National Sclerotinia Initiative

NDSU/USDA-ARS

2011 Sclerotinia Initiative Annual Meeting

Bloomington, MN

January 19-21, 2011






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Holiday Inn & Suites, Bloomington, MN

AGENDA

2011 Sclerotinia Initiative Annual Meeting
January 19-21, 2011

Wednesday – January 19, 2011

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

Thursday – January 20, 2011

7:30 am	Registration/Continental Breakfast	McIntosh/Jonathan Room
8:15 am	Welcome, Introductions & Meeting Charge – **Bill Kemp, USDA-Agricultural Research Service, Fargo, ND**	
8:20 am	Welcome from the Northern Plains Area – **Mickey McGuire, USDA-Agricultural Research Service, Fort Collins, CO**	
8:30 am	***Invited Panel: Augmenting Genetic Tolerance with Novel Fungicide Chemistries***	
9:45 am	Discussion Break	Ballroom Foyer

Sclerotinia Research Activities – Session 1 Fireside Room
Moderator – Mark Brick, Colorado State University, Fort Collins, CO

10:15 am	Improved resistance in common bean through multi-site screening and pathogen characterization throughout major production areas – **Jim Steadman, University of Nebraska, Lincoln, NE**
10:30 am	Genetic variation and virulence of *Sclerotinia sclerotiorum* on six crops in the United States **– Berlin Nelson, North Dakota State University, Fargo, ND**
10:45 am	The requirement for oxalate during pathogenesis on multiple crops – **Jeff Rollins, University of Florida, Gainesville, FL**
11:00 am	On-farm validation of cultural practice adjustments to improve white mold management in dry bean irrigation systems – **Howard Schwartz, Colorado State University, Ft. Collins, CO (Mark Brick, presenting)**
11:15 am	(1) Genetic and phenotypic diversity of *Sclerotinia sclerotiorum* on a small geographic scale and **(**2) Screening accessions of wild relatives of pea for resistance to *Sclerotinia sclerotiorum* – **Weidong Chen, USDA-ARS, Pullman, WA**

11:30 am	(1) Optimizing management of *Sclerotinia* diseases through fungicide use (2) Defining critical environmental and biological parameters required for development of *Sclerotinia* stem rot in canola and (3) Development of canola breeding populations and identification of herbicide-tolerant breeding lines with resistance to *Sclerotinia sclerotiorum* – **Luis del Rio, North Dakota State University, Fargo, ND**	
11:45 am	Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection using next generation sequencing – **Rubella Goswami, North Dakota State University, Fargo, ND**	
Noon	Working Lunch	McIntosh/Jonathan Room
	Sclerotinia Research Activities – Session 2 **Moderator – Rubella Goswami, North Dakota State University, Fargo, ND**	Fireside Room
1:30 pm	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding – **Martin Chilvers, Michigan State University, East Lansing, MI)**	
1:45 pm	Identification of novel loci for resistance to *Sclerotinia* stem rot in perennial soybean accessions **– Leslie Domier, USDA-ARS, Urbana, IL (Glen Hartman, presenting)**	
2:00 pm	Enhancing soybean for resistance to *Sclerotinia* stem rot **– Dechun Wang, Michigan State University, East Lansing, MI**	
2:15 pm	Functional verification of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and *Arabidopsis* – **Steve Clough, USDA-ARS, Urbana, IL**	
2:30 pm	Candidate genes for fungal resistance: Mapping and SNP development for LysM-domain encoding genes in soybean – **George Graef, University of Nebraska, Lincoln, NE**	
2:45 pm	Fine mapping of quantitative resistance genes to *Sclerotinia* stem rot in two soybean populations – **Glen Hartman, USDA-ARS, Urbana, IL**	
3:00 pm	Break & Poster Session	McIntosh/Jonathan Room
	Sclerotinia Research Activities – Session 3 **Moderator – Brent Hulke, USDA-ARS, Fargo, ND**	Fireside Room
3:15 pm	White mold resistance QTL: Identification, Interactions, and fine mapping in common bean – **Phil Miklas, USDA-ARS, Prosser, WA; James Myers, Oregon State University, Corvallis, OR; Phil McClean, North Dakota State University, Fargo, ND**	

3:30 pm	Gamete selection for simultaneously pyramiding and introgressing white mold resistance from Phaseolus species into pinto beans – **Shree Singh, University of Idaho, Kimberly, ID**
3:45 pm	Expression of the oxalate oxidase gene in transgenic lentils and evaluation of transgenic plants for resistance to *Sclerotinia sclerotiorum* – **George Vandemark, USDA-ARS, Pullman, WA**
4:00 pm	Identification of QTL for white mold resistance in pinto bean – **James Kelly, Michigan State University, East Lansing, MI**
4:15 pm	Genetic resistance to white mold derived from multiple sources of common bean and scarlet runner bean – **Mark Brick, Colorado State University, Fort Collins, CO**
4:30 pm	Wrap-up & Adjourn (Dinner on your own)

Friday – January 21, 2011

7:00 am	Steering Committee Breakfast Meeting	Beacon Conference Room
7:15 am	Continental Breakfast	McIntosh/Jonathan Room

***Sclerotinia* Research Activities – Session 4**
Moderator – George Vandemark, USDA-ARS, Pullman, WA — Fireside Room

8:15 am	Discovery of novel sources of resistance to head rot and stalk rot in cultivated sunflower and wild *Helianthus* **– Tom Gulya, USDA-ARS, Fargo, ND**
8:30 am	Evaluation of sunflower hybrids and germplasm for resistance to *Sclerotinia* **– Blaine Schatz, North Dakota State University, Carrington, ND (Michael Wunsch, presenting)**
8:45 am	Transferring *Sclerotinia* resistance genes from wild *Helianthus* species into cultivated sunflower – **Chao Chien Jan, USDA-ARS, Fargo, ND**
9:00 am	Use of a transformation system in sunflower for *Sclerotinia* resistance studies – **John Finer, Ohio State University, Wooster, OH**
9:15 am	Evaluation of wild *Helianthus* species for resistance to *Sclerotinia* stalk rot – **Charles Block, USDA-ARS, Ames, IA**
9:30 am	Pyramiding *Sclerotinia* head rot and stalk rot resistances into elite sunflower breeding lines with the aid of DNA markers – **Brent Hulke, USDA-ARS, Fargo, ND**

9:45 am	Characterization of the genetic basis for partial resistance to *Sclerotinia sclerotiorum* in pea – **Kevin McPhee, North Dakota State University, Fargo, ND; Lyndon Porter USDA-ARS, Prosser, WA (Behzod Tashtemirov, presenting)**	
10:00 am	Break	Ballroom Foyer
	Sclerotinia Initiative Research: The next steps **Moderator – Bill Kemp, USDA-ARS, Fargo, ND**	Fireside Room
10:15 am	***Guest Speaker*** Strategic Planning & Reporting Progress – **Rich Wilson (USDA-Agricultural Research Service, Office of National Programs–Retired, Raleigh, NC)**	
10:45 am	Strategic Plan Discussion, RFPP Process Improvement, Updating Website, On-line reprints & Fact sheets, Assignment of Additional Tasks & Wrap-up of Initiative Business	
11:45 am	Working Lunch	McIntosh/Jonathan Room
1:30 pm	Adjourn (Travel Safely!)	

2011 Sclerotinia Initiative Poster Session

Crop Germplasm Resources & Genetics		
Poster No.	Title	Author(s)
1	Searching for resistance to Sclerotinia white mold in wild relatives of pea	W. Chen, C. Coyne
2	Discovery of novel sources resistance to head rot and stalk rot in cultivated sunflower and wild *Helianthus*	T. Gulya, N. Balbyshev, B. Hulke
3	Fine mapping of quantitative resistance genes to Sclerotinia stem rot in two soybean populations	G.L. Hartman
4	Transferring Sclerotinia stalk rot resistance genes from wild *Helianthus* species into cultivated sunflower	Z. Liu, X. Cai, G.J. Seiler, T.J. Gulya, K.Y. Rashid, C.C. Jan
5	QTL analysis for white mold in pinto bean	W. Mkwaila, J.D. Kelly
6	Characterization of the genetic basis for partial resistance to *Sclerotinia sclerotiorum* in pea	K. McPhee, B. Tashtemirov, L. Porter
7	Synthesis of white mold QTL efforts in Phaseolus coccineus x P. vulgaris backcross inbred populations	J.R. Myers, S.J. Zimmerman, J.E. Haggard, J. Davis, D. Kean
8	Transferring white mold resistance from the secondary gene pool of common bean	S.P. Singh, H.F. Schwartz
9	Gamete selection for simultaneously pyramiding and introgressing white mold resistance from *phaseolus* species into pinto bean	S.P. Singh, H.F. Schwartz
10	Breeding and quantitative genetics advances in sunflower Sclerotinia research	B.S. Hulke, Z.I. Talukder, L. Qi, T.J. Gulya
11	Enhancing soybean for resistance to Sclerotinia stem rot	D. Wang, R. Kandel, C. Gu
12	Use of a transformation system in sunflower for Sclerotinia resistance studies	J. Finer, Z. Zhang

