National Sclerotinia Initiative

USDA-ARS

2012 Sclerotinia Initiative Annual Meeting

Bloomington, MN

January 18-20, 2012






2012 National Sclerotinia Initiative Annual Meeting

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Crowne Plaza Hotel & Suites, Bloomington, MN

AGENDA

2012 Sclerotinia Initiative Annual Meeting
January 18-20, 2012

Wednesday – January 18, 2012

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

Thursday – January 19, 2012

7:15 am	Registration/Continental Breakfast	McIntosh/Jonathan Room
8:15 am	Welcome, Introductions & Meeting Charge – **Bill Kemp, USDA-ARS, Fargo, ND**	
8:20 am	Welcome and Update from the Northern Plains Area – **Mickey McGuire, USDA-ARS, Fort Collins, CO**	
8:30 am	ARS Office of National Programs Update – **Roy Scott, USDA-ARS, Belstville, MD**	
8:45 am	Agreements Update – **Marcie Currie-Gross, USDA-ARS, Fort Collins, CO**	
	Sclerotinia Research Activities – Session 1 Fireside Room **Moderator –Keven McPhee, North Dakota State University, Fargo, ND**	
9:00 am	Functional verification of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and *Arabidopsis* – **Steve Clough, USDA-ARS, Urbana, IL**	
9:15 am	eQTL analysis in soybean populations to elucidate genetic architecture of host response to infection by *Sclerotinia sclerotiorum* – **George Graef, University of Nebraska, Lincoln, NE**	
9:30 am	Identification of novel loci for resistance to *Sclerotinia* stem rot in perennial soybean accessions **– Leslie Domier, USDA-ARS, Urbana, IL**	
9:45 am	Enhancing soybean for resistance to *Sclerotinia* stem rot **– Dechun Wang, Michigan State University, East Lansing, MI**	
10:00 am	Discussion Break	Ballroom Foyer
10:30 am	***Featured Speaker*** Genome structural variation and phenotypic diversity in soybean – **Robert Stupar, University of Minnesota, St. Paul, MN**	

11:30 am	Working Lunch	McIntosh/Jonathan Room

Sclerotinia Research Activities – Session 2 Fireside Room
Moderator –Thomas Gulya, USDA-ARS, Fargo, ND

12:45 pm	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**
1:00 pm	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding – **Martin Chilvers, Michigan State University, East Lansing, MI)**
1:15 pm	Transgenic expression of an oxalate oxidase gene in lentil to improve tolerance to *Sclerotinia* wilt – **George Vandemark, USDA-ARS, Pullman, WA**
1:30 pm	(1) Variation in pathogenicity and fungicide sensitivity in relation to variation of neutral markers of *Sclerotinia sclerotiorum*; (2) Searching for resistance sources to *Sclerotinia* in wild relatives of cool season grain legumes – **Weidong Chen, USDA-ARS, Pullman, WA**
1:45 pm	Identification and validation of QTL for white mold resistance in pinto bean – **James Kelly, Michigan State University, East Lansing, MI**
2:00 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**
2:15 pm	Inheritance studies of new potential sources of resistance to white mold in dry bean – **Juan Osorno, North Dakota State University, Fargo, ND**
2:30 pm	Break & Poster Session McIntosh/Jonathan Room

Sclerotinia Research Activities – Session 3 Fireside Room
Moderator – Rubella Goswami, North Dakota State University, Fargo, ND

2:45 pm	Discovery of novel sources of resistance to head rot and stalk rot in cultivated sunflower and wild *Helianthus* **– Thomas Gulya, USDA-ARS, Fargo, ND**
3:00 pm	Transferring *Sclerotinia* resistance genes from wild *Helianthus* species into cultivated sunflower – **Chao-Chien Jan, USDA-ARS, Fargo, ND**
3:15 pm	Use of a transformation system in sunflower for *Sclerotinia* resistance studies – **John Finer, The Ohio State University, Wooster, OH**

3:30 pm	Evaluation of wild *Helianthus* species for resistance to *Sclerotinia* stalk rot – **Charles Block, USDA-ARS, Ames, IA**
3:45 pm	Evaluation of sunflower hybrids and germplasm for resistance to *Sclerotinia* – **Michael Wunsch, North Dakota State University, Carrington, ND**
4:00 pm	Deployment of novel sources of *Sclerotinia* resistance in sunflower – **Lili Qi, USDA-ARS, Fargo, ND**
4:15 pm	Association mapping of *Sclerotinia* stalk rot resistance in domesticated sunflower plant introductions – **Zahirul Talukder, North Dakota State University, Fargo, ND; Brent Hulke, USDA-ARS, Fargo, ND**
4:30 pm	Evaluation of fungicides for management of *Sclerotinia* head rot in sunflowers – **Samuel Markel, North Dakota State University, Fargo, ND**
4:45 pm	Wrap-up & Adjourn (Dinner on your own)

Friday – January 20, 2012

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

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

8:15 am	On-farm validation of cultural practice adjustments to improve white mold management in dry bean irrigation systems – **Howard Schwartz & Mark Brick, Colorado State University, Fort Collins, CO**
8:30 am	Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas – **James Steadman, University of Nebraska, Lincoln, NE**
8:45 am	Genetic variation and virulence of *Sclerotinia sclerotiorum* in the United States – **Berlin Nelson, North Dakota State University, Fargo, ND**
9:00 am	Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection using next generation sequencing – **Rubella Goswami, North Dakota State University, Fargo, ND**
9:15 am	(1) Optimizing management of *Sclerotinia* diseases through fungicide use (2) 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**

9:30 am	Use of multiple pathogen isolates, inoculations, and evaluations for selecting common bean breeding lines from diverse origins with broadly effective high levels of white mold resistance – **Shree Singh, University of Idaho, Kimberly, ID**	
9:45 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-ARS, Office of National Programs–Retired, Raleigh, NC**	
10:45 am	Strategic Plan Discussion – Writing Team Input/Revisions	
11:45 am	Working Lunch	McIntosh/Jonathan Room
1:00 pm	Assignment of Additional Tasks & Wrap-up of Initiative Business	
2:00 pm	Adjourn (Travel Safely!)	

2012 Sclerotinia Initiative Poster Session

Epidemiology & Disease Management		
Poster No.	Title	Author(s)
1	Characterization of *Sclerotinia sclerotiorum* isolates from North Central US to thiophanate methyl	G. Ameen, L.E. del Rio
2	The impact on sunflower rust on hybrid performance in North Dakota	A. Friskop, S. Markell, F. Mathew, B. Schatz, P. Hendrickson, T.J. Gulya
3	Cultivar and plant spacing effects upon white mold management in dry bean	H.F. Schwartz, M.A. Brick

Genomics		
Poster No.	Title	Author(s)
4	Functional verification of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and Arabidopsis	B. Calla, L. Blahut-Beatty, L. Koziol, D.J. Neece, D. Simmonds, S.J. Clough
5	Identification of novel loci for partial resistance Sclerotinia stem rot in perennial soybean accessions	S. Chang, G.L. Hartman, L.L. Domier
6	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding	M.I. Chilvers, X. Zhuang, T. Coram, K. McPhee
7	Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection on canola	R.S. Goswami, W. Yajima, K. Chittem, L.del Rio Mendoza
8	Comparison of transcriptomes between *Sclerotinia sclerotiorum* and *S. trifoliorum*	D. Qiu, G. Vandemark, W. Chen

Pathogen Biology & Development		
Poster No.	Title	Author(s)
9	Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas	S. McCoy, R. Higgins, J. Steadman
10	Genetic variation and aggressiveness of *Sclerotinia sclerotiorum* in the United States	C. Qiu, B.D. Nelson

