for disease resistance have not exhibited individuals with increased disease resistance. Efforts to over-express a plant codon-optimized form of the *ODC2* gene and evaluate transformants for enhanced disease resistance are being pursued. Despite the finding of HSS1 degradation during infection, the identification of the *HSS1* gene represents a major step forward in identifying regulators of host defense to *S. sclerotiorum*. The Mediator complex is emerging as a master regulator of plant immunity against pathogens, especially necrotrophic fungal pathogens, which underlines our discovery of the critical role of *HSS1* in basal resistance against *S. sclerotiorum*. Efforts are now focused on identifying and developing stable forms of HSS1 and ODC2 for over-expression and evaluation of resistance.

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Identification of Major QTL Conditioning Partial Resistance to White in Dry Bean

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Funded work plan: White mold resistance-QTL: identification, interactions, and fine mapping in common bean

ABSTRACT:
Partial resistance to white mold is a useful for integrated control of white mold disease in dry bean. Our objective is to identify, verify, and find map major QTL condition partial resistance to white mold. The straw test and field reactions for two recombinant inbred populations Montrose/I9365-25 (M25, 131 F5 RILs) and UI537/I9365-25 (U25, 127 F5 RILs) were analyzed for QTL using polymorphic SNP markers from BARCBean6K_3 BeadChip. I9365-25 is a pink dry bean with moderate white mold resistance that derives from an interspecific population between *P. vulgaris* x *P. coccineus.* A major QTL WM5.4 conditioning resistance in the straw test was identified in both populations. Interesting, I9365-31, similarly derived as I9365-25 from a Pv x Pc interspecific cross, had the same WM5.4 QTL. A SNP linkage map generated for the Orion/USPT-WM-12 population consisting of 160 F5 RILs identified QTL conditioning resistance to white mold. The resistant parent is USPT-WM-12, a pinto germplasm line with a strong combination of field and greenhouse resistance. The field resistance is provided by Bunsi, but the source of the straw test resistance is unknown. Great northern Orion was the highly susceptible parent. The focus with this population is to study the nature of the greenhouse resistance. The QTL found to condition straw test reaction were WM1.2 (9%) and WM7.3 (24%). Previous RIL populations with linkage maps generated with RAPDs, SRAPs, SSRs, and other markers were revisited with SNPs generated by the BARCBean6K_3 BeadChip (5398 SNPs). The SNPs were combined with the previously generated markers to generate much denser genetic linkage maps. The QTL analysis was rerun with these denser linkage maps and the physical position of the QTL were noted. The maps include Raven/I9365-31 (R31, 100 F5 RILs) and Aztec/ND88-106-04 (AN, 80 F5 RILs). In addition, a backcross RIL population Orion*3/R31-83 (OR, 95 F5 RILs) validated QTLWM2.3, WM6.1, and WM7.1 identified in the R31 RIL population. The QTL discovered in the populations described above were compared across populations and with QTL with physical positions from recent publications. Our previous GWAS analysis of the field and straw test reaction of the Middle Diversity Panel was included. Overall, this comparative QTL analysis provides an initial visualization of the most prominent and important QTL discovered to date. It helps to sort out all the QTL results from previous studies and to reduce the number of QTL from 23 to 6 (WM2.2, WM2.3, WM3.1, WM5.4, WM6.1, WM8.3) to concentrate on first for fine mapping for marker assisted selection.

Identifying and verifying genes for defense to Sclerotinia

Wei Wei, Xing Wu, Mohammad Belaffif, and Matthew Hudson, University of Illinois, Urbana
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Funded Plan of Work: Identifying and verifying genes for defense to Sclerotinia

ABSTRACT:
Transgenic soybean carrying an oxalate oxidase gene (OxO) that encodes for an enzyme catalyzing the degradation of oxalic acid, showed enhanced resistance to Sclerotinia stem rot. A previous study characterizing the transcriptome responses of an OxO transgenic and its susceptibly parent, AC Colibri (AC), at 12, 24 and 36 hours post inoculation (hpi) by microarray analysis found that the OxO line displayed a measurably faster induction of defense responses compared to AC. To investigate the even earlier interaction between the fast growing *Sclerotinia sclerotiorum* and soybean leaves, we conducted a new RNA-Seq analysis, sampling only tissue at the site of infection, at 4 and 8 hpi. These earlier time points are expected to shed light as to when this host-pathogen interaction goes from a temporary co-existing relationship, to a hostile, combative one. By de novo assembly of soybean reads using the software program Trinity, and a generalized linear model based statistical analysis using EdgeR, we determined that 1006 soybean genes were significantly differentially expressed in at least one pairwise comparison involving time points and treatments (FDR < 0.02). These significant genes were classified into 17 functional categories according to the annotation of top BLASTx hits in NCBI (with an e-value cutoff of 10e-5). Gene expression patterns displayed by heat maps, and supported by statistical analyses, indicated that gene expression did not differ much between the two genotypes, and that most genes that were detected as changing, were consistently induced for both AC and OxO lines, with the induction increasing from 4h to 8h. Functional categories containing the largest percentages of genes are signaling (14.6%) and DNA/RNA (11.9%), such as ethylene signaling pathways and WRKY transcription factors, highlighting the importance of signaling components and transcriptional regulators in the early stages of soybean recognition and basal defense to *S. sclerotiorum*. Although most genes exhibited similar expression patterns between the two soybean genotypes, groups of genes were found to have higher fold changes at 4 hpi in OxO than in AC, including calcium signaling-related genes and ABC transporters, suggesting potential roles they play in contributing to greater resistance of OxO at later time points. Our analyses also identified 2325 Sclerotinia genes as being differentially expressed in planta versus a culture grown in liquid medium with a cut off of FDR<0.01.