Pathogen Biology & Development		
Poster No.	Title	Author(s)
13	Genetic and phenotypic diversity of *Sclerotinia sclerotiorum* on a small geographic scale	R. Attanayake, L. Porter, D. Johnson, W. Chen
14	Aggressiveness of *Sclerotinia sclerotiorum* from the north central United States on multiple crops	L. Aldrich-Wolfe, S.E. Travers, B.D. Nelson
15	The requirement for oxalate during pathogenesis on multiple crops	J. Rollins, M. Li, D. Liberti, C. Roeser
16	Characterization of Sclerotinia sclerotiorum isolates from dry and snap bean production areas across the USA	S. McCoy, J.R. Steadman, B. Higgins, L. Otto-Hanson

2011 Sclerotinia Initiative Poster Session

Pathogen & Host Genomics		
Poster No.	Title	Author(s)
17	Identification and functional analysis of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and Arabidopis	L. Blahut-Beatty, L. Koziol, D. Simmonds, B. Calla, D. Neece, O. Radwan, S.J. Clough
18	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding	M.I. Chilvers, X. Zhuang, T. Coram, K. McPhee
19	Candidate genes for fungal resistance: Mapping and SNP development for LysM-domain encoding genes in soybean	J. Jedlicka, G. Stacey, J. Specht, G. Graef
20	Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection using next-generation sequencing	W. Yajima, L.E. del Rio, R.S. Goswami

Pathogen Epidemiology & Disease Management		
Poster No.	Title	Author(s)
21	Evaluation of wild *Helianthus* species for resistance to Sclerotinia stalk rot	C.C. Block, T.J. Gulya, L.F. Marek
22	On-farm validation of cultural practice adjustments to improve white mold management	H.F. Schwartz, M.A. Brick
23	Resistance in sunflower genotypes to Sclerotinia head rot in Manitoba	K.Y. Rashid
24	Effective fungicides to reduce the impact of Sclerotinia head rot in sunflower	K.Y. Rashid
25	Evaluating fungicide tank mixtures for control of *Sclerotinia* stem rot of canola	L.E. del Rio, S. Halley
26	Modeling the influence of soil moisture on carpogenic germination of *Sclerotinia sclerotiorum*	A. Nepal, L.E. del Rio

Aggressiveness of *Sclerotinia sclerotiorum* from the north central United States on multiple crops

L. Aldrich-Wolfe, Dept. Biology, Concordia College, Moorhead, MN, and S. E. Travers, Dept. Biological Sciences and B. D. Nelson Jr., Dept. Plant Pathology, North Dakota State University, Fargo, ND, USA.

Funded Plan of Work: Genetic variation and virulence of S. sclerotiorum on six crops in the North Central Region

ABSTRACT:
Sclerotinia sclerotiorum is a pathogen of many commonly-grown crops in the north central United States, yet little is known about aggressiveness within a diverse population from multiple crops. A collection of 149 isolates were collected from multiple crops in twelve North Central states and WY, MT and CO. Isolates were evaluated for mycelial compatibility group (MCG) and microsatellite haplotype at twelve loci. Aggressiveness, as measured by lesion length, of selected isolates from this population was evaluated on six crops: dry bean, canola, lentil, pea, soybean and sunflower. Thirty isolates from six MCG with five isolates in each MCG were evaluated on all six crops in a split plot design with crops as main plot and MCG as sub-plots. Another 67 isolates were evaluated only on dry bean and sunflower. Plants were inoculated 5 to 7 weeks after planting using the cut-stem technique and placing a straw or pipette tip with mycelium of the pathogen over the cut end of the stem. Plants were maintained in a mist chamber for three days then lesion lengths measured. The experiment with the 30 isolates was repeated and data were combined and analyzed with analysis of variance. All 30 isolates were pathogenic and crop was a significant factor with the longest lesions on dry bean and the shortest on pea. However, MCG,isolate within MCG, and the interactions of crop x MCG, and crop x MCG x isolate were not significant factors in the combined analysis. Experiment x crop and experiment x MCG were significant due to longer lesions in the first experiment. The 67 isolates were only tested in one experiment. Crop was not a significant factor in the experiment, but there were significant differences among isolates and there was a significant interaction of crop x isolate. Over the two crops, the mean lesion length of the most aggressive isolate was 44 mm while the least aggressive was 19 mm.

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Genetic and Phenotypic Diversity of *Sclerotinia sclerotiorum* on a Small Geographic Scale

Renuka N. Attanayake, Department of Plant Pathology, Washington State University (WSU), Pullman; Dennis A. Johnson, Department of Plant Pathology, WSU, Pullman; Lyndon Porter, USDA- ARS, Prosser, WA., and Weidong Chen, USDA- ARS, and WSU, Pullman, WA

Funded Plan of Work: Variation in pathogenicity and fungicide sensitivity in relation to variation of neutral markers of *Sclerotinia sclerotiorum*

ABSTRACT:
The work was conducted in 2010 to better understand the genetic and phenotypic variation, and genetic structure of *Scelerotinia sclerotiorum* on a small geographic scale. A collection of 40 *Sclerotinia* isolates from one square meter top layer of soil in a Washington alfalfa field was studied for colony color, Mycelial Compatibility Groupings (MCGs), oxalic acid production, DNA haplotypes of eight microsatellite loci, fungicide sensitivity and virulence. The 40 isolates exhibited three colony colors: beige (22 isolates), dark (11 isolates) and white (7 isolates). Fifteen MCGs and 16 microsatellite haplotypes were found among the 40 isolates. However, the haplotypes did not necessarily correspond with MCGs. There were several examples that isolates within a MCG belonged to different haplotypes and that isolates of the same haplotype belonged to different MCGs. The proportion of microsatellite haplotypes over sample size (40%) was high compared to other studies where populations obtained from wider geographic areas. STRUCTURE analyses indicated there were five clusters, suggesting likely five genetic populations. All isolates showed considerable oxalic acid production except that one isolate consistently produced significantly less amount of acid, on a pH-indicating medium. The isolates also exhibited significant differences in sensitivity to fungicides benomyl, fluzinam and iprodione, and in virulence as measured by colonization on detached pea leaves. This study documents high level of genetic and phenotypic diversity of *S. sclerotiorum* on a small geographic scale, presenting challenges in managing the diseases it causes.

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Identification and functional analysis of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and Arabidopsis

Laureen Blahut-Beatty, Lisa Koziol, and Daina Simmonds, Agriculture and Agri-Food Canada, Ottawa, ON; Bernarda Calla, David Neece and Osman Radwan, Department of Crop Sciences, University of Illinois, Urbana, IL; Steven J. Clough, USDA-ARS and the Department of Crop Sciences, University of Illinois, Urbana, IL

Funded Plan of Work: Identification and functional analysis of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and Arabidopsis

ABSTRACT:
Our previous work on gene expression profiling response to *Sclerotinia sclerotiorum* infection and infiltration of purified oxalic acid (OA) in soybean has enabled the identification of hundreds of candidate defense-related genes. We added an additional microarray analysis including a later timepoint (36 hours) that will provide verification of role of some candidate genes, as well as identifying new ones. A critical component of this project is the functional characterization of the candidate genes to confirm their role in the defense response. Functional analyses are being carried out on soybean and the model plant Arabidopsis by gene over-expression or silencing (RNAi in soybean and T-DNA knockouts in Arabidopsis). At this time, 2 RNAi constructs, designed to silence a GPCR (G-protein coupled receptor) and a 14-3-3 were transformed into soybean. Recent studies have shown that G-protein signaling cascades have direct roles in plant defense responses including production of reactive oxygen species, and activation of NADPH oxidases, ion channels and phospholipases. They were also implicated in the Arabidopsis jasmonate-mediated signaling response to the necrotrophic pathogen *Alternaria brassicola* . The14-3-3 gene was selected because it showed increased expression in response to *Pseudomonas syringae* and *Sclerotinia* infection. Based on microarray results, 14 genes have been selected for functional analysis in Arabidopsis. Four Arabidopsis T-DNA knockout lines, obtained from ABRC, confirmed to be homozygous, are currently undergoing functional assays to test their response to *Sclerotinia* infection. Ten over-expression constructs were designed and transformed into Arabidopsis. Currently, these transgenics are at different stages of regeneration, recovery on the selectable marker or analysis by semi-quantitative RT-PCR to confirm over-expression of the putative defense genes. The transgenics showing a strong response to *Sclerotinia* in Arabidopsis will be advanced for functional analysis in soybean, via RNAi or over-expression studies.