2012 Sclerotinia Initiative Poster Session

Variety Development/Germplasm Enhancement		
Poster No.	Title	Author(s)
11	Evaluation of wild *Helianthus* species for resistance to Sclerotinia stalk rot	C.C. Block, L.F. Marek, T.J. Gulya
12	Reaction of bean lines to isolates of white mold from Brazil	F.F. Carneiro, J.B. dos Santos, R. S.B. Carvalho, M.E. Leite, I.A. Lima, J.D. Kelly
13	Use of a transformation system in sunflower for Sclerotinia resistance studies	J.J. Finer, Z. Zhang
14	Discovery of novel sources of resistance to head rot and stalk rot in cultivated sunflower and wild *Helianthus*	T. Gulya, N. Balbyshev, B. Hulke
15	Transferring Sclerotinia resistance genes from wild *Helianthus* species into cultivated sunflower	Z. Liu, F. Wei, X. Cai, G.J. Seiler, T.A. Gulya, K.Y. Rashid, C.C. Jan
16	eQTL analysis in soybean populations to elucidate genetic architecture of host response to infection by *Sclerotinia sclerotiorum*	O.V. Lopez, J. Jedlicka, G. Stacey, J. Specht, G. Graef
17	Identification and validation of QTL for white mold in two pinto bean RIL populations	W. Mkwaila, E. Wright, J.D. Kelly
18	Quantative trait loci analysis and find mapping of genes for resistance to Sclerotinia stem rot in soybean	C. Paul, C. Hill, G.L. Hartman
19	Deployment of novel sources of Sclerotinia resistance in sunflower	L. Qi, T.J. Gulya, C.C. Block, B.A. Vick, B.S. Hulke
20	Reaction of sunflower hybrids to Sclerotinia head rot in Manitoba	K.Y. Rashid
21	Use of multiple pathogen isolates, inoculations, and evaluations for selecting common bean breeding lines from diverse origins with broadly effective high levels of white mold resistance	S.P. Singh, H.F. Schwartz
22	Association mapping of Sclerotinia stalk rot resistance in domesticated sunflower plant introductions	Z.I. Talukder, B.S. Hulke, L. Qi, T.J. Gulya
23	Characterization of the genetic basis for partial resistance to Sclerotinia sclerotiorum in pea	B. Tashtemirov, L. Porter, K. McPhee
24	Enhancing soybean for resistance to Sclerotinia stem rot – Progress in 2011	D. Wang, M. Chilvers
25	Improving the methods used to screen sunflowers for resistance to Sclerotinia head rot: Lessons learned from screening nurseries and inoculation timing experiments in 2011	M.J. Wunsch, K. Rashid, S. Halley, L. Besemann, G. Thompson, J. Bergman, M. Schaefer, B. Schatz

Characterization of *Sclerotinia sclerotiorum* isolates from North Central US to thiophanate methyl

G. Ameen, and L. E. del Río Department of Plant Pathology, North Dakota State University, Fargo, ND 50108

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

ABSTRACT:
Sclerotinia sclerotiorum affects most broadleaf crops grown in North central US, this includes canola, chickpea, dry bean, lentils, soybean, and sunflower. In most cases growers apply fungicides as the main defense against this pathogen. Fungicides are applied once or twice usually at flowering time. Chemical compounds registered for use against *S. sclerotiorum* include azoxystrobin, pyraclostrobin, thiophanate methyl, boscalid, and metconazole. These compounds have single-site mode of action and some also have a history of resistance buildup making the monitoring of fungicide sensitivity a necessity. In this study, the sensitivity of 95*S. sclerotiorum* to thiophanate methyl was assessed in fungicide-amended potato dextrose agar. Isolates evaluated were collected from 13 states in the North Central region of the US. EC_{50} values ranged from 0.01 to 2.4 µg ml^{-1} with two isolates havingEC_{50}>2µg ml^{-1}. These two isolates are considered to be resistant to thiophanate methyl. Six other isolates had EC_{50}>1.5µg ml^{-1}. These results suggest that a shift towards resistance against this compound may be occurring. Thus, alternative ways to use this compound, like its use in combination with other fungicides should be explored.

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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**.**

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 wild annual species (1,358 accessions) and perennial species (824 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 the 2010-11 greenhouse trials, several perennial species were evaluated including *H. ciliaris* (4 acc.), *H. eggertii* (5 acc.), *H. resinosus* (14 acc.), *H.* tuberosus (38 acc.) and *H. salicifolius* (14 acc.). This was the first test of these species except for *H. resinosus,* which had 5 previously-tested accessions. All of the perennial species showed remarkable resistance. Among *H. resinosus*, 11 of 14 accessions had $\geq$ 90% plant survival and eight had 100% survival. For *H. salicifolius*, 12 of 14 accessions had $\geq$90% survival and seven had 100% survival. *Helianthus salicifolius* was the first diploid perennial species tested. The diploids may be more useful for *H. annuus* improvement than the hexaploid species because of crossing compatibility. *Helianthus tuberosus* also showed excellent resistance in the greenhouse. Of the 38 accessions tested, 30 had $\geq$90% plant survival and nine accessions had 100% survival. *Helianthus eggertii* and *H. ciliaris* were slightly lower but still impressive, with 75-96% survival among the five H. *eggertii* accessions and 62-100% survival among the four *H. ciliaris* accessions. We also tested 31 cultivated *H. annuus* accessions that had some mention of Sclerotinia head rot or stalk rot tolerance/resistance in their background narrative. One accession, PI 546353 (CM 615), was superior to the moderately resistant (tolerant) check hybrids F30294 and Croplan 305. Two accessions, PI 639163 (RHA 440) and PI 603987 (RHA 391), that were initially released as head rot tolerant restorer lines also showed some Sclerotinia stalk rot tolerance.

Twenty-one entries were planted in an inoculated field trial at Staples, MN including accessions of *H. annuus, H. argophyllus, H. debilis, H. neglectus* and *H. praecox*. Disease developed well, with the most resistant entries being found in *H. argophyllus* (PI 649865), *H. debilis* (PI 468686), and *H. praecox* (PI 435849).

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Functional verification of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and Arabidopsis

Bernarda Calla and Doris Carbajulca, University of Illinois, Urbana, IL;
Laureen Blahut-Beatty, Lisa Koziol and Daina Simmonds, AgCanada, Ottawa;
David J. Neece and Steven J. Clough, USDA-ARS and University of Illinois, Urbana

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

ABSTRACT:
Many dicots, such as soybean, are susceptible to *Sclerotinia sclerotiorum.* Soybean do not have resistance under environmental conditions that favor the pathogen. A critical virulence factor of Sclerotinia is oxalic acid (OA). Mutants that lack OA are very weak pathogens, and plants expressing oxalate oxidase (OxO), an enzyme that degrades OA are able to effectively defend against this pathogen. We have conducted a series of gene expression studies to identify genes in soybean that are responding to Sclerotinia or to pure OA. To determine soybean response to this pathogen, we have conducted microarray, RNA-seq and small RNA expression analyses from tissue infected with Sclerotinia as well as from leaves infiltrated with 5 mM OA, a concentration similar to that detected during Sclerotinia infection. Additionally, we have also determined small RNA and RNA-seq expression in Sclerotinia mycelia *in planta* and in pure culture, to allow identification of infection-specific Sclerotinia RNA. Interestingly, contrary to the predominant hypothesis that most fungi lack microRNAs, we found that Sclerotinia does produce microRNA, but as a reduced set and in low abundance. Expression data is being clustered across multiple experiments to identify putative defense-related genes. Candidate defense genes are being studied on a functional level by generation of transgenic soybeans that either over or under express the gene of interest. Additionally, homologous genes are being identified in Arabidopsis where T-DNA mutants of specific genes are available and for which the generation of transformants is much more rapid and efficient.

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Reaction of Bean Lines to Isolates of White Mold from Brazil

Flávia F. Carneiro[1,2]; João B. dos Santos[1]; Renato S. B. Carvalho [1]; Monik E. Leite[1]; Igor A. Lima[1] and James D. Kelly[2]

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[2] – Michigan State University, East Lansing MI 48824

Funded Plan of Work: Identification and validation of QTL for white mold in pinto bean

ABSTRACT:
The study was designed to determine the aggressiveness of different isolates of *Sclerotinia sclerotiorum* and to assess the level of resistance of different common bean (*Phaseolus vulgaris* L.) breeding lines to white mold. Thirteen breeding lines were inoculated with six isolates of *S. sclerotiorum* from different locations in Brazil during the 2009/2010 rainy season; which included four new isolates, plus two common to the previous season during the 2010 dry season. Each genotype was inoculated in the field with a single isolate using the straw test and evaluation was performed eight days after inoculation. Plant reaction to white mold was scored using a scale from 1 (no symptoms) to 9 (plant death). There were significant differences among lines in both seasons, indicating the presence of different resistance alleles. There was also a significant difference between isolates, suggesting the existence of genetic variation or different alleles affecting aggressiveness among isolates of *S. sclerotiorum*. The significant isolate x line interaction was small in magnitude and of the simple type, which suggests that resistance is horizontal in nature. Promising breeding lines with a carioca seed type were identified in the study.