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Identifying candidate genes for aggressiveness via association analysis of a diverse population of *Sclerotinia sclerotiorum*

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Funded Plan of Work: High-density genotyping of a diverse population of *Sclerotinia sclerotiorum*

ABSTRACT:
Sclerotinia stem rot caused by *Sclerotinia sclerotiorium* (Lib.) deBary is one of the most devastating soybean and dry bean diseases in the United States. Association mapping analysis using RAD-GBS genotypic data and lesion length phenotypic data collected on dry bean and soybean for 71 diverse isolates led to the identification of 10 significant marker trait associations (MTAs; -log10(p) >3) using the dry bean data following stringent false discovery rate p-value adjustment. To further validate these correlations and identify differentially expressed genes underlying these putative aggressiveness loci, RNAseq analysis was performed using the virulent strain 140MN of *S. sclerotiorium.* Three days post inoculation of dry bean and soybean, 779 and 571 *S. sclerotiorium* genes were differentially expressed in dry bean and soybean, respectively, during the host pathogen interaction when compared to the controls. Six differentially expressed *S. sclerotiorium* genes, five up regulated and 1 downregulated, common to both the dry bean and soybean infected RNAseq libraries aligned with MTAs identified in the association mapping. The upregulated genes were predicted to encode three glycosyl hydrolases (cell wall degrading enzymes), a MFS transporter (involved in the transport of fungal toxins), and a LicD domain protein (involved in cell adhesion and decreased choline uptake). The significantly down-regulated gene is similar to a choline dehydrogenase. These six genes currently represent our best candidate agressiveness genes. Functional analysis of the five upregulated candidate genes is being conducted using host induced gene silencing via *Agrobacterium* mediated transformation of *Arabidopsis* with a dual promoter siRNA vector containing a fragment of each candidate gene.

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Improved head rot resistance screening in sunflowers and impacts & implications of timing of Sclerotinia infection in dry bean, soybean & sunflower

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ABSTRACT:

This project characterized the impact of early versus late Sclerotinia disease development on seed yield and quality in dry beans, soybeans, and sunflowers and the associated implications for fungicide usage, and it evaluated whether screening for resistance to Sclerotinia head rot of sunflowers could be improved by including shattering of diseased head tissue in disease assessments.

Delays in Sclerotinia disease development affected Sclerotinia disease levels and the impact of Sclerotinia on seed yield differently in dry beans, soybeans, and sunflowers. In dry beans and soybeans, Sclerotinia was most severe when disease developed early in the bloom period, and in sunflowers, Sclerotinia was generally most severe when disease onset occurred late in the bloom period. The yield loss conferred by each percentage-point increase in Sclerotinia dropped sharply with delays in disease progression in dry beans, dropped relatively slowly with delays in disease progression in soybeans, and did not show any clear pattern relative to disease progression and the timing of Sclerotinia inoculations in sunflowers. When intensive irrigation highly favorable for Sclerotinia was imposed during mid- and late pod-fill after bloom was complete, new Sclerotinia infections were observed in soybeans but not in dry beans. The results suggest that soybeans may be more susceptible to Sclerotinia late in crop development than soybeans.

Optimal fungicide application timing in dry beans and soybeans was consistent irrespective of differences in disease progression, but the yield response and economic return to fungicide applications declined when disease development was delayed. Applying the fungicide boscalid at the early to full R2 growth stage optimized Sclerotinia control and yields in soybeans, and applying boscalid at early bloom before 100% of plants had an open blossom optimized Sclerotinia control in dry edible (pinto) beans.

Sclerotinia head rot assessment parameters that incorporated susceptibility to shattering of diseased head tissue resulted in a sharp improvement in the ability to accurately predict seed quality and a moderate improvement in the ability to accurately predict seed yield of sunflowers in a screening nursery conducted under conditions highly favorable for head rot in 2014. To evaluate the repeatability of these results, an expanded screening nursery was conducted in 2015. Disease establishment was successful, and differences in susceptibility to head rot and in susceptibility to shattering of diseased heads were observed. Seed yield and quality analysis is currently in progress.