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Evaluation of Wild *Helianthus* Species for Resistance to Sclerotinia Stalk Rot

Charles C. Block, USDA-ARS, Ames, IA, Laura F. Marek, North Central Regional Plant Introduction Station, Ames, IA, and Thomas J. Gulya, Jr., USDA-ARS, Fargo, ND

Funded Plan of Work: Evaluation of Wild *Helianthus* Species for Resistance to Sclerotinia Stalk Rot / Research Area: Crop Germplasm Resources & Genetics

ABSTRACT:
The objective of this project is to identify new sources of resistance to Sclerotinia stalk and root rot in wild sunflower germplasm. The USDA-ARS sunflower collection at Ames, IA contains a wide array of wild annual (1,365 accessions) and perennial species (805 accessions). Accessions are initially tested under high disease pressure in the greenhouse, with the goal of identifying accessions showing significantly better survival percentages than the most resistant hybrid checks. Accessions that are considered to be potential sources of useful resistance are further tested in the field.

In 2009-10, all available accessions of *H. anomalus*, *H. agrestis*, *H. bolanderi*, *H. deserticola*, and *H. paradoxus* were evaluated. *Helianthus agrestis* was impressive with 99.5% plant survival. Unfortunately, this species has not been crossed successfully with cultivated sunflower. The other four species were fairly susceptible, and except for three moderately resistant *H. deserticola* accessions, showed little merit in terms of further evaluation. We also evaluated a geographic cross-section of 60 wild *H. annuus* accessions to determine if a particular region (from ND to south Texas) might have a sufficiently high incidence of resistant germplasm to warrant additional screening within *H. annuus*. None of the accessions performed better than the best hybrid check, but six of the top 10 accessions originated from south Texas. Greenhouse screening moved from annuals to perennials, starting with *H. pauciflorus*, *H. californicus*, *H. resinosus*, and *H. salicifolius.* All of the perennial species showed remarkable resistance. Seven of 10 *H. californicus* accessions had 100% plant survival and the other three were above 95%. Twenty-two of the 31 *H. pauciflorus* accessions had 80% or better plant survival, equivalent to the resistant *H. argophyllus* check, PI 649863. Among *H. resinosus*, 8 of 14 accessions had 100% survival and 11 of 14 accessions were at or above 90%. For *H. salicifolius*, 7 of 14 accessions had 100% survival and 12 of 14 were at or above 90%.

Twenty-seven entries were planted in a field trial at Staples, MN along with F1 crosses of the susceptible inbred HA89 with *H. argophyllus*, *H. petiolaris* and *H. praecox*. Unfortunately, the 2010 field trial was a disappointment. Frequent, heavy rains severely limited Sclerotinia disease development and it was not possible to verify differences between the susceptible and resistant lines.

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Introgression of Resistance to White Mold Derived from Multiple Sources of Common and Scarlet Runner Bean

Mark A. Brick, Glenn Brown, Mark A. Newell, Patrick F. Byrne, & Howard F. Schwartz
Colorado State University

Funded Plan of Work: Crop Germplasm Resources and Genetics

ABSTRACT:
Genetic resistance to white mold (caused by Slerotinia sclerotiorum Lib de Bary) has been reported in both common (*Phaseolus vulgaris* L.) and scarlet runner (*P. coccineus* L.) beans. Two $F_{4:5}$ recombinant inbred line (RIL) populations were developed by crossing the pinto line USPT-WM-1 with resistant lines previously developed from common and scarlet runner beans. The two RIL populations had approximately 180 lines each. The objectives of the research were to determine if QTL previously identified in interspecific crosses could be recovered in an improved pinto breeding population. Mean white mold scores among RIL ranged from 4.2 to 8.8. Few polymorphic markers were recovered for resistant QTL previously reported. These results indicate that molecular markers from the two *Phaseolus* species can be introgressed into commercial common bean germplasm, however, early generation selection for molecular markers should probably be used for higher retention of resistant QTL in the derived lines.

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Evaluation of inoculation methods for screening of rapeseed materials for resistance against *Sclerotinia sclerotiorum*

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Funded Plan of Work: Development of canola breeding populations and identification of herbicide-tolerant breeding lines with resistance to Sclerotinia sclerotiorum

ABSTRACT:
Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is an economic fungal disease affecting rapeseed (*Brassica napus* L) worldwide. Since expression of SSR symptoms shows much variability and the trait is quantitative in nature, reliable phenotypic evaluation methods for characterization of SSR resistance are needed. Six different inoculation methods were compared for their reliability to discriminate between *S. sclerotiorum*-resistant and susceptible materials developed as part of this project. The methods were evaluated using two *S. sclerotiorum* isolates (WM031 and WM192) collected in North Dakota and 326 clonal plants derived from double haploid resistant (PI458940 x Ames 26628) and susceptible (Westar) *B. napus* materials. The methods involved mycelial inoculation on detached leaves (DL) and stems (DS), petiole inoculation (PIT), straw inoculation (ST), stem-piercing with toothpick (SP), and mycelial spray (MS). The experiment was conducted using a randomized complete block design with four replications and was repeated once. Detached materials were inoculated and incubated in laboratory at 16 hour light daily and 22°C temperature while inoculated plants were incubated in greenhouse at similar conditions. Five of the six inoculation methods, SP, ST, DL, MS , and PIT, differentiated well the resistant from the susceptible material, but only the PIT and MS discriminated between the two isolates. Results of this study also confirm that the petiole inoculation technique produced the most consistent results and was more reliable to evaluate materials for their reaction to SSR.

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Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding

Martin I. Chilvers and Xiaofeng Zhuang, Michigan State University; Tristan Coram, Dow AgroSciences, LLC; and Kevin McPhee, North Dakota State University

ABSTRACT:

The parents of a pea mapping population that Dr. McPhee established are being utilized in an RNA-seq approach to examine the expression profile of the Sclerotinia-pea host pathogen interaction. The parents, Medora a susceptible cultivar and PI169603 a partially resistant line are being inoculated with *S. sclerotiorum* under conditions similar to those under which the population was phenotyped by Dr. Lyndon Porter at Washington State. The expression profile of both pea and *S. sclerotiorum* are being examined at multiple time points to identify genes involved in the resistance response as well as *S. sclerotiorum* genes involved in pathogenicity. By utilizing similar environmental and inoculation conditions as used to phenotype the mapping population we hope to identify potential resistance genes that will map to QTL's identified by Drs. McPhee and Porter. As a reference genome is not available for pea we will utilize a software called Trans-ABySS which allows *de novo* assembly and analysis of RNA-seq data without a reference genome. Similar to the expressed sequence tag data set described below the RNA-seq approach will generate a large amount of transcript sequence data for each pea parent, which will be valuable for the development of gene-linked markers such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs).

In initial experiments a large expressed sequence tag (EST) data set was developed with massively parallel sequencing on a 454 Roche platform. Post-trimming, the data set consisted of 145,049 reads with an average read length of >200 nucleotides. The sequence reads were assigned by the use of BLAST analysis and parsing script to one of four categories; *S. sclerotiorum*, *P. sativum*, *S. sclerotiorum* and *P. sativum*, and unassigned (Figure 1). BLAST alignment was performed against the *S. sclerotiorum* and 31.6 Mbp of pea cDNA reads generated in a previous project at MSU. Fifty eight percent of reads were assigned to pea, 25% were assigned to *S. sclerotiorum*, 1% were assigned to both pea and *S. sclerotiorum* and only 16% were unassigned (Figure 1). The unassigned reads category probably still contains pea transcripts as it is unlikely that the entire pea transcriptome was represented in the reference cDNA library. The *S. sclerotiorum* cDNA reads were subsequently assembled in 1,285 isogroups which represent possible loci, 1,345 isotigs which represent putative transcript isoforms (i.e. splice variants) at those loci, and there were a remaining 22,331 singletons which are reads that could not be assembled into contigs. The pea transcripts were assembled into 11,810 contigs and 17,619 singletons. Seventy three putative SSRs with a length of repeat greater than 20 nucleotides were identified in the pea contigs with the program SSRIT[5] (Figure 1). These SSRs will be evaluated as potential makers for pea genetic mapping. We are currently completing annotation and gene ontology analysis of this data set.

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Screening Accessions of Wild Relatives of Pea for Resistance to *Sclerotinia sclerotiorum*

Clare Coyne and Weidong Chen, USDA- ARS,
and Washington State University, Pullman, WA

Funded Plan of Work: Searching for resistance sources to Sclerotinia in wild relatives of cool season grain legumes

ABSTRACT:
Practical management of white mold of pea caused by *Sclerotinia sclerotiorum* is through cultural practices, fungicide applications, and resistance when available. Cultural practices are usually inadequate in managing the disease. Fungicide applications increase production costs and reduce the competitive edge of growers in the global market place. Employing resistance is the only viable long-term approach to manage white mold of pea. However, resistance to white mold on cultivated pea (*Pisum sativum*) is rare and when available, is at low to moderate levels in cultivars and germlasms of pea. New resistance sources are needed in order to improve resistance in elite pea cultivars.