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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

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

ABSTRACT:
White mold, caused by *Sclerotinia sclerotiorum* is one of the most important diseases of pea, however little is known about the pea-*S. sclerotiorum* genetic interaction. To clarify the interaction of pathogen and host at the genetic level, whole transcriptome sequencing (RNAseq) was used.In initial experiments, a large expressed sequence tag (EST) data set from apea stem sample infected with *S. sclerotiorum* was developed by massively parallel sequencing on a 454 Roche platform.10,158 contigs were obtained by de novo assembly of 128,720 high-quality reads with an average read length of >200 nucleotides. To distinguish pea and *S. sclerotiorum* ESTs from the mixed transcriptome, a method based on the tBlastx program was developed. The method successfully separated 90.1% of an artificial mixture of pea-*S. sclerotiorum* ESTs (including 15,289 pea and 16,908 *S. sclerotiorum* ESTs) from public databases with 99.9% accuracy. Using the method, 89.4% of 454-derived EST contigs were parsedinto pea (6,299 contigs) and *S. sclerotiorum* (2,780 contigs). After annotation, 597 pea ESTs were found to be putatively involved in plant defense and response to biotic or abiotic stress, and 155 *S. sclerotiorum* ESTs were described as involved in pathogenicity or virulence. To assist in the development and refinement of pea linkage maps, 37 EST-derived SSR markers based on the 454 sequence data were developed and screened for parents of four *Pisum sativum* recombinant inbred line (RIL) mapping populations; ten SSR markers produced polymorphism in at least one population. To further investigate the interaction and quantify gene expression over time global gene expression profiling of partially resistant and susceptible pea lines infected with *S. sclerotiorum* was conducted using Illumina GA2 sequencing platform with a 75 bp paired end sequencing protocol. Pea stem samples from a susceptible pea cultivar 'Lifter' and a partially resistant cultivar 'PI240515'either inoculated with *S. sclerotiorum* or mock–inoculated were collected at 12, 24 and 36 hour after inoculation. Additionally, RNA was extracted from *S. sclerotiorum* growing on the culture medium used for inoculation was also collected. After RNA extraction, cDNA library preparation and barcoding, a total of 30 cDNA samples were sequenced in 7 lanes of one Illumina Genome Analyzer flowcell, which produced more than 300 million paired-endreads. De novo assembly of those reads resulted in60,656 Lifter contigs, 67,893 PI240515 contigs and 18,178 *S. sclerotiorum* contigs obtained from mock-inoculated samples with contigs larger than 200 nucleotides; 71,228 contigs and 81,533 contigs were assembled from the Lifter and PI240515 samples infected with *S. sclerotiorum.* Annotation, sorting and expression profiling analyses are in progress.

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Developing breeding populations with resistance to *Sclerotinia* stem rot on canola

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Funded Plan of Work: Development and evaluation of canola breeding populations for resistance to *Sclerotinia sclerotiorum*.

ABSTRACT:
Brassica napus plant introductions were used to develop a population using Ames 26628 and PI458939. In the last two years we challenged seedlings from this population using the petiole inoculation technique (PIT), allowed the survivors to produce seed, and then screened their descendants. Using this selection procedure we advanced surviving plants from this population to the F_6 generation. With each new generation plants became more tolerant of infection and those plants that died took longer and longer to do so. The percentage of surviving plants increased from 30% in the F_1 to 85% in the F_4 generation.NEP63, a DH line from the cross between Ames 26628 and PI458940, was identified as having high levels of resistance when screened using the PIT. Seeds from these two lines are being increased and will be evaluated in field conditions in 2012. Also, glyphosate-tolerant canola breeding lines were identified as having statistically less disease than the commercial controls in field trials in 2011. Sclerotinia stem rot incidence ranged between 3% and 40% although most lines evaluated had <25% incidence. Seven breeding lines had SSR incidences below 15%. Some of these lines have performed well in previous years as well and have been advanced to the next level by the breeder. The lines developed from plant introductions will also be used to develop new breeding populations to study the inheritance of resistance to *S. sclerotiorum.*

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Fungicide tank mixtures for control of *Sclerotinia* stem rot on 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:
The efficacy of Quadris (azoxystrobin), Endura (boscalid), Quash (metconazole), Proline (prothioconazole), and Topsin (thiophanate methyl) to control of Sclerotinia stem rot in canola has been evaluated in field trials at Langdon and Carrington, ND since 2009. In these trials fungicide were applied either alone or in tank combinations of two products. When in tank mixtures, each compound was added at half the doses used when applied alone. The studies were conducted using a randomized complete block design with four replications. Plants at both locations were exposed to field-produced ascospores from sclerotia spread the previous fall. Also, at flowering time plants were inoculated twice with lab-produced ascospores at a concentration of 10^3 ascospores per ml. Disease pressure as well as the efficacy of control varied from year to year, but most treatments, whether applied alone or in tank mixes with other compounds, reduced disease incidence by 40% or more when compared to the non-protected plots. In some years some fungicide mixes reduced incidence by up to 75%. Tank mixing thiophanate methyl with either boscalid or prothioconazole provided similar or better protection than either of the latter two compounds applied alone and cost less. Tank mixing fungicides seems to be a viable alternative to improve control of Sclerotinia stem rot in canola.

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

Leslie L. Domier, Glen L. Hartman, USDA-ARS, Department of Crop Sciences, University of Illinois, Urbana, IL, & Sungyul Chang, Department of Crop Sciences, University of Illinois, Urbana, IL

Funded Plan of Work: Identification of novel loci for partial resistance to Sclerotinia stem rot in perennial soybean accessions

ABSTRACT:
No sources of complete resistance to Sclerotinia stem rot (SSR) have been identified in cultivated soybean (*Glycine max*). However, high levels of resistance have been observed in some accessions of perennial *Glycine* species, which are genetically much more diverse than *G. max*. The goal of this project is to map genes for resistance to SSR on the chromosomes of perennial *Glycine* species. Accessions of *G. canescens*, *G. clandestina*, *G. latifolia*, and *G. tabacina* either highly resistant or sensitive to SSR were selected for production of recombinant inbred line (RIL) populations. During 2011, putative F_1 plants derived from resistant and susceptible accessions of *G. latifolia* were confirmed to be hybrids using SNPs that were identified in parental*DCL3* genes. Repeated attempts to make crosses with *G. canescens*, *G. clandestina*, and *G. tabacina* were not successful. Over 300 F_3*G. latifolia* lines were developed and are being advanced by single seed descent. Reduced representation libraries were prepared from genomic DNAs of *G. latifolia* accessions PI559298 and PI559300, and sequenced on an Illumina sequencer, which produced over 35 million 100-nt reads from each line. Over 350,000 SNPs were identified between the two parental lines, about 6% of which aligned to the soybean genome. To test the usefulness of the SNPs and the synteny between the G. *latifolia* and *G. max* genomes, nine SNPs were selected that aligned to soybean chromosome 4 or 13. All of the *G. latifolia* SNP markers segregated in the expected 3:1 manner in 94 lines of a *G. latifolia* F_2 population, formed two distinct linkage groups, and mapped in similar orders in *G. latifolia* and *G. max*. These results confirmed that the SNPs identified from the genome sequences of the two *G. latifolia* lines will be useful for gene mapping and comparing gene locations between *G. latifolia* and *G. max*. Plant developmental stages, *S. sclerotiorum* isolates, and inoculation methods were evaluated for high-throughput screening of *G. latifolia*l ines for resistance to SSR. When development of the RILs has been completed, loci conditioning resistance to SSR will be identified by evaluating responses of RILs to infection by *S. sclerotiorum* and segregation of SNP markers. 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

John J. Finer and Zhifen Zhang
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:
Although sunflower transformation was first reported over 20 years ago, it remains inefficient and inconsistent. Most transformation reports use *Agrobacterium*-mediated transformation of shoot producing tissues that rely on different methods of wounding to allow access of the *Agrobacterium* to the initials that give rise to shoots. In this research, sunflower line RHA280 was initially identified as exceptionally responsive to shoot induction. This line was further demonstrated to be susceptible to *Agrobacterium*, showing high levels of transgene expression in sunflower cotyledons. Over the past year, our efforts have focused on transformation targeting of the cells in the cotyledonary tissues of dry seeds, which give rise to shoot. Wounding and targeting methodologies include the following: Sonication Assisted *Agrobacterium*-mediated Transformation (SAAT), vacuum infiltration with wetting agents, vortexing with sand, bombardment with different sized tungsten particles, and bombardment with sand. The wounding treatments led to increases in gene introduction and gene expression but those treatments also led to an apparent hypersensitive response (HR), which diminished shoot production response. Efforts are underway to minimize the HR using various reducing agents and to determine if reduced wounding, along with increased throughput will provide the best balance of efforts for transgenic shoot production. Additional methods for rooting or grafting of shoots are also being developed. Classical auxin induction of roots from regenerating shoots along with the use of *Agrobacterium rhizogenes* for production of transgenic hairy roots on regenerating shoots were marginally successful. Based on success in grafting of non-transgenic seedling shoots to scions of greenhouse-grown plants, efforts are underway to evaluate grafting of *in vitro* microshoots onto *in vitro* seedlings.

