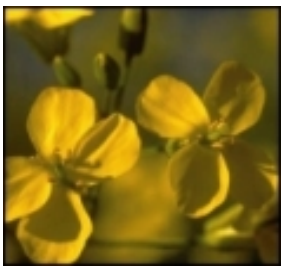
National Sclerotinia Initiative

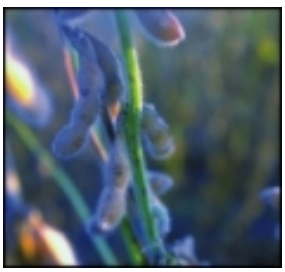
NDSU/USDA-ARS

2009 Sclerotinia Initiative Annual Meeting

Bloomington, MN

January 21-23, 2009



ASA American Soybean Association

National Sunflower Association

US Dry Bean Council

USA Dry Pea & Lentil Council

U.S. Canola Association

USB United Soybean Board

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CNSA

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The University of Georgia

University of Minnesota

Oregon State University

University of Idaho College of Agricultural and Life Sciences

Colorado State University

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IANR

University of Florida IFAS

2009 National Sclerotinia Initiative Annual Meeting

January 21-23, 2009

Holiday Inn Select

Minneapolis (Bloomington), MN

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Sclerotinia Initiative 7th Annual Meeting
January 21-23, 2009
Holiday Inn Select Minneapolis/St. Paul International Airport

Agenda

Wednesday - January 21, 2009

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

Thursday - January 22, 2009

7:30 am Registration/Continental Breakfast (**Jonathan**)

8:15 am Welcome, Introductions & Meeting Charge **- Bill Kemp, USDA-Agricultural Research Service, Fargo, ND**

8:25 am Welcome from the Northern Plains Area – **Michael McGuire, USDA-Agricultural Research Service, Fort Collins, CO**

8:30 am Thoughts on Transitions in Washington, DC – **Dale Thorenson, Gordley Associates, Washington, DC**

8:40 am ARS National Program Staff Update – **Gail Wisler, USDA-Agricultural Research Service, Beltsville, MD**

Sclerotinia Research Activities – Session 1 (**Jonathan**)
Moderator – Bill Kemp

9:00 am White mold resistance identified in common bean through multi-site screening and pathogen characterization throughout major production areas – **Jim Steadman, University of Nebraska, Lincoln, NE**

9:15 am Enhancing soybean for resistance to *Sclerotinia* stem rot – **Dechun Wang, Michigan State University, East Lansing, MI**

9:30am Cultivar, plant spacing and fungicide effects upon white mold management in dry bean – **Howard Schwartz, Colorado State University, Ft. Collins, CO**

9:45 am Discussion Break (**Ballroom Foyer**)

10:15 am (1) Characterization of the reaction of herbicide-tolerant, non herbicide-tolerant and double haploid canola lines to *Sclerotinia sclerotiorum* (2) Defining critical environmental and biological parameters needed to develop *Sclerotinia* stem rot on canola (3) Evaluation of fungicide alternatives for control of *Sclerotinia* stem rot of canola & (4) Developing a disease-warning system for *Sclerotinia* stem rot on canola – **Luis del Rio, North Dakota State University, Fargo, ND**

10:40 am	**(**1) Identifying virulence factors of *Sclerotinia sclerotiorum* through insertional mutagenesis & (2) Variation in pathogenicity and fungicide sensitivity in relation to variation of neutral markers of *Sclerotinia sclerotiorum* – **Weidong Chen, USDA-ARS, Pullman, WA**
11:00 am	*Sclerotinia* resistance enhanced by accumulation of QTL and transgenic approaches – **George Graef, University of Nebraska, Lincoln, NE**
11:15 am	Identification and functional analysis of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and *Arabidopsis* – **Steve Clough, USDA-ARS, Urbana, IL (Presenting - Daina Simmonds, Agriculture and Agri-Food Canada, Ottawa, Ontario)**
11:30 am	Identification of QTL for white mold resistance in pinto bean – **James Kelly, Michigan State University, East Lansing, MI (Presenting – Evan Wright)**
11:45 am	Working Lunch (**McIntosh**)
	Sclerotinia Research Activities – Session 2 (**Jonathan**) **Moderator – George Graef**
1:30 pm	Identifying molecular markers linked in lentil (*Lens culinaris* Medik.) to white mold resistance derived from the lentil cultivar Pennell – **George Vandemark, USDA-ARS, Pullman, WA**
1:45 pm	Mapping QTL for white mold resistance in an interspecific dry bean backcross population – **Mark Brick, Colorado State University, Fort Collins, CO**
2:00 pm	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding – **Tobin Peever, Washington State University, Pullman, WA (Presenting - Martin Chilvers, Michigan State University, East Lansing, MI)**
2:15pm	Transferring *Sclerotinia* resistance genes from wild *Helianthus* species into cultivated sunflower – **Chao Chien Jan, USDA-ARS, Fargo, ND**
2:30 pm	Development of a transformation system in sunflower for *Sclerotinia* resistance via direct gene transfer and reverse genetics approaches – **John Finer, Ohio State University, Wooster, OH**
2:45 pm	Break & Poster Session (**McIntosh**)
	Sclerotinia Research Activities – Session 3 (**Jonathan**) **Moderator – Martin Chilvers**
3:15 pm	Transfer and characterization of white mold resistance from *Phaseolus coccineus* into *P. vulgaris* – **James Myers, Oregon State University, Corvallis, OR**
3:30 pm	(1) Contribution of partial genetic resistance to white mold disease management in pinto and great northern beans & (2) Genetic characterization of scarlet-runner bean derived resistance to white mold in common bean – **Phil Miklas, USDA-ARS, Prosser, WA**

3:50 pm Characterization of the genetic basis for partial resistance to *Sclerotinia sclerotiorum* in pea – **Kevin McPhee, North Dakota State University, Fargo, ND (formerly USDA-ARS, Pullman, WA)**

4:10 pm (1) Introgressing white mold resistance from the secondary gene pool of common bean & (2) Gametic-recurrent selection for simultaneous pyramiding and introgressing white mold resistance from *Phaseolus* species into pinto beans – **Shree Singh, University of Idaho, Kimberly, ID**

4:30 pm Wrap-up & Adjourn (Dinner on your own)

Friday - January 23, 2009

7:00 am Steering Committee Breakfast Meeting (**Beacon Conference Room**)

7:15 am Continental Breakfast (**Ballroom Foyer**)

Sclerotinia Research Activities – Session 4 (**Jonathan**)
Moderator – Kevin McPhee

8:30 am Screening of sunflower for resistance to *Sclerotinia* head rot **– Blaine Schatz, North Dakota State University, Carrington, ND**