We have collected wild relatives of peas: *Pisum fulvum* (33 lines), *Pisum sativum subsp. abyssinicum* (27 lines), *Pisum sativum subsp. elatius* (45 lines), *Pisum sativum subsp. transcaucasicum* (4 lines). Ninety-five lines of wild peas have been evaluated for resistance to *Sclerotinia sclerotiorum*. Some selected cultivated pea cultivars are used in each trial for comparison. Three to four replications (pots) with 3 to 4 seeds per replication were planted in the greenhouse. Four weeks after planting, an agar plug from actively expanding colony of *S. sclerotiorum* strain WM-A1 were place at the third internode with a piece of parafilm of each plant (12 plants per line) and an plain agar plug as control on four plants. Disease progress was monitored by measuring lesion expanding on the stem every other day for eight days. Among the cultivated peas, cultivar Dark Skin Perfection is the most resistant and cultivar Columbia is among the most susceptible. Although all tested accessions of wild peas were susceptible to *Sclerotinia sclerotiorum*. There were significant differences among the accessions of wild peas. More importantly there are four accessions performed better than the most resistant cultivated pea Dark Skin Perfection. The results are being further confirmed by additional repetition of the experiment.

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Evaluating fungicide tank mixtures for control of *Sclerotinia* stem rot of canola

L. E. del Río[1] and S. Halley[2], [1]Department of Plant Pathology, North Dakota State University, Fargo, ND 50108, [2]North Dakota State University Fargo Research Extension Center, Langdon, ND 58249

Funded Plan of Work: Evaluation of fungicide alternatives for control of *Sclerotinia* stem rot of canola

ABSTRACT:
Field trials were installed in Langdon and Carrington, ND in the summer of 2010 to evaluate the efficacy of different fungicide alternatives for control of Sclerotinia stem rot on canola (SSR). The studies were conducted using a randomized complete block design with four replications. Treatments included single and/or double applications of Quadris (azoxystrobin), Endura (boscalid), Quash (metconazole), Proline (prothioconazole), and Topsin (thiophanate methyl), alone or in tank mixtures during flowering. Most tank mixtures were made by combining two products at 50 to 75% of their recommended full rate. At flowering, plants at both locations were exposed to field-produced ascospores (at Langdon, each plot was infested with 320 g of sunflower-produced sclerotia the previous fall, whereas at Carrington plots were established in a disease nursery area where sclerotia produced by the previous crop were abundant), as well as to lab-produced ascospores as they were inoculated with a suspension of 10^3 ascospores per ml. Good disease pressure was observed at the Langdon trial but not at Carrington. Most fungicide applications reduced disease incidence significantly compared to non-protected plots; however, significantly higher yields were only obtained in plots treated with a single or double application of tank mixtures of Topsin and Proline and with a single application of a mixture of Topsin and Quash. Results of this study and of previous trials conducted as part of this plan of work, suggest tank mixing fungicides at reduced doses produces equal or better control than using single products at full rates.

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Identification of novel loci for partial resistance Sclerotinia stem rot in perennial soybean accessions

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Funded Plan of Work: Development molecular markers linked to genes for partial resistance to Sclerotinia stem rot in perennial *Glycine* species

ABSTRACT:
No sources of complete resistance to Sclerotinia stem rot (SSR) have been identified in cultivated soybean (*Glycine max*). However, high levels of resistance are observed in some accessions of perennial *Glycines* species. The goal of this project is to map genes for resistance to SSR in populations of perennial *Glycines* segregating the trait. In 2010, evaluation of responses to *Sclerotinia sclerotiorum* infection was completed. Based on these data, pairs of highly resistant and sensitive lines were selected from *G. canescens*, *G. clandestina*, *G. latifolia*, and *G. tabacina* for production of recombinant inbred line (RIL) populations and selfed to reduce heterozygosity. Crosses were made between resistant and sensitive accessions of *G. latifolia*, and *G. tabacina*, and F_1 plants were produced. To aid in identification of hybrid F1 plants, portions of the soybean homologue of the *DCL3* gene were amplified from each parental line and sequenced from *G. latifolia*, and *G. tabacina*. Similar to *G. max*, the frequency of single nucleotide polymorphisms (SNPs) between the two pairs of parental lines was about one SNP per kb. Additional sets of SNP markers will be produced by sequence analysis of parental transcriptomes. The RIL populations will be evaluated for segregation of SNP markers and responses to infection by *S. sclerotiorum* to define loci conditioning resistance to SSR. Candidate genes will be selected for further analysis and eventual movement to *G. max*.

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Use of a transformation system in sunflower for Sclerotinia resistance studies

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

ABSTRACT:
In order to take full advantage of the biotechnological tools that are in place for studying Sclerotinia resistance in sunflower, more routine transformation methodologies must first be developed for this important crop. Sunflower line RHA280 was initially identified as responsive to both transformation and shoot induction. For induction of shoots, embryos were excised from mature, dry seed and embryo axes were removed. Each cotyledon was cut into 3 pieces and placed, cut side down, on a shoot induction medium containing MS salts, B5 vitamins, 1.5 mg/l BA, 0.2 mg/l NAA, 3% sucrose and 0.2% Gelrite. Shoots were initially observed as dark green region on the explant, mainly above the point of contact with the medium, located on the original surface of the cotyledon. Large masses of shoots were most often observed. The percentage of explants forming shoots increased from 46% to 100% in some cases, by using freshly-harvested seeds with visual selection of seeds with smooth surfaces. Subculture of shoot masses to media containing GA resulted in rapid elongation of shoots and leaves but the plantlets were hyperhydric and rapidly senesced. For transformation of sunflower cotyledons, *Agrobacterium tumefaciens* strain LBA4404, was selected from twelve strains as it caused only a minimal hypersensitive response on explants but yielded high transient transformation rates. For selection and monitoring of transformed cells, an *Agrobacterium* construct was used which contained a CaMV35S-driven hygromycin resistance gene and the *gfp* gene regulated by a sunflower polyubiquitin (HaUbi) promoter. The HaUbi promoter directed high levels of transgene expression in sunflower tissue. *Agrobacterium*-mediated transformation of sunflower cotyledonary tissue gave consistently high transient transformation of this target tissue and GFP-expressing shoots were occasionally recovered. *Agrobacterium* is apparently able to efficiently target a few different types of cells in sunflower cotyledons but the shoot producing cells are not preferentially targeted. Although transgenic shoots have been obtained, transgenic plants have not yet been recovered. Efforts are underway to increase the efficiency of plant recovery and improve *Agrobacterium* targeting of shoot-producing cells and selection for transgenic shoots.

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Discovery of novel sources of resistance to head rot and stalk rot in cultivated sunflower and wild *Helianthus*

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Funded Plan of Work: Discovery of novel sources of resistance to head rot and stalk rot in cultivated sunflower and wild *Helianthus*

ABSTRACT:
The 2010 growing season had abnormally high rainfall in the Red River Valley area of North Dakota and Minnesota, where many of our Sclerotinia nurseries were located. While we have successfully done field evaluations of sunflower for both Sclerotinia head rot and stalk rot for nine years, we lost virtually all of one head rot and one stalk rot nursery in 2010. Excessive rainfall eliminated stalk rot development at one of three inoculated field trials, and a severe infestation by sunflower midge, which deforms developing sunflower heads, rendering ~ 75% of a 1500 row head rot nursery data meaningless. The excessive rainfall, however, fostered Phomopsis stem canker, which enabled us to make selections for another important disease, and the midge infestation revealed some sunflower germplasm with combined tolerance to both head rot and sunflower midge. The excessive rainfall also impacted a companion project with Dr. C. Block in which we were unable to achieve stalk rot infection on wild annual sunflowers. We were able to generate good stalk rot data on 2200 rows of USDA breeding material (of 3200 rows planted at three sites) and thus progress has been made in stalk rot resistance. As a result of the continued midge infestation at the Carrington, ND research station, our entire head rot testing effort will move to Staples, MN in 2011. Field testing completed from 2008-9 in which we phenotyped 250 USDA Plant Introductions identified ~ 25 accessions with superior stalk rot resistance, and these will be planted in two locations in 2011 to assess their head rot resistance. Efforts to study the influence of root exudates from different crops on the mode of sclerotial germination in greenhouse experiments were unsuccessful and this portion of our work has been shelved. Preliminary work has begun in conjunction with Dr. Mike Boosalis (retired plant pathologist from the University of Nebraska) to define optimal parameters for large scale apothecia production. Dr. Boosalis has been producing ascospores for 20+ years for use by many Sclerotinia researchers across the U.S. and has graciously offered to collaborate with us and other U NEB scientists. The study will examine a range of Sclerotinia isolates in multiple laboratories, and the results will be published.