The project improved our understanding of economic returns to fungicide applications in dry beans and soybeans relative to the timing of Sclerotinia disease development, and it provided the first assessment of the impact of differences in susceptibility to shattering of diseased heads on seed yield and quality in sunflowers.

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Molecular characterization of partial resistance in pea (*Pisum sativum*) to *Sclerotinia sclerotiorum*.

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Funded Plan of Work: Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics assisted breeding

ABSTRACT:
The molecular bases of the interaction between two *Pisum sativum* cultivars and *Sclerotinia sclerotiorum* were investigated using RNAseq. Lesion development was delayed at least 12 hours in the partially resistant PI240515 cultivar compared with the susceptible cultivar Lifter. At 24 hpi, most of Lifter plants were lodged at the point of inoculation, while PI240515 stems were all still upright with small water soaked lesions. Accordingly, the expression profiles between cultivars were most dissimilar at 24 hpi. Based on this result, two highly differential expressed genes in inoculated PI240515, ferulate 5-hydroxylase (*PsF5H*) and chalcone synthase (*PsCHS*), were selected for further analysis. These genes have previously been linked with the reinforcement of cell walls with lignin and production of flavonoid phytoalexins in response to microbial attack. Lignin staining and quantification confirmed the higher deposition of lignin in the vascular system and interfascicular cambium cells of inoculated PI240515 plants ($P < 0.05$). Furthermore, we have started characterizing *PsF5H* and *PsCHS* genes' function. We are cloning cDNA fragments of *PsF5H* and *PsCHS* genes into the PEBV RNA2 vector of the VIGS system for pea, to silence these genes, and phenotype *S. sclerotiorum* inoculated plants. We expect to observe a decrease in resistance of PI240515 plants. Meanwhile, we tested our pea varieties using VIGS harboring the fragment of *P. sativum phytoene desaturase* (PDS), as a proof of concept. Both PI240515 and Lifter showed the expected phenotype, i.e. photo-bleached leaves (100% and 83%, respectively). Furthermore, we are cloning the full length *PsF5H* and *PsCHS* genes, and transiently expressed them in *Nicothiana benthamiana* leaves. After 12, 24, 48 and 72 hours post-inoculation, the plants will be infected with *S. sclerotiorum*, and disease index will be documented. Alongside, we have optimized the protocol to quantify diferulates using GC-MS on mock- inoculated peas. Next, we will analyze both inoculated and mock-inoculated plants and compare the levels of diferulates between the two pea varieties and how they affect disease resistance.

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Physical Mapping QTL for Resistance to White Mold from Multiple Common Bean Sources

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

ABSTRACT:
White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, adversely impacts common bean by limiting its yield potential and reducing the quality of seeds and pods. The interaction of the pathogen with its host is complex, and host tolerance is inherited as a quantitative trait with low to moderate heritability. The development of dense physical maps, as the basis for implementing genetic and molecular approaches to accelerate the rate of genetic gains in a breeding program represents a significant challenge. Towards fine mapping major QTL for partial resistance to white mold in common bean we genotyped five RIL populations Raven/I9365-31, Aztec/ND88-106-04, Montrose/I9365-25, UI-537/I9365-25, Orion/USPT-WM-12, and one backcross RIL population Orion*3/R31-83, with the BARCBean6K_3 BeadChip (5398 SNPs). These populations were characterized for reaction to white mold in replicated field and greenhouse straw test trials. The QTL analyses revealed 12 different QTL (6 QTL for field, 3 for straw test and 3 for both) on chromosomes Pv01, Pv02, Pv03, Pv05, Pv07, Pv09 and Pv10. Note that QTL found in Raven/I9365-31 and Aztec/ND88-106-04 using the dense SNP maps were comparable to the QTL previously found using partial linkage maps generated from bulked-segregant analysis. Toward meta-QTL analysis, the physical position of these 12 QTL were compared with previously reported QTL positions in the literature. Major or meta-QTL will be targeted for fine mapping and development of markers for marker-assisted selection.

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Progress on Transferring Sclerotinia Resistance Genes from Wild Perennial *Helianthus* Species into Cultivated Sunflower

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Funded Plan of Work: Transferring Sclerotinia Resistance Genes from Wild *Helianthus* Species into Cultivated Sunflower