8:45 am Uniform sunflower germplasm evaluation for resistance to *Sclerotinia* head rot in Minnesota – **Charla Hollingsworth, University of Minnesota, Crookston, MN**

9:00 am Evaluation of wild *Helianthus* species for resistance to *Sclerotinia* stalk rot – **Charles Block, USDA-ARS, Ames, IA**

9:15 am Genetic variation and virulence of *S. sclerotiorum* on six crops in the North Central Region – **Laura Aldrich-Wolfe and Berlin Nelson, North Dakota State University, Fargo, ND**

9:30 am The requirement for oxalate during pathogenesis on multiple crops – **Jeff Rollins, University of Florida, Gainesville, FL**

9:45 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**

10:00 am Break (**Ballroom Foyer**)

***Sclerotinia Initiative Research: The next steps* –** (**Jonathan**)
Moderator – Bill Kemp

10:30 am ***Guest Speaker***
Strategic Planning & Reporting Progress – **Rich Wilson (USDA-Agricultural Research Service, National Program Staff –Retired, Raleigh, NC)**

11:00 am	Strategic Plan Discussion, RFP Process Improvement, Updating Website, On-line reprints & Fact sheets, Assignment of Additional Tasks & Wrap-up of Initiative Business
Noon	Working Lunch (**McIntosh**)
1:30 pm	Adjourn (Travel Safely!)

2009 Sclerotinia Initiative Poster Session

Epidemiology & Disease Management		
Poster No.	Title	Author
1	Evaluation of fungicide alternatives for control Sclerotinia stem rot on canola	L.E. del Rio, S. Halley
2	Cultivar, plant spacing and fungicide effects upon white mold management in dry bean	H.F. Schwartz, M.A. Brick, S.P. Singh
Genomics		
Poster No.	Title	Author
3	Identification and functional analysis of candidate defense genes in soybean	B. Calla, L. Blahut-Beatty, Y. Zhang, D. Simmonds, S.J. Clough
4	Sequencing of expressed sequence tags of *Sclerotinia sclerotiorum* and *Pisum sativum*	M.I. Chilvers, T.L. Peever, T. Coram, K. McPhee, W. Chen
5	Integration of QTL for white mold resistance on the bean linkage map	M. Soule, P. Miklas, L. Porter
Pathogen Biology & Development		
Poster No.	Title	Author
6	Genetic variation of *Sclerotinia sclerotiorum* on crops in the North Central United States	L. Aldrich-Wolfe, B.D. Nelson
7	Evaluation of wild *Helianthus* species for resistance to Sclerotinia stalk rot	C.C Block, L.F. Marek, T.J. Gulya
8	Searching for genetic determinants of pathogenicity in *Sclerotinia sclerotiorum*	W. Chen, K. McPhee, G. Vandemark
9	Genetic diversity of Scl*e*rotinia *sclerotiorum* from various crops from the US Pacific Northwest	W. Chen, L. Porter, D.A. Johnson
10	Improvement in screening for resistance to *Sclerotinia sclerotiorum* in common bean through characterization of the pathogen and utilization of multi-state nurseries	L.K. Otto-Hanson, J.R. Steadman
11	The requirement for oxalate during pathogenesis on multiple crops	J.A. Rollins, M. Li
Variety Development/Germplasm Enhancement		
Poster No.	Title	Author
12	Characterization of the reaction of herbicide-tolerant, non herbicide-tolerant and double haploid canola lines to Sclerotinia	L.E. del Rio, F. Zabala
13	Transferring Sclerotinia resistance genes from diverse wild *Helianthus* species into cultivated sunflower	J. Feng, Z. Liu, X. Cai, G.J. Seiler, T.A. Gulya, K.Y. Rashid, C.C. Jan
14	Development of a transformation system in sunflower for Sclerotinia resistance via direct gene transfer and reverse genetics approaches	J.J. Finer
15	Search for new sources of stalk rot resistance in USDA sunflower plant introductions	T. Gulya, L. Marek, C.C. Block
16	Determining Sclerotinia Head Rot Resistance Responses from Sunflower Hybrids in Minnesota	C.R. Hollingsworth, C.D. Motteberg, A.J. McMechan
17	Pyramiding Sclerotinia head rot and stalk rot resistances into elite sunflower breeding lines with the aid of DNA markers	B. Hulke, B. Yue, J. Hu, T. Gulya

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Poster No.	Title	Author
18	Screening of sunflower for resistance to *Sclerotinia* head rot	D.K. Lee, B. Schatz, E. Aberle, C. Hollingsworth, S. Halley, T.J. Gulya
19	Improved resistance to *S. sclerotiorum* in pea and lentil through breeding and biotechnology	K. McPhee, W. Chen, B. Schatz
20	Characterization of the genetic basis for partial resistance to *Sclerotinia sclerotiorum* in pea	K. McPhee, L. Porter, G. Vandemark
21	QTL for white mold resistance in an interspecific dry bean backcross population	M. Newell, M.A. Brick, P. Byrne, H.F. Schwartz
22	Identifying molecular markers in lentil (*Lens culinaris* Medik.) associated with white mold resistance	G. Vandemark, W. Chen, L. Porter
23	Characterization of white mold resistance transferred into common bean from scarlet runner	S. Zimmerman, J.R. Myers

Genetic variation of *Sclerotinia sclerotiorum* on crops in the north central United States

Laura Aldrich-Wolfe and Berlin D. Nelson, North Dakota State University, Fargo, ND

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

ABSTRACT:
Sclerotinia sclerotiorum is an important pathogen of many of the most commonly-grown crops of the northern tier of states in the North Central Region, yet little is known about how this pathogen varies either genetically or in virulence across the region and across the different crop host species. In fall 2008, we obtained isolates of *S. sclerotiorum* from four crops (canola, dry bean, soybean, and sunflower) in eleven North Central states (North Dakota, South Dakota, Nebraska, Minnesota, Iowa, Missouri, Wisconsin, Michigan, Indiana, Ohio and Illinois). We also obtained isolates from Montana, Wyoming and Colorado. Of the 152 isolates collected thus far, 115 have been successfully subcultured and stored for genetic and mycelial compatibility group analyses; the remaining 37 subcultures should be completed by late January 2009. Conditions this year were unsuitable for detection of *S. sclerotiorum* on field pea or lentil. We plan to continue collecting from all six crops in 2009.

As a first step in assessing genetic variation of *S. sclerotiorum* in the region, we have begun screening for mycelial compatibility groups. We have currently screened 27 isolates from dry bean, soybean, and sunflower in the Red River Valley of North Dakota and Minnesota. These isolates form 19 mycelial compatibility groups. Mycelial compatibility groups vary in size from 1-5 isolates and, to date, do not reflect either crop from which the isolates were collected or geographic location.