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The impact on rust on sunflower hybrid performance in North Dakota

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Funded Plan of Work:

ABSTRACT:
Sunflower rust, caused by *Pucciniahelianthi* (Schwein), is an important yield-limiting disease on confection and oilseed sunflowers (*Helianthus annuus*). In recent years, the incidence of sunflower fields infected with rust has increased in North Dakota, and similarly, the number of fields thought to be experiencing yield loss (a disease severity of 3% or greater on the upper leaves) has increased. The objectives of this study were to evaluate commercial hybrids for rust resistance and determine the yield impact of the disease. Separate confection and oilseed hybrid performance trials were planted at NDSU'S Carrington Research Extension Center in Carrington, ND in 2009 and 2010. A naturally occurring epidemic of rust occurred in both years (aggregate virulence phenotype coded to race 336), and rust severity was evaluated by estimating the percent leaf area covered with rust pustules on the upper four leaves of approximately 10 randomly-selected plants per plot at approximately the R7growth stage. Disease severity in 89% of confection hybrids exceeded 3% in both years with over half of those hybrids having a disease severity of 20% or greater in 2009. Approximately half of the oilseed hybrids in 2009 had severity over 3% in 2009, but few reached that level in 2010. In 2009, correlations between rust severity and yield was significant in oilseed (r = -0.639, P=0.0001) and confection (r = -0.6886, P=0.0016) hybrids. Further, the range of yield from lowest to highest was in excess of 3-fold in confection and 5-fold in oilseed hybrid. Heads were destroyed in 2010 by an outbreak of sunflower midge, eliminating yield data. Data indicate that most hybrids may be susceptible to rust, and that yield losses can be extreme. The correlation (r) between yield loss and rust severity were similar. However, the reduction of yield in oilseed appeared to be higher at the same level of rust observed in confections. The disease will be evaluated in hybrid performance trials that experience natural rust epidemics in future years.

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Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection on canola.

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

ABSTRACT:
This project aims at identifying potential white mold resistance genes in canola through transcriptome analysis. The study uses a next-generation high throughput sequencing approach for identification of host and pathogen genes differentially expressed during infection of resistant and susceptible canola (*Brassica napus*) lines with *Sclerotinia sclerotiorum*. A double haploid homozygous progeny from a PI line of *B. napus* with significant resistance to white mold (NEP63) and with almost no resistance (NEP32) to the disease are being used in this study. Plants from these resistant and susceptible lines were inoculated with *S. sclerotiorum* using a petiole inoculation and a leaf inoculation method and RNA from infected tissue collected at different time points was used for Illumina sequencing. A total of 153,851,612 and 109,899,384, 76 bp reads were generated from petiole inoculated and leaf inoculated libraries respectively. These were aligned to the Sclerotinia genome for identification and screening of fungal sequences. Preliminary analysis of canola sequences from the petiole inoculation libraries revealed a total of 4350 differentially expressed ESTs, of which 1946 are up-regulated. Interestingly, more than 400 ESTs were detected exclusively in resistant line. Initial analysis of sequences from the leaf inoculated libraries led to the identification of a total of 477 differentially expressed ESTs in the resistant line NEP63, 320 of which were up-regulated and 157 were down-regulated. However, no genes were found to be exclusively turned on in resistant line under these conditions. Several genes involved in defense response have been identified among the up-regulated genes along with those associated with stress, hormone mediated signaling pathways, transcription factors that could also be involved in the regulation of resistance responses.

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

Thomas J. Gulya and Brent Hulke, USDA-ARS Sunflower Research Unit, Fargo ND

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

ABSTRACT:
In an attempt to both discover new sources of resistance and to broaden the genetic base of sunflower project breeding material, we have continued to phenotype a large group (250) of USDA Plant Introductions, and compare them with elite USDA released germplasms. These Plant Introductions (PIs) are genetically very diverse, and originate from 30 countries. Over a two-year period, four sets of stalk rot data were generated and in 2011, head rot was evaluated in two inoculated trials. This same group of PIs was also tested for *Phomopsis* resistance in four field trials in 2011, so that we will ultimately be able to do association mapping for all three diseases. A sister project has genotyped this set of PIs with 8,700 SNP markers and several re-sequenced resistance gene candidates, which has been used to map stalk rot resistance and can now be applied to head rot. While our head rot field nurseries were successful in 2011 following a move from Carrington, ND to Staples, MN to escape severe insect damage, we lost two stalk rot nurseries due to flooding and hail in one case and drought at another location, thus losing 2,000 rows of stalk rot tests. Our breeding efforts for combining head rot and stalk rot resistance have continued and we introduced new populations to our breeding program. There were hundreds of lines that underwent multiple years of evaluation in stalk rot, head rot, and yield tests, and this resulted in the 2011 release of four oilseed lines, RHA 472, 473, 474, and 475. Current lines under multiple years of selection include lines with resistance to multiple races of rust and downy mildew so that subsequent releases will embody resistance to Sclerotinia head and stalk rot plus other disease resistances, yield, and quality traits. 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 25 years to many Sclerotinia researchers across the U.S. and has graciously offered to collaborate. Work is in progress at Fargo, ND; Carrington, ND; Lincoln, NE; and Scottsbluff, NE, to optimize this ascospore production method. Publication of a method facilitating ascospore production will benefit *Sclerotinia* researchers on all crops.

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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:
Due to the lack of highly tolerant cultivated sunflower germplasm, new sources of Sclerotinia resistance from wild *Helianthus* species need to be identified and incorporated into a cultivated background. Wild perennial *Helianthus* species are highly resistant to Sclerotinia and have provided good sources of resistance for this project. Backcross BC_4F_3/BC_4F_4 progenies of stalk rot resistant hexaploid perennial *H. californicus* crossed with HA 410 were evaluated in replicated field trials in 2009-2011. Backcross progenies of five stalk and head rot resistant interspecific amphiploids crossed with HA 410 were established in the field, and BC_2F_4/BC_3F_3 families were evaluated in replicated field trials in 2009-2011. Crosses between NMS HA89 and head rot resistant diploid perennials *H. maximiliani* and *H. nuttallii* were advanced to BC_1F_4 and BC_2F_4 families for replicated field trials in 2009-2011. Stalk rot resistant diploid perennials *H. maximiliani, H. giganteus,* and *H. grosseserratus* were crossed with HA 410 in 2007, and their BC_1F_4/BC_2F_3 families evaluated in replicated field trials in 2009-2011. Replicated field tests in 2009 and 2011 for head and stalk rot resistance indicated moderate to good resistance indicating successful gene introgression. Progeny families susceptible for two years were eliminated and those resistant for two years will be further tested. However, field trials in 2010 screening for head and stalk rot resistance failed to produce usable results due to the complications of unexpected midge damage and adverse environmental conditions. Likewise, field trials in 2011 for stalk rot resistance failed due to hail damage and an extremely wet growing season at Carrington. A genomic *in situ* hybridization technique (GISH) distinguishing chromosomes of the perennials and cultivated sunflower has been developed. This technique will be used to assess meiotic chromosome pairing between chromosomes of wild perennials and the cultivated line, and the chromosome segments introgressed into the cultivated background. New perennials of *H. silphioides, H. salicifolius, H. hirsutus, H. occidentalis, H. divaricatus,* and *H. resinosus* were established to further diversify the resistance genes and to increase the probability of identifying useful major resistance QTLs. Fifty-nine alien addition progeny plants from backcrosses of interspecific amphiploids with HA 410 have been grown in the greenhouse and are being characterized. The addition lines will provide materials for identifying major QTLs for Sclerotinia resistance on specific chromosomes. Over 200 advanced backcross progeny families were grown in Fargo for seed increase for the 2012 replicated field tests.