ABSTRACT:
Wild perennial *Helianthus* species are highly resistant to Sclerotinia stalk and head rot. Six interspecific amphiploids have shown excellent stalk and head rot resistance. In order to introgress the resistance genes from wild *Helianthus* species, crosses and backcrosses have been conducted between amphiploid, hexaploid, tetraploid and diploid perennials with cultivated sunflower (HA 410, HA 441, HA 451 or NMS HA 89). The backcross progenies with 2n=34 chromosomes derived from different crosses were evaluated in replicated trials in 2009-2015. In 2015, 411 families were tested for stalk rot at Carrington, ND and Grandin, ND, and 336 families for head rot at Carrington, ND and Staples, MN. Families with better resistance than the recurrent parents identified in the different trials will be retested. Families derived from amphiploids and wild perennial species, including *H. californicus*, *H. maximiliani*, *H. nuttallii*, *H. giganteus*, *H. grosseserratus* and *H. strumosus*, indicated moderate to excellent stalk and head rot resistance, further confirming successful gene introgression. Nearly 400 early generation families of *H. hirsutus*, *H. salicifolius*, *H. occidentalis*, and *H. divaricatus* tested or retested in replicated stalk rot trials in 2015 indicated excellent stalk rot resistance. Seed was increased in the field for more than 500 progeny families derived from different crosses in 2015, including eight Sclerotinia stalk and head rot tolerant germplasm bulks based on field screening from 2009-2015. Agronomic data were collected for the germplasms to be released. Progenies with 2n =34-37 chromosome number derived from various crosses were backcrossed with HA 410, HA 451 or NMS HA 89 to stabilize chromosome numbers and enhance further recombination. These materials will provide additional potential pools of resistance genes and increase the probability of identifying useful resistance QTLs. The BC_2F_1 generation for the new crosses involving *H. strumosus*, *H. tuberosus*, and *H. simulans* with improved seed set percentage were obtained by backcrossing with HA 410 or NMS HA 89 at BC_1F_1. Further backcrossing is in progress to reduce the chromosome numbers, and to improve the pollen fertility and seed set. Eight Sclerotinia head and stalk rot resistant germplasm bulks derived from five perennial species will be released.

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Quantitative Trait Loci Analysis of White Mold Avoidance in Pinto Bean

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Funded Plan of Work: Validating QTL for White Mold Resistance in Mesoamerican Beans

ABSTRACT:
White mold is a major disease of common bean (*Phaseolus vulgaris* L.) grown in temperate production areas. The objective of this study was to use single nucleotide polymorphism (SNP) markers from the BARCBean6K_3 BeadChip to identify quantitative trait loci (QTL) associated with traits related to white mold resistance in common bean. A recombinant inbred line (RIL) population from a cross of disease-tolerant pinto line AN-37 and disease-susceptible line P02630 was evaluated in Michigan for 4 yr under white mold pressure. Traits evaluated included disease incidence, the numbers of days to flower and to maturity, canopy height, lodging, seed yield, and 100-seed weight. A linkage map of the RIL population spanning 1499 cM was constructed using 447 SNP markers. The map covered all 11 bean chromosomes with an average distance of 3.6 cM between markers. A total of 13 QTL for agronomic and disease traits were consistently identified in different years. A major QTL WM3.1^{AN} associated with white mold avoidance was validated on chromosome Pv03. The QTL is associated with disease avoidance traits such as canopy porosity, plant height, stay green stem trait, and maturity. Quantitative trait loci for maturity and canopy height also mapped to the same genomic region on Pv03. Finding strong associations between maturity, lodging, canopy height, and disease incidence offers alternative strategies to improve levels of white mold avoidance over greenhouse screening. The validation of the WM3.1$^{AN, AP630}$ QTL for white mold avoidance should provide bean breeders with the opportunity to introgress avoidance traits into their germplasm.

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Relationship between environmental conditions and *Sclerotinia sclerotiorum* apothecia production, ascospore release and primary plant infection of soybean

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ABSTRACT:
Fungicides applications are a component of an integrated management system of white mold in soybean. However, a difficulty that most growers face is identifying the best timing for fungicide applications to get the optimum level of control against white mold. In 2014 Michigan fungicide trials, it was noted that an application at the beginning of pod development (R3) tended to control disease and increase yield over an application at beginning of flower (R1). The main objective of this study is to improve knowledge of the timing for apothecia development and ascospore release to determine optimal timing of fungicide application in soybean. In 2015, plots of soybean cultivar AG2535 were established at the MSU Montcalm Research Farm in Entrican. To study timing for apothecia development and ascospore release, eight 14-inch and eight 30-inch row soybean plots (12m x 6m) were used for this study. Also, to assess efficacy of foliar fungicides for white mold management, eight 14-inch and eight 30-inch row soybean plots (12m x 2.3m) were established in the same location. In each row spacing field, experimental design was a randomized complete block, four treated plots with Endura applied at the beginning of flowering (R1 growth stage) and four control plots (non-treated). The number of apothecia, airborne ascospores and the disease intensity (incidence and severity) were monitored two times per week. In addition, five plants were randomly collected in each plot of the fungicide trial. Apothecia were first observed in the 14-ich row (growth stage R1-R2), eight days before they were observed in the 30-inch soybean plots (growth stage R3). The maximum number of apothecia was 50 times higher in the 14-inch plots than in the 30-inch plots. Also, incubated plants were infected up to 13 and 18 days prior to symptom development in the 14-inch and 30-inch soybean fields, respectively. White mold pressure was very high (untreated plot averaging 0.97 disease incidence) and there were no significant differences between untreated and treated plot in term of disease incidence and disease severity index. However, there is a significant difference between treated and untreated plots for the first 5 and 10 days after application in the 14-inch and 30-inch soybean fields, respectively. Data collected are being used in a collaborative modelling project between Wisconsin and Michigan.