We have also initiated the screening of published microsatellite markers to use for determining haplotypes of *S. sclerotiorum*. We are currently extracting DNA from 30 isolates representing the geographic and crop diversity found in our collection. We will screen for polymorphisms using these isolates to pick the best 10-12 primer pairs. Characterizing levels of genetic variation in *S. sclerotiorum* across the northern tier of states will permit us to test for differences in virulence among isolates collected from different crops and a broad geographic area where Sclerotinia diseases are important. Knowledge of these differences should improve our ability to screen for resistance.

Contact Information: Laura Aldrich-Wolfe, Dept #7660 Plant Pathology, North Dakota State University, P.O. Box 6050, Fargo, ND 58108-6050; 701-231-5134; laura.aldrich-wolfe@ndsu.edu

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, USDA-ARS, Fargo, ND.

Funded Plan of Work: Evaluation of Wild *Helianthus* Species for Resistance to Sclerotinia Stalk Rot

ABSTRACT:
The evaluation efforts are focused primarily on the annual diploid *Helianthus* species in the USDA sunflower germplasm collection. A greenhouse screening method was employed, using soil-applied *Sclerotinia*-infested millet as inoculum. Greenhouse screening facilitates the rapid evaluation of much larger numbers of plants than could be managed in field plots. With this approach, much of the susceptible germplasm can be filtered out, making better use of the follow-up field screening trials. In 2008, 255 accessions were evaluated in the greenhouse, including all available accessions of *H. argophyllus*, *H. debilis*, *H. exilis*, *H. neglectus*, and *H. praecox* plus 45 accessions of *H. annuus*. Accessions with superior wilt resistance were identified in all species except for *H. exilis*. Twenty accessions were selected for a 2008 North Dakota field trial. The entries included susceptible and resistant germplasm from nine *H. debilis* accessions, three *H. argophyllus*, six *H. annuus* and two *H. resinosus* (perennial) accessions. Extensive flooding killed the *H. debilis* plants, but data was obtained from most of the other plots. The three most Sclerotinia-resistant entries were two *H. resinosus* accessions, PI 650079 and PI 650082, both with 100% survival and one *H. argophyllus* accession, PI 649863, with 94% survival. The resistant check, Croplan 305, had 88% survival while the susceptible check, Cargill 270, had 59% survival. Three of the *H. annuus* accessions had survival percentages equivalent to Croplan 305, PI 653604 (87%), PI 435434 (86%) and PI 435417 (85%). The excellent wilt resistance (near immunity) confirmed in the hexaploid species *Helianthus resinosus* deserves further exploration, but genetic incompatibilities may hinder attempts to transfer resistance from this species into cultivated sunflower.

Contact Information – Dr. Charles C. Block, USDA-ARS, G-212 Agronomy Bldg, Iowa State University, Ames, IA 50011; 515-294-4379; ccblock@iastate.edu

Identification and functional analysis of candidate defense genes in soybean

Bernarda Calla, Department of Crop Sciences, University of Illinois, Urbana, IL; Laureen Blahut-Beatty, Agriculture and Agri-Food Canada, Ottawa, ON; Yunfang Zhang, Agriculture and Agri-Food Canada, Ottawa, ON; Daina Simmonds, Agriculture and Agri-Food Canada, Ottawa, ON; Steven J. Clough, Department of Crop Sciences, University of Illinois and USDA-ARS, Urbana, IL

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

ABSTRACT:
Microarray studies have been conducted that enabled the identification of significantly differentially expressed genes in soybean plants in response to *Sclerotinia sclerotiorum*. These studies were expanded to include the effects of oxalic acid, a major virulence factor of *S. sclerotiorum*. Candidate defense genes were identified by placing them into functional categories, based on the annotation of their closest sequence match in public databases, and clustering the genes across multiple experiments. Defense genes will be verified firstly by quantitative real-time RT-PCR and secondly by obtaining knockouts of these genes in soybean and/or *Arabidopsis thaliana*. For Arabidopsis, we will use T-DNA insertion mutants of genes of interest that have high sequence identity with the soybean gene. To obtain knockouts in soybean, we will examine the feasibility of using a viral induced gene silencing system (VIGS). Alternatively, stable transgenics, utilizing RNAi constructs, will be generated. Additionally, overexpression of candidate defense genes will be studied in Arabidopsis and/or soybean. Promising candidate defense genes will be mapped to see if they map to known QTLs related to defense against *S. sclerotiorum*.

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Daina Simmonds, Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, ON, K1A 0C6; 613-759-1320, Daina.Simmonds@agr.gc.ca

Searching for genetic determinants of pathogenicity in *Sclerotinia sclerotiorum*

W. Chen, USDA-ARS, Grain Legume Genetics and Physiology Research Unit, Pullman, WA., K. McPhee, NDSU, Fargo, ND. and G. Vandemark USDA-ARS, Grain Legume Genetics and Physiology Research Unit, Pullman, WA

Funded Plan of Work: Identify Virulence Factors of *Scleortinia sclerotiorum* through Insertional Mutagenesis

ABSTRACT:
Sclerotinia sclerotiorum is a ubiquitous plant pathogen causing white mold and other diseases on more than 400 plant species including many economically important crops. White mold diseases occur worldwide, inflicting considerable damage. Management practices of the disease include cultural practices, fungicide applications and planting resistant cultivars when available. Host resistance to this fungus has been inadequate. One knowledge gap that hampers effective management of the disease is that we don't have an adequate understanding of the mechanistic interactions between the pathogen and host plant. Because S. sclerotiorum has a wide host range, it is speculated that the pathogen must possess rather general pathogenic mechanisms such as production and secretion of oxalic acid and cell wall-degrading enzymes. However, very little is known about the pathogenic genetic determinants of this devastating pathogen. This research is focused at increasing our understanding of pathogenic determinants of *S. sclerotiorum*. Recent research advancement has allowed us to identify four putative genes in pathogenesis.

We used insertional mutagenesis to generate random mutations in the nuclear genome of *S. sclerotiorum* using *Agrobacterium tumefaciens*-mediated transformation, screened the transformants to identify mutants that have lost or reduced pathogenicity. The disrupted genes in the identified mutants were located through reverse PCR, and sequenced. The determined sequences were used to search the genome sequence of S. sclerotiorum. Through this process, we identified four putative genes of pathogenicity factors. The four putative genes are a haloacid dehalogenase-like hydrolase gene, a nucleoside phosphatase gene, a gene coding for esterase of the alpha-beta hydrolase, and a GTPase gene. These putative genes provided needed information for gene knock-out experiments and complementation tests. The main impact is on increasing our understanding of pathogen biology and development, specifically pathogenicity genes.