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eQTL analysis in soybean populations to elucidate genetic architecture of host response to infection by *Sclerotinia sclerotiorum*

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Funded Plan of Work: eQTL analysis in soybean populations to elucidate genetic architecture of host response to infection by *Sclerotinia sclerotiorum*

ABSTRACT:
Phenotypic analysis using a detached leaf assay of recombinant inbred lines (RILs) from 5 related soybean populations with the cultivar Williams 82 as the common susceptible parent identified QTL in multiple populations on 16 of the 20 chromosomes. In a recent follow-up study we mapped six LysM-domain encoding genes to locations where we identified QTL on chromosomes 2, 9, 13, 15, 18, and 19. LysM genes on chromosomes 5 and 11 were not associated with our identified QTL. LysM-containing receptor-like kinases have been 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. To obtain a more complete picture of the soybean response to infection with *Sclerotinia sclerotiorum*, we proposed to evaluate gene expression in the parental lines and selected RIL populations to identify differentially expressed genes and map them in these same populations. The five parental lines were treated with either crab shell chitin (chitin) or bacterial flagellin 22 (flg22) and the PAMP-triggered oxidative burst was measured. We report the results as either total production of reactive oxygen species (ROS) or nitric oxide (NO) relative to the mock treatment. Results of the parental assays showed Vinton 81 had significantly greater ROS production than the other 5 parent lines after flg22 treatment. For the chitin treatment, Williams 82 had significantly greater ROS production than the 5 "resistant" parents, which did not differ from each other. When NO production was measured, the chitin treatment showed a significant difference between Williams 82 and each of the other parental lines except NKS 19-90. The results of the parental lines with the chitin treatment agree well with their grouping based on the detached leaf assay and field results for reaction to Sclerotinia. To date we have completed one replication of the Williams 82 x Vinton 81 RIL population (92 lines) for ROS production following flg22 treatment. Preliminary results of the QTL analysis to correlate specific genetic regions with the variation that we see in ROS production indicate that there are some genomic regions associated with ROS production that correlate with our previously identified QTL for response to Sclerotinia infection, or with regions where other identified resistance genes are mapped. We are obtaining data for NO production as well on the first replication of the population, and will report on these results at the meeting.

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Evaluation of fungicides for management of Sclerotinia head rot in sunflowers

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Funded Plan of Work: Evaluation of Fungicides for Control of Sclerotinia Head Rot of Sunflower and Determination of Yield Impacts

ABSTRACT:
Sclerotinia head rot, caused by *Sclerotinia sclerotiorum*, is one of the most serious diseases to the U.S. sunflower crop. Management of this disease is complicated by limited genetic resistance in commercial hybrids, the broad host range and long-lived nature of the pathogen, and an absence of fungicides labeled for sunflower head rot. Recently, two independent studies demonstrated a reduction in disease from a fungicide application with chemicals not labeled in sunflower, suggesting that managing this disease with fungicides may be possible. The objectives of this study were to 1) establish uniform fungicide trials in multiple environments in multiple states in order to identify fungicides that are efficacious against the sclerotinia head rot phase of the sunflower pathogen *Sclerotinia sclerotiorum*, and 2) to develop a yield loss model for head rot. Misted and Inoculated fungicide trials were established at Langdon and Carrington, ND (North Dakota State University) and Scotts Bluff, NE (University of Nebraska). In each trial, 11 to 13 treatments and a non-treated control were evaluated in an RCBD with four replications. The non-treated controls had disease incidence of approximately 0%, 35% and 67% in Scottsbluff, Langdon, and Carrington, respectively, in mid-to-late-September. No differences in disease severity between treatments and the non-treated control were observed in trials, however, differences among some treatments were observed at Langdon. Yield and quality data in the Carrington trial were compromised because of a significant hail storm, no differences in yield or quality were observed at Langdon, and yield data from Scotts Bluff has limited value without head rot development. With limited disease data the development of a yield-loss model was not possible. Historically, development of head rot has been reasonably consistent at Langdon and Carrington, and the development of Sclerotinia on other crops has been successful at Scottsbluff. Future plans include a repeat of the experiment at all locations and an increase in plot size.

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

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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:
Multiple sites and cooperators in major dry and snap bean production areas were used to validate putative new sources of resistance to white mold (WM). The SI support allowed development of adapted WM resistant bean lines form wide crosses. The WM pathogen isolates used to screen for resistance in the greenhouse and isolates found in field screening nurseries in major bean production areas exhibited a wide range of aggressiveness. Eighty-six mycelial compatibility groupings (MCGs) were found in 362 isolates. Only 15% of MCGs were sampled frequently over multiple screening locations and 85% were unique to locations. Data supports the hypothesis that differences in isolate aggressiveness are associated with MCGs; no aggressiveness differences were found within an MCG, only between MCGs. Isolates were also collected from three producer fields from each of four major bean production regions in the USA and were characterized using MCGs and the straw test for aggressiveness. When isolates from screening nurseries were compared with producer field isolates from three locations, there were significant differences in isolate aggressiveness. More sampling will be done to determine other relationships. Sixteen microsatellite markers have been tested and shown to be polymorphic with the isolates collected from our WM nurseries and growers' fields. Data from the microsatellites supports the conclusion that 70% of the total molecular variance of the current isolate haplotypes is from differences within the geographic areas. These populations demonstrate heterozygosity and high population differentiation. Characterized isolates for specific traits will be available for 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:
Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of white mold, can cause severe yield losses in pea. Partially resistant pea accessions have been previously identified. Utilization of these accessions in breeding program is challenging due to poor knowledge of genes involved in resistance. The current research project is the next step in an attempt to lessen the impact of white mold on the pea industry. The specific objectives of this study were to, l) identify accessions of *Pisum sativum* L. with a high level of resistance to *S. sclerotiorum*, 2) develop a genetic linkage map based on a population from the cross, 'Lifter'/PI240515 and 3) identify markers linked to quantitative trait loci (QTL) conferring partial resistance to white mold. The *P. sativum* core collection composed of 504 accessions and maintained by the U.S. Department of Agriculture Western Regional Plant Introduction Station (WRPIS) located at Pullman, WA, was screened for reaction to the pathogen *S. sclerotiorum* following a mini agar plug technique. In this technique two distinct mechanisms of partial resistance to white mold in pea were identified and described - lesion expansion inhibition (LEI) and nodal transmission inhibition (NTI). In a second greenhouse experiment, forty-nine accessions were identified to have LEI, forty-one accessions were identified to have NTI, and thirteen accessions possessed both. Future breeding projects will combine genetic factors contributing to partial resistance mechanisms from these accessions. In our mapping project, DNA from a population of 190 F_2 plants developed from the cross 'Lifter'/PI240515, were tested with SSR, CAPS and RAPD markers. A total of 78 markers were used to create a genetic linkage map with nine linkage groups that spanned 734 cM. Two QTL associated with resistance traits were identified and placed on this map. One QTL associated with LEI explained a total of 34% of the phenotypic variation and another QTL relating to partial resistance of NTI explained a total of 19% of the phenotypic variation. This is the first report of QTL for *S. sclerotiorum* resistance in pea. Future work will involve genetic mapping and QTL in F_7-derived RILs from the same population. Identification of additional QTL will provide better understanding of genetic resistance to white mold in pea. Ultimately, development of resistant pea varieties will be enhanced through marker assisted selection utilizing the identified QTL.

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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 provide new knowledge and tools to facilitate development of resistant cultivars. For 2011, we entered a new phase in the transfer of white mold resistance from *Phaseolus coccineus* into common bean where the validation and characterization of identified QTL is underway. During the winter of 2011 a greenhouse straw test was performed on twenty of the most resistant interspecific lines. Eighteen lines were more resistant than 91G, and one was more resistant than the resistant check G122. Results also indicated that the majority of lines were fixed for resistance. Crosses were made during the spring of 2011 with 13 interspecific lines possessing markers for identified interspecific QTL to produce 55 cross combinations. The populations will be used to fine map and validate the QTL. Inbred populations segregating for different QTL combinations were successfully characterized for disease reaction by the straw test and in the field. Clearly the WM2.2 and WM8.3 QTL have different modes of action, with WM2.2 having the greatest effect in the field and WM8.3 in the straw test, which was expected given WM2.2 from Bunsi is not expressed in the straw test. The benefit of combining WM8.3 with WM2.2 may not be worthwhile given the negative 'linkage drag' effect on yield associated with WM8.3 in a pinto bean background. However, the negative effect of WM8.3 on yield in snap bean has been overcome. We continue to use marker-assisted backcrossing, implementing some of the new indel markers identified by fine mapping in Objective 4, to move WM7.1 and WM8.3 QTL into a higher yielding commercially acceptable pinto bean market type. A new pinto germplasm USPT-WM-12 with partial resistance and high yield potential will be released. USPT-WM-12 had the highest yield out of 64 entries in the Michigan white mold nursery (data courtesy of Jim Kelly) for two consecutive years (2010 and 2011). To date we have discovered 25 indels that map within the WM7.1 QTL region. Of those, 16 are polymorphic within the mapping population we are using, and mapping is currently underway. Fine mapping WM8.3 QTL is nearly complete, as 61 additional indels have been added to the region, resulting in a 3.1 cM interval for the QTL which is narrower than the published 25 cM interval. Utilizing the gene models for that chromosome, 37 genes were differentially expressed at 24-48 hrs, after inoculation. Two defense-related genes, *LRR* and *PUB13* 2), are of particular interest because they mapped just outside the WM8.3 QTL peak. The tissue for RNAseq analysis for WM7.1 QTL has been collected and await next-gen sequencing (100 nt reads).