RNA-Seq data analysis of soybean leaves infected with Sclerotinia

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Steven J. Clough, USDA-ARS and University of Illinois, Urbana
Laureen Blahut-Beatty, Lisa Koziol and Daina Simmonds, AAFC, Ottawa

Funded Plan of Work: Identifying and verifying genes for defense to Sclerotinia

ABSTRACT:
Oxalic acid (OA) is a major virulence factor for *Sclerotinia sclerotiorum*, which causes Sclerotinia stem rot. OA has been suggested to play multiple roles during disease development, especially in the early stage of infection when this necrotrophic pathogen and host maintain a temporarily co-existing relationship. This study involved an RNA-Seq experiment to explore the difference of transcriptome level regulation between a susceptible cultivar (AC-Colibri) and a transgenic resistant line that contained the oxalate oxidase (OxO) gene at 4h and 8h post inoculation. The initial step of the analysis was removing sequence reads that originated from the pathogen (nearly 2% of total reads) by aligning each library to the Sclerotinia genome. The remaining sequence reads were assumed to be from soybean, and they were assembled into de novo transcriptome contigs using De Bruijn graph oriented program Trinity. The N50 for the Trinity assembly was 2103 nt, and the alignment rate for each library was nearly 95% in pair-end mode, using Bowtie2 and RSEM for alignment and counting, respectively. Differential gene expression analysis was done in EdgeR. Overall 1139 trinity genes were selected with at least 1 CPM in one of the treatments, and a FDR value less than 0.02. Annotation for each differentially expressed gene was given according to the BLASTx results in the NCBI database. A total of 1006 Differential expressed genes were assigned as soybean genes, and the rest 133 genes were assigned as Sclerotinia genes based on NCBI annotation. Gene set enrichment analysis was done and all soybean genes were fitted into a total of 17 functional categories, in which signaling and DNA/RNA categories were overrepresented. Heatmaps were generated in R to help represent the overall expression profiles.

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Sclerotinia stalk rot resistance in sunflower: QTL mapping and gene introgression from wild *Helianthus* species

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Research Project: Identification of major genes-QTL for Sclerotinia resistance in cultivated sunflower and wild *Helianthus*

ABSTRACT:
Stalk rot (SR) caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is a serious disease of sunflower (*Helianthus annuus* L.) in the cool and humid areas of the world. A recombinant inbred line (RIL) population of 106 lines derived from a cross between the two highly SR tolerant sunflower lines, HA 441/RHA 439 were evaluated in inoculated fields trials at multiple locations in North Dakota and Minnesota during 2012-2014. Highly significant genetic variation (p <0.001) for SR disease incidence (DI) was observed in the RIL population. The phenotypic variation of SR resistance revealed transgressive segregation in the RIL population across all environments, where some of the progeny showed more extreme phenotypes than either of the parents. Genotyping-by-sequencing (GBS) approach was adopted to discover single-nucleotide polymorphism (SNP) markers and simultaneously genotyping the RIL population. A genetic linkage map was developed comprised of 1,049 SNP markers on 17 linkage groups (LGs) spanning 1,431.05 cM. Quantitative trait loci (QTL) for SR resistance were identified in each environment separately, with consensus reached on the number and precise position of the repeatedly mapped QTL across environments using a QTL-meta analysis. Ten significant QTL were identified; four meta-QTL spanning 15.27 cM on LG10, each explaining between 18 and 36% of the observed phenotypic variance. The remaining six significant QTL, two each on LGs 11 and 17 and one each on LGs 9 and 16 were identified in only one environment, each explaining between 9 and 26% of the phenotypic variance. Alleles conferring increased resistance were contributed by both parents. The potential utility of the important QTL for marker assisted selection (MAS) was analyzed and the SNP markers flanking these QTL were converted into allele-specific PCR based markers for immediate use.
We have successfully transferred SR resistance from three wild annual *Helianthus* species into cultivated sunflower and finally selected two *H. petiolaris*, six *H. argophyllus*, and five *H. praecox* introgression lines as potential candidate for germplasm release based on their higher levels of SR resistance in multiple environments during 2012-2015. A whole genome scan was performed using genotyping-by-sequencing (GBS) approach to detect the presence of the wild introgression segments in the selected lines. Polymorphic SNP markers revealed the presence of wild segments in the cultivated sunflower background located on LGs1, 3, 8, 9, 10, and 11; some of these might be associate with SR resistance.
The advance backcross recombinant inbred (AB-RIL) population of 150 lines derived from HA 89/*H. argophyllus* (PI 494573) was evaluated for SR resistance in the field screening trials at Carrington and Grandin, ND in 2015. Highly significant variation (p <0.001) for SR disease incidence (DI) was observed in both the environments. The other AB-RIL population derived from HA 89/*H. praecox* was advanced to the BC_2F_5 generation for future evaluation.