Contact Information - Weidong Chen, USDA-ARS, Grain Legume Genetics and Physiology Research Unit, Pullman, WA 99164. 509-335-9178, Weidong.chen@ars.usda.gov

Genetic diversity of *Sclerotinia sclerotiorum* from various crops from the US Pacific Northwest

W. Chen, USDA-ARS, Pullman, WA., L. Porter, USDA-ARS, Prosser, WA., and D.A. Johnson, Washington State University, Pullman, WA.

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

ABSTRACT:
White mold disease caused by *Sclerotinia sclerotiorum* on many crops result in significant economical losses. Despite extensive studies on population variation of this pathogen in many crops, the populations of *S. sclerotiorum* in the US Pacific Northwest (PNW) have not been extensively studied. The PNW harbors diverse cropping systems including irrigated and dry land agriculture on various geographical terrains with generally mild winter conditions. The different agricultural practices and cropping systems may impact population structure of *S. sclerotiorum*. This study was to examine genetic variation and population structure of *S. sclerotiorum* from different cropping systems in the PNW. Mycelial compatibility grouping was used to measure genetic diversity of 88 sclerotial isolates of *S. sclerotiorum* from three states (22 isolates from a potato field in Bonners Ferry, ID; 32 isolates from a potato field in Hermiston, OR; and 34 isolates from a pea field in Walla Walla, WA), to compare with isolates previously obtained from lentil. Each isolate was obtained at least 1.8 m away from other isolates collected within a field. All isolates from the same field were paired in all possible combinations. High levels of MCG diversity were found among the populations: 12 MCGs were found among 22 isolates from Bonners Ferry, ID, 20 MCGs among 32 isolates from Hermiston, OR, and 23 MCGs among 34 isolates from Walla Walla, WA. Relationship of genetic variation in neutral marker loci and variation in the quantitative phenotypic traits, pathogenicity and fungicide sensitivity, of the same populations are being investigated. The main impact of this research is on increasing our understanding of pathogen biology and development.

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

Martin I. Chilvers, Michigan State University; Tobin L. Peever, Washington State University; Tristan Coram, North Carolina State University; and Kevin McPhee, North Dakota State University

The aim of the project was to utilize expressed sequence tags (ESTs) that we have developed for genome-wide gene expression studies of the *S. sclerotiorum* and *Pisum sativum* interaction. As detailed below we now have a large collection of ESTs that are being analyzed prior to the expression profiling studies that we have proposed.

We adopted a novel approach to generating EST data for the interaction between *Sclerotinia* and pea. Briefly, the pea cv. 'Lifter', identified as having partial resistance to *S. sclerotiorum*, has been inoculated with an isolate of *S. sclerotiorum*. Total RNA was isolated from advancing lesions to capture expressed transcripts from both pathogen and host, which was confirmed with quantitative RT-PCR. The quality of the RNA sample was qualified on an Agilent Bioanalyzer and was converted into a 'normalized' cDNA pool. The normalization process reduces the abundance of highly expressed transcripts so that rare transcripts are better represented by the cDNA pool. The cDNA pool was sequenced using massively parallel sequencing on a 454 - GS FLX pyrosequencing platform (Roche). A total of over 40Mb of sequence data was generated, this is a large amount of EST data and far more than we would have generated using a traditional cDNA library approach. If we had used a traditional cDNA library approach we may have sequenced 2,000 to 4,000 clones. The 40Mb of sequence data corresponds to approximately 50,000 clones of a traditional cDNA library.

It is important to develop genomic resources for *S. sclerotiorum* that are relevant to the interaction between *S. sclerotiorum* and *P. sativum*. Currently, nothing is known about the genetic mechanisms that control the basic biology and pathology of *S. sclerotiorum* interacting with pea. The development of ESTs will enable the identification and characterization of resistance and pathogenicity genes from host and pathogen. Additionally, ESTs from *S. sclerotiorum* will be useful to refine the annotation of the *S. sclerotiorum* genome sequence.

We predict that analysis of the EST data set will yield a substantial number of genes involved in pathogenicity and resistance responses. The plant and fungal transcripts will be sorted by mapping the reads to the *S. sclerotiorum* genome and where possible ESTs will be assembled into larger contiguous reads. The EST data set will then be used to conduct expression profiling of the pea-*S. sclerotiorum* interaction.

Contact Information – Dr. Martin I. Chilvers, Dept. of Plant Pathology, Michigan State University, 107 CIPS bldg, East Lansing, 48824; Phone: 517-353-9967; chilvers@msu.edu

Defining critical environmental and biological parameters needed to develop *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: Defining critical environmental and biological parameters needed to develop *Sclerotinia* stem rot on canola.

ABSTRACT:
Three field trials were installed in Langdon, ND in the summer of 2008 to study the epidemiology of Sclerotinia stem rot of canola (SSR). The first two studies evaluated the impact of three watering regimes [natural precipitation (NP) which was considered a control, NP + misting, and NP + soil irrigation] on SSR development on two canola cultivars (HyClass '906' and Pioneer 45H21). The inoculum for one of these studies was lab-produced ascospores sprayed on plants when they were at 40% bloom; the inoculum for the other study was produced in the field by sclerotia deposited on the plots in the fall of 2007. A misting system was activated three times each night starting at 10:30 PM during the flowering period to provide supplemental leaf moisture to some plots (NP+misting); nozzles located in plots that received irrigation (NP+ soil irrigation) were activated five times per day during the three weeks prior to flowering to provide supplemental moisture to the soil. The misting system was inactivated on rainy days to prevent excess soil moisture. The third study evaluated the effect of three inoculum concentrations (10^3, $2.5x10^3$, and $5x10^3$ ascospores per ml) and five inoculation timings on the development of SSR epidemics. All studies were conducted using a randomized complete block design; the first two with a split plot arrangement of treatments and the third as a 3x5 factorial. Misting plots during flowering time significantly increased the intensity of SSR epidemics independently of the type of inoculum used. Supplementing soil moisture did not have a significant impact on SSR intensity compared to natural precipitation. Significant differences in SSR intensity were detected between the two cultivars used, especially when canopy moisture was supplemented with misting. In general, SSR intensity increased with the amount of inoculum applied. Canola plants were equally susceptible to SSR throughout the flowering period, although the highest intensity was obtained when plants were inoculation twice at 50% and 100% bloom. Inoculating once four days after plants had reached 100% bloom resulted in the lowest incidence.