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Identification and Validation of QTL for White Mold in Two Pinto Bean RIL Populations

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Funded Plan of Work: Identification and validation of QTL for white mold in pinto bean

ABSTRACT:
Pinto beans (*Phaseolus vulgaris*) are the most widely grown commercial class of dry beans in the U.S. and are among the most susceptible to white mold (*Sclerotinia sclerotiorum*). In order to enhance resistance the goal of this project was to identify quantitative trait loci (QTL) that were associated with resistance to white mold and to other agronomic traits associated with disease avoidance in pinto bean. Using a 94 RIL population (AP630) generated between AN-37 and P02630 cross, QTLs controlling disease incidence in the field and greenhouse and yield were identified. Heritability estimates were low for traits like lodging (0.23), days to maturity (0.21), plant canopy height (0.25) and field disease incidence (0.23), whereas traits like yield, seed weight and the straw test had moderately high heritability estimates of 0.53, 0.74 and 0.46 respectively. A linkage map was developed using 107 SSR markers, covering 399 cM across six bean linkage groups. Interval mapping analysis detected four 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. In 2010 the marker IAC 90 was significantly associated with field resistance in single marker analysis ($p = 0.05$). In 2008 QTL for resistance were detected on B3 and B7. Yield QTL were detected on B2 and B5 accounting for up to 39% of observed variation in yield. These QTL were contributed by alleles from the adapted parent P02630, whereas the QTL for the straw test on B2 B3 and B8 came from the resistant parent AN-37. The QTL on B2 was consistent in three separate evaluations while the QTL on B3 overlapped with that for field disease resistance in 2008 which could imply that the disease score in 2008 were not severely confounded by environmental conditions. Microsatellite marker screening revealed that 43 polymorphic markers associated with resistance from the first population (AP630) were also segregating in AN-37/P02647 half-sib population (AP647). These 34 SSR's represented 31% polymorphism rate. Single marker analysis with significant markers in the population showed that the AN-37 allele of BMd-34 which was previously unlinked but now mapped near markers on bean linkage group B2 increased field resistance by 15%. The QTL on B2 is likely the same QTL as was previously mapped by other authors. The favorable alleles of marker BMd-1 in the QTL interval on B3 were from the AN-37 parent and contributed to an average of 10% increase in resistance in the greenhouse straw test in three separate tests. The QTL on B3 could be the same one that was mapped in the Aztec/ND population from which AN-37 parent was originally selected.

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Inheritance Studies of New Potential Sources of Resistance to White Mold in Dry Bean.

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Funded Plan of Work: Inheritance Studies of New Potential Sources of Resistance to White Mold in Dry Bean.

ABSTRACT:
White mold (*Sclerotinia sclerotiorum* (Lib) de Bary.) is one of the most common and problematic diseases in dry bean (*Phaseolus vulgaris* L.), especially in regions such as North Dakota and Minnesota, where more than 50% of the total U.S. dry bean production is located. Sources of resistance are scarce and not very effective against the disease, at least from the physiological standpoint. In addition, some of these sources are difficult to use due to low combining ability and poor agronomic performance. Limited progress has been achieved, obtaining improved lines containing some of the genes for white mold resistance and better agronomic performance through germplasm enhancement. Intense breeding efforts are underway in order to incorporate this resistance into elite commercial cultivars with very limited results so far. The main bottleneck has been in transferring the genetic resistance into commercial cultivars with good yield, high seed quality, and good agronomic performance. Linkage drag is often the main limitation for successful progenies. Because of the pervasiveness of the disease and the lack of good resistance, it is important to discover as many resistance loci as possible.

Two breeding lines from the NDSU dry bean breeding program have been recently identified as potential sources of resistance to white mold. A molecular approach will be used to compare these new sources with other genes and genomic regions previously reported, in order to determine if they contain new and unique resistance or if the resistance can be traced back to sources of resistance previously known. These lines are at advanced breeding stages and could be released in the near future given its competitive yield and good agronomic performance. In the meantime, this project will focus on the study of the gene or genes in these lines and the possible origins of the high levels of resistance to white mold observed.

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Quantitative trait loci analysis and fine mapping of genes for resistance to Sclerotinia stem rot in soybean

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Funded Plan of work: Variety Development/Germplasm Enhancement in soybean

ABSTRACT:
Soybean Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum* is considered to be one of the most important diseases of soybeans in the North-Central United States. Absence of high levels of resistance dictates the continued search for new sources of resistance. To date, only partial resistance to this disease has been found, and little genetic gain has been made thorough breeding for quantitative resistance. The objectives of this study were to identify new quantitative trait loci (QTL) associated with resistance to S. sclerotiorum in PI 194639 and subsequently fine map the major QTLs. Significant QTLs were mapped in the population of 155 $F_{4:5}$ RILs developed from a partially resistant parent PI 194639 to susceptible cultivar Merit. Resistance to Sclerotinia stem rot was assessed by inoculating the population with a cut stem inoculation technique and measuring the lesion length at 7 and 14 days after inoculation. These same lines were genotyped with184 SSR including TRAP markers. Two major QTLs explained the total phenotypic variation of 23.3% in linkage groups (LG) A2 and B2 and four new quantitative trait loci (QTLs) were identified in addition to the reported QTLs in LG L. These new QTLs were located in LGE (8.5%), LGF (8.1%), LGI (6.7%), and LGO (7.6%). The total phenotypic variation explained by these QTLs was 30.9%. Furthermore, recently released SSR and SNPs markers were selected in the major QTL lineage intervals of A2 and B2 for fine mapping. Resistance QTLs identified in this study could be incorporated into germplasm releases that both private and public breeders could use in developing cultivars with improved Sclerotinia stem rot resistance.

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Deployment of novel sources of Sclerotinia resistance in sunflower

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Funded Plan of Work: Deployment of novel sources of Sclerotinia resistance and tools for breeding resistance in sunflower

ABSTRACT:
Wild sunflower and the sunflower crop are native to North America. There are 51 species of wild *Helianthus*, of which 14 are annual and 37 are perennial. Sunflower wild species are an important source of genetic variation for Sclerotinia resistance. Sclerotinia stalk rot resistance was identified in wild annual species. The resistant plants were selected from 21 accessions of four wild annual species, *H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris*, and were first crossed in 2009 to a nuclear male-sterile line, NMS HA 89, which is susceptible to Sclerotinia stalk rot. Twenty F_1 hybrids were obtained, and seven of them had fewer than ten seeds. Thirteen F_1 hybrids were screened for their reaction to inoculation with *Sclerotinia sclerotiorum* in greenhouse trials in 2010. Susceptibility was determined by the percentage of dead or severely wilted plants (disease incidence) 14 days after inoculation. The hybrids showed a resistance level similar to or higher than the resistant checks, HA 441 (14%), and Croplan 305 (18%), with a disease incidence range from 0 to 25%. Among the 13 F_1 hybrids, nine had disease incidence lower that 10%, whereas HA 89 had a disease incidence of 26%, and the susceptible check Cargill 270 and Seeds 2000 4129 (both commercial hybrids) had disease incidences of 100 and 96%, respectively. We optimized infection techniques in the greenhouse trials in order to better differentiate HA 89 from resistant checks. The amount of the infected millet as inoculum was increased from 80 g (F_1 screening) to 120 g (BC_1 and BC_2 screening). The selected F_1 resistant plants were backcrossed to HA 458, and their BC_1s were again backcrossed to HA 89. A total of 575 BC_2F_1 plants from eight crosses were screened for their reaction to stalk rot in the greenhouse in 2011, and the 68 most resistant plants were advanced to the BC_2F_2 generation. Given the quantitative nature of stalk rot resistance, we are currently in the process of screening 3000-4000 plants from the BC_2F_2 populations in greenhouse trials, and will select 100-200 most resistant plants to advance to the BC_2F_3 generation for field testing to validate the greenhouse results in the 2012 growing season. To molecular map Sclerotinia stalk rot QTLs transferred from exotic germplasm, an advanced backcross (AB) population is being developed. Fifty-one BC_1F_1 plants derived from the cross of HA 89 (susceptible parent) with *H. argophyllus* (PI 494573, resistant parent) were screened for their reaction to Sclerotinia stalk rot, and 14 resistant BC_1 plants selected were again backcrossed to HA 89. A total of 300 plants from the 14 BC_2F_1 plants were advanced to the BC_2F_2 generation by single-seed descent in 2011. Our goal is to produce an AB population of $BC_2F_{2:6}$ lines by an additional four cycles of single-seed descent.