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Sclerotial development and pathogenicity of *Sclerotinia sclerotiorum* depend on low pH, independent of oxalic acid

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Funded Plan of Work: Comparative transcriptomics of *Sclerotinia sclerotiorum* infecting grain legumes for genomics assisted breeding.

ABSTRATCT

The fungus *Sclerotinia sclerotiorum* causes the devastating whit mold disease on more than 400 plant species including many economically important crops such as beans, canola, sunflower, soybean and cool season legumes, and produces copious (up to 50 mM) amounts of oxalic acid, which, for over a quarter century, has been claimed as the pathogenicity determinant. The claim was based on UV-induced mutants that concomitantly lost oxalic acid production and pathogenicity. The evidence so far supporting such a claim has not fulfilled the molecular Koch's postulates because the UV mutants are genetically undefined and harbor a developmental defect in sclerotial production. The inability to develop sclerotia is a developmental defect, which alone could explain lack of pathogenicity. In this study, using two independent mutagenesis techniques, we generated mutants of *S. sclerotiorum* that completely lost oxalic acid production, and tested the resulting mutants for growth at different pHs and for pathogenicity on four host plants (faba bean, pea, green bean and soybean). The oxalate-minus mutants accumulated fumaric acid, produced functional sclerotia and have reduced ability to acidify the environment. The oxalate-minus mutants retained pathogenicity on plants, but their virulence varied depending on the pH and buffering capacity of host tissue. Acidifying the host tissue enhanced virulence of the oxalate-minus mutants, whereas supplementing with oxalate did not. These results suggest that it is low pH, not oxalic acid *per se*, that establishes the optimum conditions for growth, reproduction, pathogenicity and virulence expression of *S. sclerotiorum*. Exonerating oxalic acid as the primary pathogenicity determinant will stimulate research into identifying additional candidates as pathogenicity factors towards better understanding and managing Sclerotinia diseases.

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Small RNA processing in *Sclerotinia sclerotiorum*

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Funded Plan of Work (2013): Identification of viruses infecting *Sclerotinia sclerotiorum* and their potential use as a biological fungicide

ABSTRACT:
RNA silencing pathways are under-studied in plant pathogenic fungi. Once elucidated, they can be utilized to improve plant health by more effectively silencing fungal genes from host plants or viral vectors. In this study, we characterized changes in gene expression and small RNA accumulation in *Sclerotinia sclerotiorum*, a damaging and widely distributed fungal plant pathogen, in response to hypovirus infection. Our results indicated that the fungus produced small RNAs of predominantly 22 nt. The *S. sclerotiorum* genome was predicted to contain 333 families of microRNA-like (milRNA) genes, which similar to other recently characterized fungal species, were not conserved with other eukaryotes. Degradome analyses confirmed that 39 of the predicted milRNAs mediated cleavage of *S. sclerotiorum* mRNAs. Consistent with the role of RNA silencing in defense against mobile and infectious genetic elements, most of the small RNA sequences in virus-free *S. sclerotiorum* were derived from retrotransposons. Similarly, large amounts of predominantly 22 nt sequences were detected from the hypovirus genome, indicating that it also was targeted for degradation by host RNA silencing pathways. These findings suggest that virus-induced hypovirulence could result from disruption of normal RNA silencing pathways and that a robust RNA silencing pathway exists in *S. sclerotiorum* that can be exploited for RNA-based pathogen resistance strategies.

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Sources of white mold resistance derived from wide crosses in common bean and progress in characterization of relevant pathogen isolates