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Characterization of the reaction of herbicide-tolerant, non herbicide-tolerant and double haploid canola lines to *Sclerotinia sclerotiorum*

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Funded Plan of Work: Characterization of the reaction of herbicide-tolerant, non herbicide-tolerant and double haploid canola lines to *Sclerotinia sclerotiorum*

ABSTRACT:
Plants from accessions 426281,163497, 175050, and Ames 21738, were identified as having the highest resistance levels against *Sclerotinia sclerotiorum* among 406 *Brassica rapa* accessions evaluated using the petiole inoculation technique. Screening procedures were conducted in three stages that included greenhouse and field evaluations. Starting in the second stage, plants that survived the inoculation were transplanted and self-pollinated. Plants from 42 accessions survived inoculations in the second stage, but only the best four were advanced to the final stage. The average survival rate for *B. rapa* S1 plants in the third screening stage was 14% compared to an average of 8% for two commercial controls (Hyola 357 Magnum and Hyola 440). S2 seeds have been produced from plants that survived inoculation in the third stage. Two additional cycles of inoculation and self-pollination of surviving plants will be conducted before plants materials are entered in a breeding program. Three NDSU canola breeding lines, 0330416, 30457, and 0427681 were identified as having significantly higher levels of resistance to *S. sclerotiorum* than a commercial control (Invigor 5550). These lines had the highest levels of resistance among 45 glyphosate-tolerant lines that were evaluated in field conditions. This information has been shared with the canola breeder at NDSU. The protocol for development of canola double haploid lines using microspore cultures has been established. Haploid plants from two *B. napus* accessions identified as having high levels of resistance to *S. sclerotiorum* (PI 458940 x Ames 26628) were. Efforts to double chromosome count are underway.

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Transferring Sclerotinia Resistance Genes from Diverse Wild *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:
Since the present-day hybrids and cultivated lines lack sufficient Sclerotinia tolerance, searching for new resistance sources in wild *Helianthus* species and incorporating the resistance genes into cultivated backgrounds becomes a necessity for sunflower breeding. Recent screening efforts of the wild *Helianthus* species for both Sclerotinia head and stalk rot suggest an abundance of resistance genes in populations of wild perennial species. The objectives of this project were to transfer Sclerotinia head and stalk rot resistance from diverse wild *Helianthus* accessions into cultivated sunflower by the backcrossing method. Our first approach utilizing resistant hexaploids was started in 2004. Stalk rot resistant hexaploid *H. californicus* and *H. schweinitzii* were crossed with HA 410. Continued backcrossing with HA 410 and selfing resulted in BC_4F_3 plants with improved pollen and seed fertility and with 2n chromosome numbers between 34 and 37. Those progenies will be further backcrossed or seeds increased in the field in 2009. Our second approach utilizing interspecific amphiploids was started in 2005. Five amphiploids highly resistant to stalk and head rot were crossed with HA 410, and BC_2F_2 plants with 2n=34 to 36 were established in the greenhouse for further backcrosses or seed increase in 2009. Our third approach utilizing diploid perennials started in 2006. NMS HA89 was crossed with head rot resistant *H. maximiliani* and *H. nuttallii* accessions and progenies backcrossed with HA 441. BC_1F_4 and BC_2F_4 seed increased in the field in 2008 will be evaluated for resistance in 2009. Additional crosses were made between NMS HA89 and stalk rot resistant diploid perennials *H. maximiliani, H. giganteus,* and *H. grosseserratus,* and backcrossed with HA 410 in 2007. Their BC_1F_2 progenies with 2n=34-35 chromosomes were obtained in 2008, and selfed BC_1F_3 progeny will be grown in the field in 2009 for seed increase. Furthermore, our recent success in developing GISH and BAC-FISH techniques provides tools to distinguish cultivated and perennial species chromosomes as well as to identify individual chromosomes to specific linkage groups. In summary, we have progressed well during the past four years preparing progenies for field seed increase and for the following field disease resistance evaluation. Resistant lines identified in 2009 and 2010 are expected to provide new resistance genes to further enhance Sclerotinia resistance of the sunflower crop.

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Development of a transformation system in sunflower for Sclerotinia resistance via direct gene transfer and reverse genetics approaches.

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Funded Plan of Work: Development of a transformation system in sunflower for Sclerotinia resistance via direct gene transfer and reverse genetics approaches.

ABSTRACT:
Efforts were initiated to develop routine and straightforward transformation methodology for sunflower using *Agrobacterium*-mediated transformation of cotyledonary tissues. This research is based on the identification of sunflower line RHA280, which was confirmed to be very responsive to shoot induction. Initial media optimizations and explant preparation modifications were largely unsuccessful as seed-to-seed variation in response existed. The percent tissues that formed adventitious shoots increased from 44% to 98% when freshly-harvested seed from greenhouse-grown plants were utilized. Shoots were initially induced from cotyledonary tissues of the mature embryo, which was excised from the seed coat, prior to sterilization. Following the removal of the apical and basal end of the dry embryo, the remaining cotyledonary tissues were cut into 1 mm wide sections (~3 sections per dry embryo). Cotyledonary tissues were placed, cut side down, on a shoot induction medium (SIM) containing MS salts, B5 vitamins, 1.5 mg/l BA, 0.2 mg/l NAA, 3% sucrose and solidified with 0.2% Gelrite. After one day, paired cotyledon pieces, from the two cotyledons, were separated and replaced on SIM for an additional 2-3 weeks. Shoot formation occurred from surface tissues, along the basal end of the cotyledon piece, in contact with the medium. To induce shoot development, shoot-forming tissues were transferred to a shoot development medium (SDM), which was similar to SIM but contained no NAA, 0.5 mg/l BA, and Nobel agar as the solidifying agent. For *Agrobacterium*-mediated transformation of cotyledon pieces, excised cotyledon pieces were treated using Sonication-Assisted *Agrobacterium*-mediated Transformation (SAAT) and co-cultured on SIM with *Agrobacterium* for 2-3 days. For rapid observation of transformation efficiency, tissues were then placed on SIM with Timentin for monitoring GFP expression. High levels of GFP expression was observed in cotyledon surface tissues, along the basal end of the cotyledon piece, which is the same area that gives rise to shoots. Stably-transformed GFP-expressing sunflower callus has been obtained but effort to generate whole transformed shoots and plants are still underway.