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Genetic Variation and Aggressiveness of *Sclerotinia sclerotiorum* in the United States

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Funded Plan of Work: Genetic variation and virulence of *S. sclerotiorum* in the United States

ABSTRACT:
A study of the genetic variation and virulence of S. sclerotiorum from crops in the United States is in progress using 68 isolates from 14 states outside of the north central region. A previous study examined isolates within the north central region. The isolates were from the following states: AR, AZ, CA, CT, FL, GA, ID, MA, NC, NY, OK, OR, SC and WA. These were collected from 17 different crops: broccoli, butter nut squash, cabbage, canola, carrot, cucumber, cauliflower, common bean, gourd, lentil, lettuce, parsley, pea, petunia, potato, soybean and tomato. Isolates were evaluated for mycelial compatibility group (MCG) and microsatellite haplotype at twelve loci. There were 50 MCG's identified within the 68 isolates. Thirty of the isolates were paired with 33 previously identified MCG from the north central region and seven of the previously identified MCG were identified within the isolates. Confirmation of these results and more pairing with previously identified MCG's is currently in progress. Fifty of the isolates have been genotyped with microsatellite markers, and genotyping of the remaining isolates is in progress. Aggressiveness of all isolates will be tested on multiple crops within the next year. The results from the 68 isolates will be compared to those obtained with isolates from the north central region of the United States.

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Comparison of transcriptomes between *Sclerotinia sclerotiorum* and *S. trifoliorum*

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Funded Plan of Work: Sequencing of Expressed Sequence Tags of *Sclerotinia sclerotiorum*

ABSTRACT:
Both *Sclerotinia sclerotiorum* and *S. trifoliorum* cause Sclerotinia stem and crown rot of chickpea and white mold on many economically important crops. The host range of *S. trifoliorum* is mainly on cool season forage and grain legumes of about 40 plant species, whereas the host range of *S. sclerotiorum* encompasses more than 400 plant species including all the host plant species of *S. trifoliorum*. Despite of morphological and ecological differences between the two species, both species are equally pathogenic on chickpea. Extensive research has been conducted on *S. sclerotiorum* and its genome sequences are available. However, relatively very little is known about *S. trifoliorum*. To take advantages of the genomic information of *S. sclerotiorum*, we compared the transcriptome of *S. trifoliorum* with that of *S. sclerotiorum* in order to gain a better understanding of the biology of both species. A total of 23133 unique transcripts with average length of 439bases (10.1Mb genome coverage) were obtained from *S. sclerotiorum*, whereas 21043 unique transcripts with average length of 418bases (8.8 Mb genome coverage) were obtained from *S. trifoliorum*. Approximately 43% of the transcripts were genes with known functions for both species, and approximately 60% of the transcripts were found between the two species. Among 1411 orthologcontigs (transcripts with more than one read), 147 (10%)were more highly (> 3 folds) expressed in *S. trifoliorum* than in *S. sclerotiorum*, and173 (12%) were more highly expressed in *S. sclerotiorum* than in *S. trifoliorum*. The supercontig 35 had 7-fold higher levels of average RPKM value than any of the other supercontigs for both species. Approximately 140 transcripts from each species were found in DNA regions that are not considered as coding regions, and 15 of those transcripts were the same in both species. Additionally, differences in expressed genes involved in pathogenesis like oxalate biosynthesis and endopolygalacturonases were detected between the two species.

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Effective fungicides for reducing Sclerotinia head rot in sunflower

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ABSTRACT:
Field trials have been conducted for several years at the Agriculture and Agri-Food Canada Research Station at Morden, Manitoba to test the efficacy of fungicides in reducing the sclerotinia head rot and yield losses in sunflower. Randomized complete block design trials with four replications were established every year using a susceptible oilseed hybrid with three treatments of each fungicide, at early flowering, at late flowering, and two applications at both dates followed manufacturer's recommendations. Sunflower heads were inoculated with ascospore suspension supplemented with ground sclerotinia-infected millet seed under a misting system operated at 5 mn/ 30 mn for 4 weeks. Head rot incidence was assessed weekly, and severity at the end of the season. The efficacy of fungicides varied from year to year with changes in head rot incidences, higher in 2009 and 2010 than in 2011 with dry conditions. Most fungicides reduced the head rot by 20-50% in 2009, by 50-90% in 2010, and by 20-80% in 2011, and improved the yield by 20-100%, 10-45%, and 10-40%, respectively. A few fungicides significantly reduced head rot and improved yield over the 3-years. Fungicide applications at early flowering were more effective than at late flowering, and two applications were better than one.

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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 was the final year) investigated 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 Research Initiative (SRI) 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 SRI.

During 2009 - 2011, we encountered low white mold pressure in fields with a history of the disease due to delayed plantings (early-spring rains) which delayed flowering until late July when weather conditions were warm and dry. In addition, one or more of the commercial fields was severely damaged by common rust and/or bacterial brown spot which opened up the canopy of the commercial cultivar surrounding the trial and significantly reduced the production of ascospore inoculum. Analysis of data from the multiple locations revealed that there were no interactions between fungicide treatment or nitrogen by entries or locations during 2009 and 2010; nitrogen was not compared in 2011. Yields of the 4 entries averaged 1135-3641 lb/acre at the research station and 2966-3433 lb/acre at the better commercial fields in the absence of white mold and with varying presence of bacterial diseases and rust in the surrounding fields of commercial pinto beans. When combined over locations, yield ($P < 0.01$) and seed size ($P < 0.001$) differences between entries were significant. Common rust was not a problem since the 4 entries possess genetic resistance against the common races of rust present in this region during the 2009 study.

Plant canopy monitoring at the research station site during late vegetative to seed fill periods of crop growth showed that average daily relative humidity was higher in a prostrate vine cultivar (Montrose) than an upright semivine type (Stampede); and canopy temperature showed the reverse trend during 2010-2011. These preliminary observations have led our project to a renewed focus during 2012-2013 on improving our understanding of the effects of plant spacing and architecture (prostrate versus upright) upon pathogen development and forecasting with the goal of enhancing disease forecast models and IPM approaches to deal with white mold.

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Use of Multiple Pathogen Isolates, Inoculations, and Evaluations for Selecting Common Bean Breeding Lines from Diverse Origins with Broadly Effective High Levels of White Mold Resistance

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Funded Plan of Work: Use of Multiple Pathogen Isolates, Inoculations, and Evaluations for Selecting Common Bean Breeding Lines from Diverse Origins with Broadly Effective High Levels of White Mold Resistance

ABSTRACT:
A low level of resistance to white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is found in small-seeded Middle American common bean (*Phaseolus vulgaris* L.). Comparatively higher level of resistance is found in large-seeded Andean common bean. Yet higher levels of resistance occur in the secondary gene pool species, namely *P. coccineus* and *P. costaricensis*. White mold resistance is controlled by >20 genes and quantitative trait loci (QTL). Over the past 10 years we have introgressed white mold resistance from three different *P. coccineus* (23 interspecific breeding lines) and one *P. costaricensis* (one interspecific breeding line) germplasm accessions. Also, we developed six breeding lines by combining or pyramiding white mold resistance from four large-seeded Andean common bean, using a double-cross population, and 12 breeding lines from broad-based multiple-parent crosses between the Andean and Middle American common bean and interspecific breeding lines derived from the secondary gene pool. The objective of this capstone phase of our research is to determine the effectiveness of the use of multiple pathogen isolates, inoculations, and evaluations for selecting breeding lines with broadly effective high levels of white mold resistance. Five susceptible and seven resistant controls, and the 42 white mold resistant breeding lines that we developed within the past 10 years through the National Sclerotinia Initiative projects will be evaluated in two greenhouse environments in Colorado and Idaho, using a randomized complete block design with three replicates. One less aggressive and one aggressive pathogen isolates will be used in each greenhouse. Also, three sequential inoculations will be made on the same plants, and disease severity index will then be determined at 7, 14, 21, 28, and 35 days post inoculations. Thus, breeding lines with broadly effective high levels of white mold resistance derived from *Phaseolus* species of the primary and secondary gene pools will be identified. Subsequently, highly white mold resistant breeding lines will be submitted to the National Bean White Mold Nursery to further determine their stability and effectiveness for combating white mold in the USA and North America. Also, research results and seed of highly white mold resistant breeding lines will be distributed to private and public researchers and other clienteles through state, regional, and national trials, individual requests, progress reports, refereed publications, conferences, and field trips. Subsequently, highly white mold resistant breeding lines could be used in further genetics and breeding studies and to develop common bean cultivars of different market classes to combat white mold disease problems in the USA. This project encourages inter-state and inter-disciplinary collaboration. This objective supports the Sclerotinia Initiative area of Crop Germplasm Resources and Genetics.
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Association mapping of Sclerotinia stalk rot resistance in domesticated sunflower plant introductions