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Fine mapping of quantitative resistance genes to Sclerotinia stem rot in two soybean populations

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Funded Plan of Work: Variety Development/Germplasm Enhancement

ABSTRACT:
Sclerotinia stem rot (*Sclerotinia sclerotiorum*) is an important disease in many soybean production areas of the Midwestern United States. Some efforts have been made to find resistance sources useful for breeding and genetic studies; however, no sources of complete resistance have been identified. Genetic studies have shown that the resistance to this pathogen was complex and inherited in multigenic fashion. Because of the complexity of the trait and the lack of tight markers associated with quantitative genes, it is difficult to breed for and develop, partially resistant cultivars; hence there are no commercial cultivars available with high levels of Sclerotinia stem rot resistance. In 2008, we reported on the development of recombinant inbred lines (RIL) populations, and mapped resistance QTL in a population of 155 $F_{4:5}$ RIL developed from the hybridization of the partially resistant parent, PI194639, to the susceptible cultivar Merit. This population also was genotyped with 134 simple sequence repeat (SSR) markers. Two putative QTL controlling lesion lengths were identified by composite interval mapping (CIM) and mapped to linkage groups (LGs) A2 and B2. The LG A2 QTL was linked to the marker Sat_138 and explained 12% of the phenotypic variation for LL. The LG B2 QTL was proximal to the marker Satt126 and explained 11% of the phenotypic variance. Two minor QTL also were mapped onto LGs K and L, explaining 6% of the total phenotypic variation. A multivariate model that included all significant QTL explained 27% of the observed phenotypic variation of LL. Large gaps exist between the markers in the map, resulting in potentially some important chromosome lineages containing QTL being overlooked. The goal of this project is to discover new quantitative resistance genes that can be compiled and used for selecting higher levels of Sclerotinia stem rot resistance in soybean. The objective is to fine map the location of Sclerotinia stem rot resistance genes from PI194639 and PI194634 by identifying SNP markers that are significantly associated with QTL for resistance. For population 1 (Merit x PI 194639), phenotyping was repeated and50 additional SSR markers were added (making a total of 184 polymorphic SSR markers). For population 2 (Merit x PI 194634), an initial evaluation of Sclerotinia stem rot resistance in the greenhouse was completed, parents were screened for polymorphic markers, and DNA was extracted from RILs. Over 100 SSR markers were used for genotyping with 40 markers being polymorphic. Ultimately, this work will allow for the development of accumulating quantitative resistant genes for the control of Sclerotinia stem rot. This will provide new novel resistance genes that could be incorporated into germplasm releases that both private and public breeders would use in developing resistant cultivars.

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Breeding and quantitative genetics advances in sunflower Sclerotinia research

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Funded Plan of Work: Pyramiding Sclerotinia head rot and stalk rot resistances into elite sunflower breeding lines with the aid of DNA markers

ABSTRACT:
In 2010, we continued the process of backcrossing the head rot QTL from the HA 441 x RHA 439 population into confectionery and elite oilseed backgrounds. Progress is slow due to complexities in scoring of alleles in breeding progenies (dominant markers sometimes in repulsion phase, and many gel bands with TRAP). BC_2F_1 hybrids will be produced this winter. We will again select progeny based on their marker profiles at 6 major QTL loci for advancement into another backcross this next summer. About 50% of the markers are already fixed in the genetic background of the recurrent parent, despite the fact that these lines are susceptible to moderately resistant. We hope that additional QTL will result in more head rot resistant lines by backcrossing.

Our efforts to perform association mapping with the 260 Plant Introductions (PIs) obtained from the North Central Regional Plant Introduction Station of USDA-ARS in Ames, IA, are moving forward. We have finished data analysis on the phenotypic data Based on the distribution of data, which is more broadly and normally distributed than we were expecting, we believe that the phenotypes will be adequate for mapping using the association mapping model. We are currently completing the development of a marker set for our resistance candidate genes and 10,000 random SNPs from a companion project. This will contribute the necessary genotypes with which we can complete our association model. As soon as all the genotyping is complete, we can run our association analysis, discover marker-trait associations for stalk rot, and prepare publications on the work.

Our traditional breeding program had a successful season in 2010, with breeding lines in the F_4 to F_7 selfing generations being tested for Sclerotinia head and stalk rots, each at two locations. Our goal here is to continue introgression of new, minor resistance loci from many domesticated sources into elite germplasm by conventional means. We plan a release of germplasm this winter.

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Candidate genes for fungal resistance: Mapping and SNP development for LysM-domain encoding genes in soybean

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Funded Plan of Work: Candidate genes for fungal resistance: Mapping and SNP development for LysM-domain encoding genes in soybean

ABSTRACT:
Recently in Arabidopsis, LysM-containing receptor-like kinases were genetically defined as receptors for chitin. Chitin is a major component of fungal cell walls and is an established pathogen-associated molecular pattern (PAMP). This information suggests that the LysM genes represent promising candidate genes that could explain some of the resistance to white mold in selected genotypes. Analyses of soybean sequence databases indicated that soybean has 13 unique LysM-RLKs and at least another 17 LysM-domain containing proteins. The genes were cloned and sequenced. The objectives of this study are to (1) map LysM-domain encoding genes in soybean and correlate their map locations to known white mold QTL, and (2) develop SNP markers for those LysM-domain genes mapping close to known white mold QTL. Five soybean RIL populations used previously to identify QTL for sclerotinia resistance were used in this analysis. The common susceptible parent is the cultivar Williams 82. We identified 37 LysM-domain genes that were located on Linkage Groups that contained significant QTL from our previous work. We have determined that at least six of the LysM genes map to locations where we identified QTL on LGs D1b, E, F, G, K, and L. LysM genes on LG A1 and B1 were not associated with our identified QTL. For the Dassel population, the LysMe11 gene is associated with an identified QTL on LG I. The LysMe11 locus was significant in the QTL analysis, and the allele associated with smaller lesion size comes from Dassel, the resistant parent. The SNP markers that are developed could be used for marker-assisted breeding to develop near-isogenic lines with different combinations of LysM alleles for further study, and potentially cultivars with improved resistance.

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Progress on the introgression of 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:
The necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary attacks sunflower (*Helianthus annuus* L.) causing root, stalk, and head rot, and is one of the most damaging and difficult-to-control sunflower diseases. Some wild perennial *Helianthus* species have been identified to contain abundant resistance genes to this fungus. The objectives of this project were to transfer Sclerotinia head and stalk rot resistance from resistant wild perennial haxaploid and diploid *Helianthus* accessions, and interspecific amphiploids into cultivated sunflower, via the traditional backcross method. BC_4F_3 plants (2n=34-37) of *H. californicus*/HA 410 crosses were obtained with improved pollen fertility and seed set in 2008 for hexaploid species. The backcrossing of hexaploid *H. schweinitzii* with HA 410 obtained more than 40 BC_3F_2 and BC_4F_1 plants in 2010. Five amphiploids with high resistance to stalk and head rot were crossed with HA 410, and BC_2F_2 /BC_3F_1 plants (2n=34-36) were obtained in the greenhouse in 2008, and with further backcrossing and selfing in 2008 and 2009 produced seed for the 2009 and 2010 field evaluations. For Sclerotinia resistant diploid perennial species, *H. maximiliani*, *H. giganteus*, *H. grosseserratus*, and *H. nuttallii*, selfed and/or backcrossed progenies with 2n=34-35 chromosomes were obtained in 2008 with seed increased in the field in 2009 and 2010. In 2009, replicated field tests with 163 and 313 progeny families screened for head and stalk rot resistance at Carrington, ND, respectively, showed good introgression of resistance genes. These materials were planted in 2010 for a second year of field evaluation, as well as the new families with seed increased in 2009. In 2010, replicated field tests with 309 and 413 progeny families were screened for head and stalk rot resistance, respectively. However, due to unexpected midge damage and adverse environmental conditions at Carrington, most of the tests for head rot failed to produce usable results in 2010. For stalk rot, we have decided to eliminate the heavily infected families from both years, and further evaluate only the lightly infected families in 2011. Molecular tracking using SSR markers suggested a higher frequency of gene introgression when perennial diploids species were used. In 2010, eight accessions from three diploid and one tetraploid perennial species were established in the greenhouse for crosses with HA 410 and HA 451. HA 451 is tolerant to both head and stalk rot. These new crosses will provide more diverse resistance genes for developing Sclerotinia resistant germplasm.