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Sclerotinia resistance enhanced by accumulation of QTL and transgenic approaches

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Funded Plan of Work: Sclerotinika resistance enhanced by accumulation of QTL and transgenic approaches

ABSTRACT:

This research is being conducted to increase the level of resistance to *Sclerotinia sclerotiorum* in soybean cultivars and to develop and evaluate improved disease control and resistance options for producers. The goal is to increase the level of resistance to *S. sclerotiorum* in soybean. Objective 1 is to combine quantitative trait loci (QTL) that were previously mapped and identified with the resistance phenotype into single breeding lines. We identified 40 F5:6 lines with the smallest lesion size that were evaluated during 2006 for reaction to *S. sclerotiorum* in 12 replications of a lattice design using the detached leaf test (DLT). Nineteen of the lines that contained new combinations of multiple QTLs had a lesion size equal to or smaller than the best parent in the cross, and better than the resistant check NKS19-90. The 19 selected F5:7 lines were evaluated again during 2007 using the DLT, and for seed increase. A multi-location yield test was conducted during 2008, with QTL lines, parents, and checks evaluated in sets according to maturity of the lines. Yield of the most resistant lines from a cross were in the same range as the parental QTL lines, but none yielded better than the parents or the yield checks. A second year of multi-location yield tests will be conducted during 2009. What is also needed is a good field evaluation of and verification of resistance for these lines. Objective 2 is to determine if a novel antifungal synthetic peptide expressed in soybean will confer resistance to *S. sclerotiorum.* We developed transformed plants with a codon-optimized gene-expression cassette for the antifungal peptide that contains the barley alpha-amylase signal sequence to export the peptide to the apoplast. We conducted the DLT on T2 populations from seven independent transformation events. Results indicated no significant difference between the plants with the lytic peptide and those without the inserted gene expression cassette. No further studies are planned with the lytic peptide.

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Determining Sclerotinia Head Rot Resistance Responses from Sunflower Hybrids in Minnesota

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Funded Plan of Work: 2008 Uniform Sunflower Germplasm Evaluation for Resistance to Sclerotinia Head Rot in Minnesota

ABSTRACT:
Sclerotinia head rot, caused by *Sclerotinia sclerotiorum*, is a severe disease issue for sunflower producers. Effective disease management currently relies on hybrid resistance. A uniform germplasm trial identifying sunflower hybrids with resistance is conducted yearly in Minnesota, North Dakota, and Canada. Data reported here represent the Minnesota research effort.

On 22 May 08, a total of 82 entries (74 hybrids, four known susceptible controls, and four resistant controls) were planted into single row plots 7.6 m in length in a randomized complete block design with four replications. Plants were hand thinned to 25/row at the R1 growth stage (terminal bud formation). Daily water misting of the test area was initiated on 5 Aug (2 min misted, 13 min not misted, continuous). Plots having at least ten plants at approximately the R5.5 growth stage (disk flowers 50% flowered) were hand inoculated with a spore suspension delivering a total of 30,000 *S. sclerotiorum* ascospores/head to promote uniform disease development. Plots were inoculated 5, 6, 7, 8, 9, 12, 13, 14, or 18 Aug as entries matured. Misting continued on diminishingly aggressive schedules until 18 Sep. Even then, environmental conditions promoted continued disease development. The trial accumulated 4.9 cm of rain on six nonconsecutive days after the misting system was disabled and before the final disease severity rating (DSR) was recorded (8 Oct).

Sclerotinia head rot was severe and damaging. On 8 Oct, hybrid DSR means ranged from 1.0 to 5.0 on a visual rating scale from 0 (no symptoms) to 5 (100% head rot), while the experiment grand mean was 3.8. Using a Fisher's Protected Least Significant Difference mean separation test from PROC GLM in SAS, Proseed 7052 had the least DSR (0.98), but was not significantly different from PANNAR PEX 3426, Seeds2000 X4994, ProSeed 6007, and ProSeed 7016 ($P < 0.0001$). Six hybrids, including one susceptible control entry, had DSRs of 5. However, the group was not significantly more diseased than 34 other entries. Later maturing sunflower entries appeared to have less severe disease than earlier maturing hybrids. Simple linear regressions (PROC REG) determined that the days after inoculation (DAI) explained 37% of the total variation in DSR between entries from data collected on 24 Sep ($P < 0.0001$) while 24% of the variation was explained by the DAI from the 8 Oct rating ($P = 0.0002$). This indicates that the experimental design is selecting for Sclerotinia head rot resistance as well as later maturing hybrids.

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Pyramiding *Sclerotinia* head rot and stalk rot resistances into elite sunflower breeding lines with the aid of DNA markers

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Funded plan of work: Develop novel germplasm and varieties with field resistance to *Sclerotinia sclerotiorum*.

ABSTRACT:
Work was conducted in 2008 to determine the stalk rot resistance of RILs from the RHA 280 x RHA 801 population, as well as to begin introgression of previously identified QTL for head rot resistance into elite sunflower germplasm lines.

The stalk rot RILs and their testcrosses with cms HA 89 were tested in 2 environments in 2008. Only one environment produced good differentiation of lines and testcrosses, the other was uninformative. This data, taken together with previous data from 2006 and the greenhouse, is not enough to release lines from this population. Analysis with molecular markers in this population has found 6 putative QTL for stalk rot resistance, although the stalk rot resistance data going into the model had a large error variance.

The work to begin introgression of the head rot QTL into elite germplasm began this year with the final purification of a RIL line with a large number of the QTL alleles of interest, and the formation of F_1 populations in both confection and oilseed backgrounds. As of December, work was underway to genotype the F_1 plants and parent lines to confirm genomic constitution at the loci of interest, as well as to produce BC_1F_1 seed to continue population development. An additional backcross will be attempted in 2009, with line development and field testing to follow.

Identification of QTL for White Mold Resistance in Pinto Bean

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Funded Plan of Work: Variety Development/Germplasm Enhancement

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. In order to enhance resistance the goal of this project funded by the National Sclerotinia Initiative is to identify quantitative trait loci (QTL) that are associated with resistance to white mold in pinto beans. The source of resistance was the pinto bean line AN-37 that has shown consistently high levels of white mold resistance in field trials in Michigan. However, the seed quality and agronomic characteristics of AN-37 would not meet the commercial standards for pinto beans. In the MSU breeding program, pinto breeding line P02630 was identified for combination of high yield, superior seed quality, and upright plant architecture. The upright architecture exhibited by this line is likely responsible for its avoidance to white mold in field trials and is also desirable to growers who are interested in narrow row production systems suitable for direct harvest. P02630 was crossed to AN-37 to develop a 94-entry $F_{4:7}$ recombinant inbred line (RIL) population that was evaluated under white mold pressure in the field for two years (2007-08) and data was collected on percent white mold, yield, maturity and lodging. Twenty-two RILs exceeded yield of 40 cwt/acre and the top entry, P07863, had the lowest white mold rating (11%) in the trial in 2007. Based on the superior performance, P07863 was entered in 2008 National Sclerotinia trials and the Midwest Regional Performance Nursery -MRPN. Fifty RILs exceeded 40 cwt/acre and the same RIL, P07863 topped the trial at 130% of the mean in 2008. In addition P07863 topped the NSI trial in Michigan yielding over 53 cwt/acre or 136% of the mean. The RIL population will continue to be evaluated for reaction to white mold in a replicated field white mold nursery in 2009 and using the greenhouse straw test. The population will also be genotyped in the laboratory, using SSR, TRAP and SRAP markers, to identify regions of the genome, or QTL, which are associated with white mold resistance. This work will identify agronomically superior pinto breeding lines with improved white mold resistance and will also discover QTL for resistance and avoidance to white mold. The QTL will be validated in other mapping populations and compared with previously identified QTL to identify the most robust QTL for future improvement of resistance to white mold in pinto and other medium-sized dry bean classes.