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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:
Sclerotinia sclerotiorum (Lib.) de Bary is the most destructive pest of Sunflower (*Helianthus annuus* L.) worldwide. Markers based on the Sclerotinia resistance gene will enable efficient marker-assisted selection (MAS). We sequenced five candidate genes, known to associate with Sclerotinia disease resistance, in a set of 104 sunflower genotypes comprised of plant introductions (PIs) and inbred lines. This set of sunflower lines is a subset of a larger association mapping population evaluated for Sclerotinia stalk rot resistance in two-year multilocation trials. The total candidate gene sequence regions analyzed covered a concatenated length of 3,791bp per individual. The number of haplotypes per gene ranged from 5 to 31 with an average of 19.6. A total of 187 polymorphic sites were detected for all candidate gene sequences, 149 of which were SNPs and 38 were indels. Coding regions of the candidate genes identified 66 SNPs with a frequency of 1 SNP per 41 bp, while the non-coding regions identified 83 SNPs with a frequency of 1 SNP per 13 bp. Fifteen SNPs in the coding regions led to changes in amino acid codons. Two single-base indels found in the coding regions of COI24 and DET1N1 gene sequences resulted in frame-shifts and substantial amino acid changes. Intragenic LD varied between candidate genes and was generally moderate to low. LD decay throughout the candidate gene regions declined to an $r^2 = 0.2$ for genetic intervals as long as 150 bp, but extended up to 500 bp with $r^2 = 0.1$. Preliminary result revealed strong association of *COI1, EIN1* and *ABI1* and *ABI2* genes with the Sclerotinia stalk rot resistance. Further work is in progress in the development of Infinium chip for the significant SNP markers in order to genotype the remaining lines of the association mapping population using Illumina's Golden Gate assay.

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Expression of the oxalate oxidase gene in transgenic lentils

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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 is a destructive disease of lentils in the North Central US and also is responsible for episodic outbreaks of disease on lentils grown in the Pacific Northwest. The most cost effective and sustainable approach for controlling losses in lentil due to white mold is to develop new varieties that have genetic resistance to the disease. However, no reports exist in the literature of the identification of lentil genotypes with acceptable levels of resistance to white mold, which suggests that it may be very challenging to develop resistant varieties of lentils using conventional breeding methods. Transgenic approaches should be examined as part of the effort to develop lentil lines with enhanced tolerance to white mold. The goal of this project is to develop transgenic lentils that express the barley oxalate oxidase gene and evaluate these plants for resistance to *S. sclerotiorum*. Previously tested oxalate oxidase gene constructs were sequenced and several 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). PCR was done to amplify the oxalate oxidase gene from barley DNA and this gene was sequenced to confirm fidelity to the wild type sequence. The gene was cloned so that it is under the transcriptional control of the CAMV 35S Promoter and the NOS Poly-A, and this construct was cloned into the binary expression vector pCambia 1301, which carries a *hpt* gene that confers resistance to hygromycin. The recombinant vector was precipitated onto tungsten particles and introduced into the lentil line LC01602300R and the cultivar Morena by biolistics. Putative transformed shoots are currently regenerating in plant tissue culture media supplemented with the selectable agent hygromycin. Several physical parameters that influence biolistic transformation including the distance of the sample from the stopping plate, the size of tungsten particles, and the amount of helium pressure used to deliver the DNA coated particles need to be examined to develop an efficient lentil transformation system.

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Enhancing soybean for resistance to Sclerotinia stem rot – Progress in 2011

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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. Using resistant cultivars is the most effective method to control the disease. The goal of this research is to improve soybean for resistance to the disease. Multiple sources of resistance to the disease were used as parents in our breeding program. The progenies from these crosses are at various generations and were evaluated in 2011 at different scales according to their generations and stages of selections in the breeding program. Four hundred seventy one F4:5 lines were evaluated at one location for maturity, lodging, and yield. One hundred fifty four F4:6 lines selected based on 2010 data were evaluated in 2011 at two locations for yield and other agronomic traits. Thirty one F4:7 lines were evaluated at eight locations for their agronomic performance and yield under natural disease pressure. The 31 F4:7 lines were also evaluated in an inoculated Sclerotinia disease nursery with other 43 advanced selections. The disease severity index (DSI) in the disease nursery ranged from 6.7% to 73.3% and the four most resistant lines with DSI less than the 10% threshold were among the 31 F4:7 lines derived from a Sclerotinia resistant parent.

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Evaluation of sunflower hybrids and germplasm for resistance to *Sclerotinia*

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Funded Plan of Work: Evaluation of sunflower hybrids for resistance to Sclerotinia head rot

ABSTRACT:

Sclerotinia head rot, caused by *Sclerotinia sclerotiorum*, is a severe disease of sunflowers that has compromised the viability of sunflower production in parts of the upper Midwest. To facilitate the development of commercial sunflower hybrids with improved resistance to this disease, nurseries for screening hybrids for resistance were established at multiple locations. In addition, in an effort to improve screening methodologies, inoculation timing experiments were conducted to assess the susceptibility of sunflowers to head rot during and after flowering. To facilitate disease establishment, sunflower heads were inoculated with ascospores, and trials were misted with microsprinklers. Sunflower midge, which was a problem in Carrington in 2010, was successfully managed with a late planting date and insecticides.

The combined analysis across screening locations identified multiple entries that exhibited reduced Sclerotinia head rot relative to the most susceptible lines. Disease pressure was high in Carrington but optimal in other locations; disease ranged from 0 to 40% across entries in Morden,67 to 100% in Carrington, 19 to 89% in Langdon, 24 to 94% in Crookston, 3 to 90% in Oakes, and 32 to 99% in Sidney. Incidence of Sclerotinia head rot across entries was highly correlated for Oakes, Langdon, and Crookston, where moderate levels of disease were established and all sunflower heads were inoculated during flowering. Results from Carrington, Morden and Sidney were less consistent due to hail damage and high disease pressure (Carrington) and different inoculation strategies (Morden and Sidney). The results emphasize the need to limit misting to moderate levels and to inoculate all plants at the identical growth stage when screening for head rot.

The inoculation timing experiments conducted in Carrington, Langdon, and Oakes, ND suggest that sunflowers may be susceptible to head rot both during and after flowering. Inoculations at R5 (flowering) and R6 (immediately after flowering) resulted in significant increases in disease incidence relative to the control in Carrington and Langdon, respectively. However, susceptibility to head rot at the R6 growth stage may be highly dependent on environmental conditions; in Carrington, head rot incidence was sharply lower when plants were inoculated at R6 relative to R5.

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Insertional mutation at the Cu-Zn-superoxide dismutase gene reduces virulence of *Sclerotinia sclerotiorum*

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Funded Plan of Work: Identifying Virulence Factors of *Sclerotinia sclerotiorum* Through Transformation

ABSTRACT:
Sclerotinia sclerotiorum is a necroptrophic plant pathogen causing white mold and stem rot disease on many economically important pulse, vegetable and field crops, showing a non-host-specific pathogenic mechanism. Extensive studies on this pathogen have been carried out, but the pathogenic mechanisms of this pathogen are still incompletely understood. In order to gain insight in understanding its non-specific host-pathogen interactions, Agrobacterium-mediated transformation (AMT) was used to generate random mutations and to identify potential virulence/pathogenicity factors in *S. sclerotiorum*. About 900 AMT transformants were generated, purified by repeated hyphal tip isolation, and screened for virulence. This abstract is to describe the research on characterizing two of the mutants with reduced virulence. The two stable mutants showed significantly less virulence in comparison with the wild type strain as measured by colonizing pea leaves in detached leaf assays. Southern hybridization analysis showed that the mutation was due to a single T-DNA insertion, and inverse PCR and DNA sequencing identified that the T-DNA insertion site was in the gene of Cu-Zn-superoxide dismutase (SOD, SS1G00699) of *S. sclerotiorum*. This SOD gene consists of an open reading frame of 465 bps, and its expression levels were significantly induced under oxidative stresses or during infection of pea plants, but could not be detected in the mutants. Complete cDNA of the SsSOD1 functionally complemented the Cu, Zn superoxide dismutase gene in a Δsod1 *Saccharomyces cerevisiae* mutant. The SOD mutant had increased sensitivity to heavy metal toxicity and oxidative stress in culture conditions and reduced ability to detoxify superoxide in infected pea leaves. The mutation at the SsSOD1 gene also reduced expression levels of a known pathogenicity gene endo-polygalacturanase 3. These results suggest that this SOD gene plays critical roles in detoxification of reactive oxygen species during host-pathogen interactions and is an important virulence factor of *S. sclerotiorum* in pathogenesis.

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