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Improved resistance in common bean through multi-site screening and pathogen characterization throughout major production areas

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Funded Plan of Work: Improved resistance in common bean through multi-site screening and pathogen characterization throughout major production areas

ABSTRACT:
Bean cultivars with intermediate resistance and/or avoidance to white mold (WM) would reduce disease losses and require no input costs for growers. Thus, one project goal was to identify sources of resistance in adapted and nonadapted common bean lines utilizing standardized greenhouse screening methods and field nurseries across major bean production regions. Multi-site testing provided data for identifying three kidney bean lines and a breeding line with moderately high resistance and two pinto lines with field and greenhouse intermediate resistance. Three adapted lines also exhibited avoidance to WM. In the past four years, a snap bean and 9 lines with pinto, kidney and bayo seed were released. A second project goal was to assess variation in common bean isolates of *Sclerotinia sclerotiorum*. To address this goal, we devised a unique study on pathogen variation across bean-production areas that tests the hypothesis that pathogen variation within and between test sites influences identification of WM resistance. Mycelial compatibility groupings (MCGs), aggressiveness, and microsatellites (SSRs) are used to identify genotype and phenotype differences in the isolates that can influence stability of identified WM resistance over time and location. Collecting isolates from specific bean host lines replicated at each resistance screening site permitted us to assess within and between location variations. High variation in aggressiveness and genetic variability (measured by MCGs and microsatellites) of pathogen isolates within and between field screening nursery locations and greenhouse test isolates has been found. Another hypothesis we are testing is that isolates collected from screening nursery sites and greenhouse tests show similar phenotype and genotype variability as isolates collected from growers' fields in the same region. In other words, that the isolates involved in screening reflect characteristics of those found in local grower fields. When isolates from screening nurseries in each of 3 states were compared with grower field isolates in the same state, there were significant differences in aggressiveness. Our database of characterized isolates facilitates new isolate characterization. The expected outcome is a set of characterized isolates for breeders and pathologists searching for unique and common clones with more or less aggressiveness to use in screening for resistance.

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Characterization of the Genetic Basis for Partial Resistance to *Sclerotinia sclerotiorum* in Pea

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Funded Plan of Work: Characterization of the Genetic Basis for Partial Resistance to *Sclerotinia sclerotiorum* in Pea

ABSTRACT:
As a universal pathogen, *Sclerotinia sclerotiorum* Lib, infects all major broadleaf crops in the Northern Plains region and limits crop rotation. It is a serious threat to field pea (*Pisum sativum* L.) production in North Dakota. Sustainability of the pea industry in ND will maintain its competitiveness by reducing the economic impact of Sclerotinia on the crop. Pea accessions with physiological resistance to the pathogen have been identified previously. Identification and incorporation of genetic resistance into locally adapted cultivars is the main goal of this project. Specific objectives include 1) identification of major QTL on pea genetic map that are associated with partial resistance to white mold, 2) stacking identified resistance genes into adapted cultivars. Progress over in the past twelve months has been to advance Population 4 'Lifter'(PRIL-5)/PI240515 and Population 6, PI169603/'Medora'(PRIL-2) populations from the F_5 to F_7. These seeds will be increased for replicated field trial evaluations. Genotypic analysis using molecular markers tested on DNA extracted from the F_2 population is being performed. Two experiments conducted to phenotype Population 4 and Population 6 using F_3 and F_4 family seed have been completed. Distribution of reduced lesion expansion data from both generations follows a normal curve indicating additive effect of genetic loci for the trait. Nodal resistance is significantly skewed toward greater disease development. The results from the genetic analyses, greenhouse tests and field trials will be used to develop crossing blocks in an effort to pyramid the resistance mechanisms.

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White Mold Resistance-QTL: Identification, Interactions, and Fine Mapping in Common Bean

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Funded Plan of Work: Resistance-QTL: Identification, Interactions, and Fine Mapping in Common Bean

ABSTRACT:
Identification, characterization and fine mapping major QTL conditioning resistance to white mold in common bean (*Phaseolus vulgaris* L.) will fill up the plant breeder's tool box with germplasm, knowledge, genetic markers and other technologies leading to the development of resistant cultivars. Work conducted in 2010 revealed new QTL conferring physiological resistance to white mold from *P. coccineus*. Three backcross inbred interspecific populations [91G/PI 433251B (Gx43) (264 lines); MO162/PI433251B (Mx430 (120 lines); 91G/PI 255956 (Gx25) population (115 lines)], have been populated with markers and tested for disease reaction, and putative QTL have been mapped to Pv02, Pv05, Pv06, Pv09, and Pv10. Although QTL have been identified in these populations, entire chromosomes were missing from the maps. The most logical explanation is that homozygous *P. coccineus* alleles in a *P. vulgaris* background reduce fitness compared to heterozygous alleles. Validation and transfer of these QTL into dry and snap bean breeding lines was initiated. Inbred dry bean populations segregating for WM7.1 and WM8.3 QTL, and WM2.2 and WM8.3 QTL were successfully characterized for disease reaction in the straw test and field. The WM7.1 and WM8.3 QTL exhibited an additive effect in the field and greenhouse. The WM2.2 and WM8.3 QTL exhibited an additive effect solely in the field. These inbred populations were used to map 53 new indel markers to the WM8.3 QTL region in the fine-mapping experiment. A region of eight markers originally was expanded to 61 markers over a 56.7 cM distance. A few markers more tightly linked with the QTL will be tested for marker-assisted breeding applications. The analysis of RNA sequence expression patterns associated with WM8.3 QTL was conducted by evaluating near isogenic lines with and without the QTL. Using the straw test, RNA was collected from pools of WM8.3 resistant and susceptible lines 24 and 48 hrs after inoculations. Mock inoculation and non-wounding controls were included. cDNA libraries were developed and sequenced using the Illumina GAIIx technology, with 131 million reads of 32 nt (4.2 bp billion) collected. The sequencing was completed in late November 2010. A preliminary analysis at 24 hr post inoculation discovered 53 genes that were up-regulated and 64 that were down regulated between resistant and susceptible lines. Work is in progress to locate those expressed genes which map within the WM8.3 region.

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Identification of QTL for agronomic traits and resistance to white mold in wild and landrace germplasm of common bean

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Funded Plan of Work: Crop Germplasm Resources and Genetics

ABSTRACT:
White mold, caused by *Sclerotinia sclerotiorum* (Lib.) De Bary, is a serious yield reducing fungal pathogen of common bean (*Phaseolus vulgaris* L.). Our objective was to identify QTL for resistance to white mold from wild and landrace accessions of common bean using two inbred backcross line (IBL) populations derived from the recurrent black bean parent 'Tacana'. Selective phenotyping failed to detect QTL for field disease resistance but other agronomic traits less sensitive to environmental conditions or population size were detected. Three novel QTLs for white mold resistance WM7.4^{TL}, WM9.2^{TW} and WM11.1^{TL} were identified in the greenhouse straw test on linkage groups B7, B9 and B11 and three previously mapped QTL were also validated on B2, B3 and B4. A major QTL, SY2.1^{TL} that accounted for more than 35% variation for yield under white mold pressure in all three years was detected on B2 in the TL population. Enhanced resistance to white mold in common bean could be achieved by combining different QTL associated with physiological resistance with yield under disease pressure.

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Identification of QTL for white mold and agronomic traits in pinto bean

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Funded Plan of Work: Crop Germplasm Resources and Genetics

ABSTRACT:
Pinto beans are the most widely grown commercial class of dry beans in the U.S. and are among the most susceptible to white mold. The goal of this project was to identify quantitative trait loci that are associated with resistance to white mold and agronomic traits of economic importance in pinto bean. Using a 94 RIL population generated between AN-37 and P02630 parents, QTLs controlling disease incidence in the field and greenhouse and yield were identified. Heritability estimates were low for traits such as lodging (0.16), days to maturity (0.21), plant height (0.22) and field disease incidence (0.29). Traits such as yield, seed weight and the straw test had moderately higher heritability estimates of 0.48, 0.63 and 0.43, respectively. A linkage map was developed using 66 SSR markers, covering 200 cM across six bean chromosomes and interval mapping analysis revealed four major QTLs. A QTL for resistance to white mold in the field in 2007 and 2009 was identified near markers BM157 and IAC90, respectively on bean linkage group B1 and on B3 and B7 in 2008. Yield QTL were detected on B2 and B5 accounting for up to 39% of observed variation over 3-years. These QTL were contributed by alleles from the MSU parent P02630. Three QTL for resistance in the straw test on B2 B3 and B8 came from the resistant parent AN37. The QTL on B2 was consistent in three separate evaluations whereas the QTL on B3 did overlap with field disease resistance in 2008. These QTL will be used to select for white mold resistance and yield in a second AP647 pinto population.