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White mold resistance in pea and lentil through breeding and biotechnology

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Funded Plan of Work: Improved resistance to *S. sclerotiorum* in pea and lentil through breeding and biotechnology

ABSTRACT:
White mold caused by *Sclerotinia sclerotiorum* can be a devastating disease on pea and lentil. Absence of identifiable resistance in genetic resources has hindered breeders' progress toward development of resistant varieties. This project aimed to use two approaches to develop resistance to *Sclerotinia sclerotiorum* in pea and lentil. The first approach involved screening available cultivars and plant introduction accessions for resistance under field conditions. Thirty-seven pea genotypes and 294 plant introductions were evaluated under field conditions at Carrington, ND. One hundred fifty accessions were evaluated in a single replicate evaluation in 2006 and 142 were evaluated in 2007. Two hundred seventy-seven accessions were evaluated for a second year in 2008. Research plots for advanced lines and cultivars consisted of seven rows spaced 18 cm apart and 7.6 m long and were arranged in a randomized complete block design with 4 replicates. At early bloom all plots were inoculated with ascospores. Immediately after inoculation a misting system was used to maintain high humidity and favor disease development. The misting system was run for 2-4 minutes every half hour, 24 hours/day, for 4 weeks. Disease was scored periodically and growth and development data were recorded. Disease scores at Carrington in 2006 were not as severe as in the other three years (2005, 2007 and 2008) due to dry and unfavorable conditions. Six accessions were common to all three years (2005-2008) and among these, 'DS-Admiral' had the lowest average score for disease incidence (3.0) and 'Majoret' had the highest score (4.8). A highly significant negative correlation was observed between disease rating and yield, days to beginning and days to end bloom. Disease reaction scores for accessions evaluated in 2006 ranged from 0.0 to 5.0 while ratings in 2007 and 2008 ranged from 0 to 9 on a scale of 0 to 10 where, 0 = no disease and 10 = all plants showing symptoms. No correlation between disease severity scores was detected between the first year, either 2006 or 2007, and the 2008 scores. One accession, PI 118501, had low scores both years it was evaluated and will be used as a possible source of resistance. Other accessions with low disease ratings in both years should be further evaluated in field and controlled conditions to verify the level of resistance. The second approach involved introducing the oxalate oxidase gene from barley (*Hordeum vulgare* L.) into pea and lentil through *Agrobacterium tumefaciens*-mediated transformation. The oxalate oxidase gene was successfully cloned from barley cDNA and incorporated into a twin binary vector. Transformation experiments using two pea cultivars, 'Mukta' and 'Joel', and one lentil cultivar, 'Pardina', have been completed; however, now transformants have been recovered. The twin binary vector will allow the selectable marker gene, *nptII*, to be separated from the oxalate oxidase gene through natural Mendelian segregation. It is expected that incorporation of the most resistant germplasm into breeding programs will contribute to development of cultivars with improved natural genetic 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 is an important disease pest of many crops including pea (*Pisum sativum* L.) and crop losses have been significant when environmental conditions were conducive to disease development. Limitation regarding available germplasm with resistance has hampered development of resistant pea cultivars. The goal of this project is to reduce the economic impact of the white mold pathogen on the pea crop. Specific objectives are to, 1) place the genetic factors (QTL) controlling partial resistance to white mold on the pea map and 2) to pyramid the available mechanisms of resistance in an effort to develop durable resistance. The recent description of partial genetic resistance to *S. sclerotiorum* among pea accessions provides an opportunity to study the genetic control of resistance. Four sources of resistance, PI 103709, PI 169603, PI 240515 and ICI 1204-3 represent two distinct mechanisms of resistance (inhibited lesion expansion and nodal resistance). Six genetic mapping populations involving these resistant sources are in development and two populations, PRIL-17 and PRIL-19, have been advanced toward development of recombinant inbred line (RIL) populations. F_2 seed from PRIL-17 and PRIL-19 were grown in the greenhouse and produced F_3 family seed for initial screening for reaction to Sclerotinia infection in Prosser, WA. Several morphological data were collected on the individual F_2 plants to contribute to mapping the resistant phenotype based on F_3 family data. Leaf tissue was also collected from each plant for genotyping and genetic map development. A total of 355 F_3 family lines from the mapping population PRIL-19 [PI169603/Medora(PRIL-2)] were screened twice in replicated growth chamber trials for resistance to *Sclerotinia sclerotiorum* at 21.1ºC and 100% RH. A maximum of four plants were screened per line per screening trial by inoculating the plants at the fourth node. Lesions that developed on individual plants were measured 48 hours after inoculation. Plants were then placed in a growth chamber at 21.1ºC and 70% RH for one week. Lesion advancement was classified according to the following severity values: 0 = plant did not survive, 1 = lesion expanded from the 4^{th} to the 1^{st} node, 2 = lesion expanded from the 4^{th} to the 2nd node, 3 = lesion expanded from the 4^{th} to the 3^{rd} node, and 4 = lesions did not expand beyond the initial inoculation point at the 4^{th} node. Approximately fifty percent of the lines survived when assessed nine days after inoculation per screening trial. This may indicate that there is a major resistance gene associated with the survival of plants inoculated with *S. sclerotiorum*. Resistant information gathered from each pea line will be compared to DNA extractions from each line to identify genes and QTLs associated with the resistance. DNA markers associated with the resistance will be made available to breeding programs to aid selection of resistant progeny.