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Collaborators: J. Kelly (MI), H. Rietman (Bel), M. Wunsch (ND), J. Myers (OR), P. Miklas (WA), M. Brick (CO), 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:
One of our goals is to test putative sources of resistance in adapted backgrounds at multiple sites located in most of the major bean-production areas of the northern states. Multi-site testing allows annual bean evaluation data when some sites have no data due to weather. A straw test that consistently identifies sources of resistance in adapted and unadapted bean germplasm is used for greenhouse tests where only 240 bean seeds are needed to confirm resistance. Best performing lines are released to public and private breeders. A number of bean lines with intermediate resistance to white mold are poised to be released in 2016. Remaining inconsistencies in verifying WM resistance levels may be due to genetic variation of the pathogen between locations. The only previous study on pathogen variation in screening nurseries in bean production (or any crop system) areas in the U.S. was published in 2011 by our lab. No other relevant studies exist. Aggressiveness differences and genetic variation using molecular markers have identified *S. sclerotiorum* isolate variation that could impact resistance evaluation studies. Low number of grower isolates has negatively affected our ability to compare pathogen variability from screening sites and growers' fields within production areas. New producer field isolate collections are planned. Sensitivity of *S. sclerotiorum* isolates to a number of fungicides is a new character we are testing on both mycelial growth using a high-throughput approach and on ascospores. Most isolates have shown less sensitivity to the fungicide thiophenate-methyl. Genetic analysis of 366 isolates using a subset of 16 microsatellite markers revealed that geographically and temporally distant populations showed little to no significant population differentiation. For example, populations from France and Australia were not genetically distinct from U.S. populations. This is a surprising result considering the soil-borne nature and infrequent sporulation of this pathogen, which would lead us to expect differentiation of populations between fields. Although our results are congruent with previous studies that indicated populations of *S. sclerotiorum* are mostly clonal, lack of differentiation may be due to existing SSR markers not providing sufficient resolution. A second goal is to make available, through the NSI or other websites, information on characterized isolates that we can supply to plant breeders and pathologists to use in screening for resistance. This would give private and public plant breeders and pathologists flexibility in selecting resistance screening isolates to match their desired level of resistance and use across local or diverse production regions. The overall approach we are using in our bean research is also applicable to facilitate identification of white mold resistance in other susceptible crops such as canola, pulses, soybean and sunflower.

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Strategies to identify functionally significant soybean defense genes against *Sclerotinia sclerotiorum*

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Funded Plan of Work: Identifying and verifying genes for defense to Sclerotinia

ABSTRACT:
Our goal is to identify soybean genes that confer resistance to *Sclerotinia sclerotiorum* to facilitate breeding applications and to gain a better understanding of plant-pathogen interactions. We have previously shown that a wheat enzyme, oxalate oxidase (OxO), confers superior resistance when expressed in soybean as it catalyzes the degradation of oxalic acid (OA), the major virulence factor of the fungus. This OxO resistant line and its susceptible parent were used to study changes in gene expression following fungal infection and OA infiltration in order to identify candidate defense genes. Bioinformatic analysis of microarrays and RNA-seq identified hundreds of candidate defense genes. The definitive validation of the defensive roles of these candidate genes is best carried out in soybean by silencing or overexpression. However, because soybean transformation is resource and time intensive, high-throughput screening systems are being examined to narrow down the numbers of genes for final analysis in soybean. We are exploring three systems, 1) a viral vector system using bean pod mottle virus, 2) a transient expression system by Agro-infiltration of *Nicotiana benthamiana,* and 3) a systemic silencing system. The viral vector system is effective in silencing a gene of interest, whereas Agro-infiltration is efficient to examine over-expression. Progresses and the challenges of each system will be presented. To date, soybean transformation has been used for functional analysis of three genes and identified a regulatory protein (14-3-3) as a potential resistance gene. We noted that constitutive silencing or overexpression of some candidate defense-related genes resulted in abnormal and/or infertile plants. As some of these genes may function in other plant processes, we are considering promoters that are inducible, i.e. DEX- (dexamethasone), JA- (jasmonic acid), pathogen-, and estrogen-inducible promoters. We observed that the DEX- and JA- promoters were induced 2-10 fold upon induction in soybean, however this level of expression is much lower than that of a constitutive CaMV 35S promoter. This suggests that such inducible promoters may be useful in expressing regulatory molecules that act upstream of defense signaling.

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The potential of genomic selection to improve white mold resistance in soybean

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

Abstract
Genomic Selection (GS) is a new breeding method in which genome-wide markers are used to predict breeding values of complex traits for individuals in a breeding population. The objective of our research was to investigate the potential of genomic selection to improve white mold resistance in soybean. The research was based on experimental data of two soybean populations consisting of 432 elite soybean cultivars and 280 soybean plant introductions (PIs). The two populations were phenotyped for white mold resistance in disease nursery and were genotyped with 52,041 markers using SoySNP50K Illumina BeadChip. Genomic best linear unbiased prediction (gBLUP) method was used to predict genomic estimated breeding values (GEBVs) of disease severity index (DSI) in combination with fivefold cross validation. Prediction accuracies of DSI were 0.64 and 0.56 for elite soybean lines and PIs, respectively. Genomic prediction models outperformed over the prediction based on the significantly associated marker alone. Analyses using subsets of the full marker set suggest that using one marker every 500kb (about 2000 genome-wide SNPs) is sufficient for genomic selection in both of the two populations. Our results suggest that GS could become an effective tool for increasing the efficiency of developing soybean cultivars with white mold resistance as the costs of genotyping continue to decline.