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Modeling the influence of soil moisture on carpogenic germination of *Sclerotinia sclerotiorum*

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Funded Plan of Work: Defining critical environmental and biological parameters needed to develop *Sclerotinia* stem rot on canola

ABSTRACT:
Ascospores of *S. sclerotiorum* are considered the primary inoculum for development of Sclerotinia stem rot of canola. Ascospores are produced by apothecia when sclerotia undergo carpogenic germination (CG). The amount of soil moisture in soil is perhaps one of the most important factors affecting CG. Moisture availability in turn can be affected by soil texture. Thus, studies were conducted with the objective of characterizing soil moisture and soil texture play on carpogenic germination. A Fargo Silty clay soil (92% clay, 7% silt, and1% sand) and an Aylmer-Bantry fine sand soil (4% sand, 52% silt, and 44% clay were mixed in proportions of 2:1, 1:1, and 1:2 v/v to create three additional textures. Sclerotia were buried in samples from each soil texture and the samples were set at constant 100%, 75%, 50% or 25% soil saturation or to conditions fluctuating back and forth between 100 and 0; 75 and 0; 50 and 0 or 25 and 0% saturation at 25 unit intervals. Samples were incubated at 14/18^{o}C day/night for 82 days. CG was recorded at five-day intervals. The area under cumulative moisture curve (AUCMC) and rate of moisture accumulation were calculated for all treatments. These variables, along with percentage of clay and silt were used as predictor variables. Binary data created using CG thresholds ranging from 15 to 35% were used as dependent variables. Data was split into two arbitrary fractions, one for model development and the other for model validation. AUCMC and rate of moisture accumulation had a significant effect on CG but soil texture did not. Field validation of this model will be discussed.

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The requirement for oxalate during pathogenesis on multiple crops

Jeffrey Rollins, University of Florida, Gainesville, FL

Funded Plan of Work: The requirement for oxalate during pathogenesis on multiple crops

ABSTRACT:
This project began in July, 2009. The Specific Objectives are: (1) Conduct whole plant bioassays with culture filtrates from the wild-type and Δ*oah1* mutants to determine if filtrate activity correlates with virulence level. (2) Identify the compound(s) associated with leaf-necrosis activity. We have completed specific objective one and are currently repeating experiments associated with specific objective two. The significant finding from specific objective one is that the oxalate minus (Δ*oah1*) mutant retains significant necrosis-inducing activity within its culture filtrate. There appears to be no direct correlation between observed necrosis activity and virulence level as Δ*oah1*mutants are severely attenuated in their virulence. We conclude that the observed activity may be involved in establishing initial compatibility between *Sclerotinia* and its hosts. We are following up with biochemical fractionation experiments to determine if more than one activity is present in the wild-type culture filtrates. This finding would support our hypothesis that the Δ*oah1* mutant retains at least one necrosis-inducing activity in common with the wild type and these activities may play differing roles in establishing compatibility and colonization. From specific objective number two we have determined that the Δ*oah1* mutant necrosis-inducing activity is in the molecular weight range of 3 to 10 kDaltons. We have not been successful in further purifying the activity by ion exchange chromatography but are continuing our efforts on this front to make a biochemical identification of the activity.

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On-Farm Validation of Cultural Practice Adjustments to Improve White Mold Management in Dry Bean Irrigation Systems

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Funded Plan of Work: On-Farm Validation of Cultural Practice Adjustments to Improve White Mold Management in Dry Bean Irrigation Systems

ABSTRACT:
This 3-year project (2011 will be the 3rd year) will investigate the roles of cultural practices and timely application of a fungicide in reducing damage from *Sclerotinia sclerotiorum* to *Phaseolus vulgaris* cultivars with varying degrees of resistance (plant architecture – disease avoidance, interspecific resistance) when grown under different irrigation systems. These objectives support the Sclerotinia Initiative (SI) area of Pathogen Epidemiology & Disease Management (including crop production practices and chemical control); and are relevant to PM 4.0.7 of the Strategic Plan for the Sclerotinia Research Initiative.

During 2010, this project conducted a replicated field trial in white mold-infested grower fields to investigate the role and value of cultural practice modification within an Integrated Pest Management context that compares the effects of added fertilizer and/or fungicide when promising varieties are grown under varying irrigation systems. During 2009-10, we encountered low white mold pressure in fields with a history of the disease apparently due to delayed plantings (early-spring rains both years) which delayed flowering until late July when weather conditions were warm and dry. Yields of the 4 entries averaged 1135 lb/acre (1281 seed/lb) at the research station and 2966 lb/acre (1204 seed/lb) at the better commercial field in the absence of white mold and with moderate bacterial disease at the commercial field in 2010; similar to results obtained in 2009. When combined over locations, yield ($P < 0.05$) and seed size ($P < 0.05$) differences between entries were significant. Plant canopy monitoring during late vegetative to seed fill periods of crop growth showed that average daily relative humidity was higher in a prostrate variety (Montrose) than an upright type (Stampede); and canopy temperature showed the reverse trend. Field data during the 2011 study will include microclimatic monitoring, soil fertility, plant stand, disease intensity, yield, seed size, and economic impacts. Results will be shared with colleagues and growers via progress reports, refereed publications, extension releases, web sites, and meetings. Agronomic and chemical (especially fungicide) implications from this IPM approach will be applicable to other host cropping systems affected by foliar phases of white mold.

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Gamete Selection for Simultaneously Pyramiding and Introgressing White Mold Resistance from *Phaseolus* Species into Pinto Bean

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Funded Plan of Work: Gamete Selection for Simultaneously Pyramiding and Introgressing White Mold Resistance from *Phaseolus* Species into Pinto Bean

ABSTRACT:
Partial white mold [WM, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] resistance is found in large-seeded Andean and small and medium-seeded Middle American dry and green common bean (*Phaseolus vulgaris* L.) and in interspecific breeding lines (IBL) derived from *P. coccineus* and *P. costaricensis* of the secondary gene pool. Resistance of individual genotype, irrespective of its evolutionary origin, is inadequate for combating WM in the USA. Furthermore, no effort has been made for pyramiding resistance from *Phaseolus* species and introgressing pyramided resistance (PWMR) into cultivars. The goal of this research is to pyramid WM resistance from *Phaseolus* species of the primary and secondary gene pools and introgress the highest levels of the PWMR into pinto bean, the largest market class in the USA and North America. The effectiveness of PWMR across environments also will be determined. These objectives support the Sclerotinia Initiative area of Crop Germplasm Resources and Genetics. White mold reaction of 11 common bean (A 195, CORN 501, CORN 601, G 122, 'ICA Bunsi', L 192, MO 162, PC 50, VA 19, USPT-WM-1, and 'Chase') and eight IBL derived from *P. coccineus* and *P. costaricensis* (VCW 54, VCW 55, VRW 32, 92BG-7, I9365-25, 0785-120-1, 0785-121-1, and 0785-227-1) was verified in two greenhouse environments in Idaho and Colorado (June 2008 to December 2010). The complementation test among five Andean dry bean (A 195, G 122, MO 162, PC 50, and VA 19) and three IBL derived from *P. coccineus* (VCW 54, 92BG-7, and 0785-220-1) was performed (June 2008 to May 2009). Four single-crosses among selected WM resistant parents of diverse evolutionary origins were made (June to September 2010), which were used to make three-way and double-crosses (October to December 2010). The latter will be used to make multiple-parent crosses (January to May, 2011). These crosses should allow simultaneous pyramiding of high levels of WM resistance from across *Phaseolus* species of the primary and secondary gene pools and transfer into pinto bean. Also, 78 F_5 families developed from two double-crosses, namely USPT-WM-1/CORNELL 601//USPT-CBB-1/92BG-7 and Chase/I9365-25//ABL 15/A 195 made for a doctoral dissertation, and over two thousand early generation progenies from additional crosses were screened in the greenhouse (June to December 2010). From the initial screenings it is encouraging to note that some of the recombinants exhibited higher levels of WM resistance than the individual parents. Also, some of these recombinants had pinto-like seed. However, because these are only in early segregating generations it will take several selection and progeny testing cycles to develop breeding lines uniform for WM resistance reaction and assess their true potential.

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Transferring White Mold Resistance from the Secondary Gene Pool of Common Bean

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Funded Plan of Work (June 2009 to May 2010): Introgressing White Mold Resistance from the Secondary Gene Pool of Common Bean

ABSTRACT:
White mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] is a severe and widespread disease of dry and green common bean (*Phaseolus vulgaris* L.). Low levels of white mold resistance occur in the common bean. But, higher levels of resistance occur in the secondary gene pool (SGP) species such as *P. coccineus* and *P. costaricensis*. The objectives in 2009-2010 were to (1) complete the evaluation and selection of interspecific breeding lines (IBL) derived from crosses of common bean with the *Phaseolus* species of the SGP, and (2) compare the white mold reaction of the resistant IBL with known sources of white mold resistance. These objectives support the Sclerotinia Initiative area of Crop Germplasm Resources and Genetics. Twenty of 915 IBL derived from three different accessions of *P. coccineus* that survived sequential screenings until May 2009 along with the three previously developed IBL (VCW 54, VCW 55, VRW 32) and eight partially resistant and susceptible checks were compared in the greenhouse in Colorado and Idaho in 2009-2010. A randomized complete block design with three replicates (6 plants per replicate) was used. Each plant was inoculated two times and white mold disease severity was scored on a 1 to 9 scale (where 1 = healthy with no disease symptoms and 9 = severely diseased or dead plants) at 14 and 28 days post the first inoculation using a modified cut-stem method. The mean white mold score of all genotypes was lower at 14 d compared to 28 d evaluation in both greenhouses. Also, white mold scores were lower at both 14 and 28 d evaluations post the first inoculation in Colorado than in Idaho. The IBL derived from *P. coccineus* and *P. costaricensis*, in general, had lower white mold scores than other resistant checks. Furthermore, six of 20 IBL had lower white mold scores than previously developed IBL. But, all new IBL were still variable for their white mold reaction, and will require additional screenings and selection. Seed of white mold resistant IBL VRW 32 derived from recurrent backcrossing of ICA Pijao with *P. costaricensis* accession S 33720 was produced in the greenhouse. The first field increase was made at Kimberly, Idaho in May-September planting in 2010. Harvested seed of VRW 32 and more than a dozen new IBL derived from *P. coccineus* accessions G 35006 and PI 433246 has been sent to Puerto Rico for additional seed increases during the off-season. Thus, we hope to have sufficient quantity of seed for further testing in regional and national nurseries by public and private researchers to determine their usefulness for combating white mold disease in the USA. Subsequently, the most promising and contrasting IBL will be released and registered in the Journal of Plant Registrations.

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Expression of the oxalate oxidase gene in transgenic lentils and evaluation of transgenic plants for resistance to *Sclerotinia sclerotiorum*

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Funded Plan of Work: Expression of the oxalate oxidase gene in transgenic lentils and evaluation of transgenic plants for resistance to *Sclerotinia sclerotiorum*

ABSTRACT:
White mold disease, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a particularly destructive disease of lentils in the US. Screenings of lentil varieties and germplasm for resistance to white mold has failed to identify any genotypes with acceptable levels of resistance. The lack of appreciable genetic resistance among lentils tested suggests that it may be very challenging to develop resistant varieties of lentils using conventional breeding methods. The oxalate oxidase gene from barley is known to detoxify the effects of oxalic acid, a primary determinant of pathogenicity of *S. sclerotiorum*. Expression of a transgenic oxalate oxidase gene construct has been shown to confer tolerance to white mold in soybean. The objective of this work is to express this gene in transgenic lentil and examine levels of resistance to white mold in these transgenic lines. We have developed protocols regenerate shoots in the presence of either kanamycin or hygromycin from lentil cotyledonary nodes using *A. rhizogenes* 18r12v and the binary vector pAKK 1444B. Successful regeneration protocols have been developed for two high yielding lentil breeding lines LC01602062T, a Turkish Red lentil, and LC02601144P, a Spanish Brown (Pardina) lentil. The oxalate oxidase gene construct from pDW25 was sequenced. Five point mutations in this sequence were detected when compared to the wild type gene sequence of the oxalate oxidase gene from barley (GenBank accession # Y14203). Four mismatches are in 'wobble' positions but the mutation at position 2 of codon 79 resulted in the substitution of valine for alanine (Fig. 4). PCR was done to amplify the oxalate oxidase gene from barley DNA. The gene was cloned into pCR® 2.1-TOPO and several individual clones were sequenced. A single clone (pEVOO1) was identified that has 100% sequence homology to the wild type oxalate oxidase gene. We are currently performing recombinant DNA procedures to excise the GUS gene from pCAMBIA 1301 and in its place ligate the oxalate oxidase gene from pEVOO1 between the 35S Promoter and NOS-Poly A domains. Expression constructs in which the oxalate oxidase gene under the control of the potato superubiquitin promoter in pBINARS will also be developed using appropriate recombinant DNA approaches. Realizing these immediate objectives are critical prerequisites for the ultimate objectives of this study, namely the development of lentil lines that express resistance to Sclerotinia white mold.

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Enhancing soybean for resistance to Sclerotinia stem rot

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

ABSTRACT:
Sclerotinia stem rot is a major soybean disease in Michigan and other states where cool and wet weather prevails during the soybean flowering time. The most economic practice to control the disease is to use resistant varieties. The long-term goal of this research is to improve soybean for resistance to the disease. The current objectives are to evaluate advanced breeding lines derived from different resistance sources for resistance to the disease and for yield and other agronomic traits and to determine if reported quantitative trait loci (QTLs) associated with resistance to Sclerotinia stem rot are also associated with the resistance in the new resistance sources used in our breeding program.

Over 1,000 advanced breeding lines derived from seven different sources of resistance to Sclerotinia stem rot were evaluated for yield and other agronomic traits in multiple locations and multiple years in Michigan. Five lines were selected and were further evaluated for yield and other agronomic traits in regional trials across northern US states in 2010.

Of the over 1,000 lines evaluated for yield, 392 lines were derived from five new resistant sources. These lines were evaluated for resistance to Sclerotinia stem rot in the greenhouse with the drop-mycelium method. These 392 lines were also tested with simple sequence repeat (SSR) markers linked to 33 reported resistance QTLs. Markers from 8 of the 33 QTL regions were significantly associated resistance to Sclerotinia stem rot in the 392 lines.

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Screening of Sunflower for Resistance to Sclerotinia Head Rot

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Funded Plan of Work: Evaluation of Sunflower Hybrids and Germplasm for Resistance to Sclerotinia

ABSTRACT:

In order to support efforts to improve Sclerotinia head rot resistance of sunflowers, commercial hybrids and breeding lines were screened for resistance at five locations across Manitoba, Minnesota, and North Dakota in 2010. Two sets of screenings were conducted: an initial screening of 73 sunflower hybrids and breeding lines that had not been previously evaluated publically, and a repeat screening of 25 hybrids and breeding lines previously evaluated in 2009. The repeat screening was designed to confirm resistance identified in sunflower head rot screenings conducted in 2009. To facilitate disease establishment, sunflower heads were inoculated with approximately 15,000 ascospores at early to mid-flowering (approximately R5.2 to R5.8 growth stage), and a favorable microclimate for disease was created withmicrosprinklers. Misting was initiated at flowering and continued as necessary to promote disease development.

Sclerotinia head rot establishment was moderate to excellent across sites. In the initial screenings, average head rot incidence ranged from 4 to 81% in Carrington, ND and 0 to 29% in Morden, MB. In the repeat screenings, average head rot incidence ranged from 10 to 81% in Carrington, ND, 7 to 70% in Langdon, ND, 5 to 78% in Crookston, MN, 26 to 93% in Oaks, ND, and 0 to 45% in Morden.

Sclerotinia head rot incidence of hybrids was moderately correlated across sites in the 2010 screenings. The Pearson correlation coefficient ranged from 0.14 to 0.69 between repeat screens, with the correlation exceeding 0.50 in five of the ten pairs of repeat screens. The Pearson correlation coefficient between the initial screens conducted in Carrington and Morden was 0.27. The relationship was significant in six of ten pairs of repeat screens and between the initial screens conducted in Carrington and Morden ($P < 0.05$). Unusually severe sunflower midge (*Contariniaschulzi*) pressure at the Morden, MB, Carrington, ND, and Crookston, MN screening sites complicated research efforts, with the lowest correlation in Sclerotinia head rot susceptibility of hybrids occurring between midge-infested and midge-free screening sites.

The combined analysis of both initial screens and the combined analysis of all five repeat screens each identified multiple entries that exhibited reduced Sclerotinia head rot incidence relative to the most susceptible entry.

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Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection using next-generation sequencing

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Funded Plan of Work: Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection using next-generation sequencing

ABSTRACT:
The objective of the current study is to identify fungal and plant host genes differentially expressed during resistant and susceptible interactions. Selected doubled haploid canola lines susceptible and resistant to *Sclerotinia sclerotiorum* infection were inoculated in growth chambers using the NE162 isolate from beans that had been used for genome sequencing. A petiole inoculation technique was used and infected petioles, along with appropriate controls, were harvested at 8, 16, 24, and 48 hours post inoculation. RNA extracted from harvested material was used to generate cDNA libraries. These libraries have been submitted for high-throughput DNA sequencing using Illumina/Solexa technology. Analysis of expression patterns of selected *S. sclerotiorum* genes expected to be involved in the disease process is being conducted using reverse transcription PCR to determine if there are differences in gene expression during resistant and susceptible interactions. Results from these initial experiments will be presented and on-going efforts aimed at identification of novel resistance and pathogenicity genes will be discussed.

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