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Using Common Bean Seedlings for White Mold Resistance Evaluation via the Straw Test

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

ABSTRACT:
Common bean (*Phaseolus vulgaris* L.) is one of the most significant legume crops which have been grown for its dry seeds and edible pods worldwide. White mold, caused by *Sclerotinia sclerotiorum* (Lib.), is considered one of the most pathogens of bean that can cause up to 100% yield loss under certain conditions. Generally, field trials and the straw test have been used to evaluate accessions for white mold resistance by measuring disease incidence and severity, while for straw test, only severity is normally measured. This experiment was conducted to modify the existing straw test protocol by using seedlings, at about 16 days from sowing seeds, instead plants with four to five nodes, at about 30 days from sowing seeds. Six common bean accessions were used to compare both methods. G122 and NY6020-5 are both highly resistance, Ex Rico has moderate resistance, and Oregon 5630, Oregon 91G, and Beryl are highly susceptible. Both conventional and seedling test methods showed similar results when analyzed separately. G122 and NY6020-5 were highly significantly more resistant compared to the other accessions; the severity score were 2.9 and 3.0 for G122 and NY6020-5, respectively using seedling straw test and 5.08 for both of them using conventional straw test. While no significant difference was observed between G122 and NY6020-5 in both methods, mycelia invaded 97.4, 100.0, 100.0, and 94.2% of the main stem of the Ex Rico, Oregon 5630, Oregon 91G, and Beryl seedlings, respectively after seven days. However, only 36.7 and 23.5% of the main stem of the G122 and NY6020-5 seedling were colonized. In addition, the rate of colonization was on average 0.58 mm/h for the susceptible group and 0.3 mm/h for the resistant accessions. The significant benefits of using seedlings to evaluate white mold resistance by conducting straw test are reduced time, efforts and resources compared to the conventional method.

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Using genomic selection to optimize prediction of *Sclerotinia* and agronomic phenotypes for more efficient breeding

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Funded Plan of Work: Using GS to optimize prediction of Sclerotinia and agronomic phenotypes for more efficient breeding.

ABSTRACT:
Sunflower breeding has made huge gains in disease resistance and quality traits that are simply inherited, but lacks efficiency to adequately deal with Sclerotinia resistance, because of the complex genetic architecture. Many genes of small effect must work in concert to facilitate partial resistance. Lines exist with high levels of resistance in the field, as demonstrated by recent germplasm evaluations. They do not, however, bring the most favorable yield and agronomic characteristics to sunflower hybrids. The primary goal of this work is to better balance the intensity and efficiency of selection for Sclerotinia resistance and other agronomic traits, to make more breeding progress per generation on all traits proportional to their actual value to the producer. Genomic Selection (GS) is a new statistical technology we would like to investigate for this purpose. In the past year, we have conducted resequencing of all 2009 to current experimental inbred lines from the USDA sunflower breeding program that have both yield and Sclerotinia resistance data from specialized nurseries. We have also developed a relational database system that will allow us easy access to nursery, field phenotyping trial, and genomic datasets to conduct model fitting. In the next several months, we will begin analyzing the phenotypic data for each line with respect to its genotype to determine whether random effect predictors in the form of molecular markers could improve accuracy of selection for Sclerotinia and yield in early generations of inbred lines. This could potentially replace unreplicated testing in early generations or, in other words, allow for preliminary line performance prediction in the absence of field data.

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Meeting Room Specifications

	Orchard Ballroom					The Grove Room					The Empire			Apple Terrace	Executive Conference Room	Pippins Room	Fuji Room	Taylor
	Cortland	Fireside	Jonathan	McIntosh	Combined	Beacon	Duchess	Regent	Waldorf	Combined	Braeburn	Melrose	Combined					
Dimensions	31' x 47'	28' x 47'	22' x 47'	22' x 47'	103' x 47'	20' x 25'	20' x 25'	20' x 25'	18' x 25'	93' x 20'	25' x 25'	31' x 25'	62' x 25'	75' x 16'	24' x 23'	39' x 16' x 36'	19' x 25'	20' x 38'
Square Feet	1,457	1,316	1,034	1,034	4,841	500	500	500	360	1,860	625	775	1,400	1,200	550	1,400	475	760
Seating Style	Seating Capacity																	
Theater	190	170	140	140	750	60	60	60	30	210	70	80	160	120	60	150	40	70
Classroom	100	80	60	60	300	27	27	27	18	110	38	42	84	72	30	64	27	40
U-Shape	48	40	32	32	140	18	18	18	12	66	22	24	48	40	18	N / A	16	24
Hollow Square	62	54	48	48	172	30	30	30	28	78	28	40	66	72	30	40	19	30
Conference	62	54	48	48	176	24	24	24	16	88	20	30	60	44	24	24	18	26
Banquet Rounds	130	110	80	80	450	40	40	40	20	150	30	50	100	110	40	120	30	60
Ceiling Height	10'8"	10'8"	10'8"	10'8"	10'8"	8'	8'	8'	8'	8'	9'	9'	9'	10'	8'	10'	9'	9'

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