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Contribution of Partial Genetic Resistance to White Mold Disease Management in Pinto and Great Northern Beans

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Funded Plan of Work: Contribution of partial genetic resistance to white mold disease management in pinto and great northern beans

ABSTRACT:
Beans with intermediate seed size, like pinto, great northern, pink, and small red, belong to race Durango of the Middle American gene pool. Developing partial resistance to white mold for the "Durango" market classes is a major goal for bean breeding programs in the U.S. Our objective is to document what effect the partially resistant or less susceptible pinto and great northern lines being developed have on overall white mold disease control. Pinto PT7-8 with potential disease avoidance, 'Quincy' pinto and 'Orion' great northern as susceptible checks, and three lines: pinto USPT-WM-1, pinto 11A-39, and 29C-6 great northern, with partial resistance to white mold derived form diverse sources, Bunsi navy bean, G122 red-mottled landrace from India, and NY6020-4 snap bean breeding line, respectively, were selected for this study. The select lines were grown in a replicated field trial under white mold disease pressure. A commercial fungicide Topsin M was applied at recommended rates and bloom stages. Three spray treatments were used, 0, 1, and 2, applications. Disease severity score (1=best to 9=worst) and yield (lbs/A) were the primary parameters used to assess disease response and fungicide efficacy. USPT-WM-1 and 11A-39 exhibited the greatest resistance and did not require a fungicide application to achieve full yield potential. Although 11A-39 was the most resistant based on disease score (2.6, compared to 7.4 for Quincy), it had the lowest yield potential among the lines tested, which can be attributable in part to the derivation of its resistance from the Andean gene pool (landrace G122). Quincy and Orion were the most susceptible as expected and had a significant yield increase and decrease in disease severity score in response to a single fungicide application. This years study exposed problems with late maturity and/or low yield potential in the partially resistant lines. These likely negative linkage drag effects from introgression of partial resistance from exotic sources must be reduced if development of useful germplasm lines and cultivars with partial resistance is to be realized.

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Transfer and characterization of white mold resistance from *Phaseolus coccineus* into *P. vulgaris*

James R. Myers, Oregon State University, Corvallis, OR

Funded Plan of Work: Transfer and characterization of white mold resistance from *Phaseolus coccineus* into *P. vulgaris*

ABSTRACT:

P. coccineus accessions with high levels of resistance were crossed and backcrossed to common bean cultivars to transfer of resistance into *P. vulgaris*. Certain parental combinations were more productive than others, leading to a focus on four populations. These are 91G/PI 255956, 91G/PI 433251B, G122/PI 433251B, and MO162/PI433251B. We first characterized the 91G/PI 255956 population by straw testing three times, once with the oxalate test, and once in the field as individual lines. In this population, 77 SSR and 59 AFLP markers were scoreable. A linkage map was constructed consisting of 11 linkage groups (LGs) that corresponded to 9 of the 11 core map LGs based on known SSR marker locations, plus a single LG with no anchoring loci. Reanalysis of the mapping population with the newly released version of MapQTL 6.0 using composite interval mapping revealed QTL on B02, B03, B09 and an unlinked fragment that collectively explained 34.7% of the phenotypic variation. The QTL on B02 and the unlinked group were associated with resistance in the straw test and explained 18.6% of phenotypic variation. One possible QTL was observed on B07 for oxalate tolerance. Linkage groups B01, B05, and B08 were not represented in this population and a high level of segregation distortion was observed, with more heterozygotes than expected. In 2007/2008, the 91G/PI 433251B and MO162/PI 433251B populations were advanced to the BC_2F_5 generation in the greenhouse, and to the BC_2F_6 generation in the field during the summer. DNA was extracted from BC_2F_4 plants in the greenhouse. Straw tests were performed on remnant BC_2F_5 seed (summer) and on the BC_2F_6 (fall). In general, the M0162/PI 433251B population had a higher frequency of lines similar to or better than G122, while the 91G/PI433251B population was skewed towards a greater number of susceptible lines. Field data were obtained using a repeated check - nearest neighbor design. Parent lines were screened with 172 SSR primers, 76 of which were polymorphic on 3% agarose gels. Primers that amplified but did not appear polymorphic are being rescreened on polyacylamide gels. Primers with polymorphism detectable on agarose gels were scored on the entire population. A total of 169 of 250 RAPD primers have shown polymorphism. Primers for candidate genes have also been developed and preliminary screening on parental lines shows polymorphism for seven candidate genes. These include genes for a WRKY transcription factor, chitinase, phosphatase-2-C, defensin, COS1, lippoxygenase and phenylalanine ammonia lyase.

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QTL for White Mold Resistance in an Interspecific Backcross Dry Bean Population

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Funded Plan of Work: Mapping QTL for White Mold Resistance in an Interspecific Dry Bean Backcross Population

ABSTRACT:
Genetic resistance to white mold has been reported in both common (*Phaseolus vulgaris* L.) and scarlet runner (*P. coccineus* L.) beans. From funding received from the USDA Sclerotinia Initiative, we developed a common bean RIL population derived from the cross G122/CO72548. In this RIL population, we identified a line, WM67, which had moderate levels of resistance to WM and possessed important QTL linked to resistance. We then utilized WM67 and scarlet runner bean accession PI255956 as parents to develop an interspecific inbred backcross line (IBL) population to pyramid QTL and resistance genes from scarlet runner bean with common bean. In 2008, we reported the effect of previously reported QTL associated with white mold resistance in the IBL population. Three IBL lines had Straw Test ratings significantly ($P<0.05$) lower than G122 (3.14, 3.29, and 3.32 *vs* 5.24). Markers linked to the B2b, B7, and B8 in common bean and one QTL from *P. coccineus* accounted for 9.7 ($P<0.05$), 12.8 ($P<0.01$), 10.8 ($P<0.01$), and 7.0% ($P<0.05$) of the phenotypic variation in resistance, respectively. A total of eleven molecular markers contributed by scarlet runner bean parent PI 255956 accounted for a significant proportion ($P<0.05$) of the phenotypic variation in resistance. Four markers previously associated with resistance in the scarlet runner bean population could not be evaluated for their associations with WM resistance because they were not polymorphic in the IBL population. Mean Straw Test ratings were higher for lines that had >25% scarlet runner alleles compared with lines that had <25% scarlet runner alleles (4.8 *vs* 3.8, respectively), indicating that lines with higher proportion of scarlet runner alleles also had higher levels of resistance to WM. We observed severe segregation distortion in the IBL population for all polymorphic molecular markers, and only 16 of the 65 IBL possessed any scarlet runner alleles. Two potential recombination events were detected. One on linkage group B2b between SSR markers BM152 and BM160, and a second on linkage group B7 between SSR marker BM160 and the *Phs* SCAR marker. No other examples of recombination were detected. The results from this study suggest that QTL associated with white mold resistance from common and scarlet runner bean can be combined in an IBL population however due to segregation distortion recombination between common and scarlet runner bean chromosomes is limited. Our results suggest that MAS for resistance QTL from scarlet runner bean into common bean may be a viable method to pyramid QTL from scarlet runner bean and common bean to improve white mold resistance in common bean

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The requirement for oxalate during pathogenesis on multiple crops

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

ABSTRACT:
The importance of oxalic acid production and polygalacturonase synergy during colonization and symptom development in diseases caused by *Sclerotinia* spp., has been well established through a number of independent physiological experiments. Using a mutant-based genetic approach, oxalic acid production has also been reported to be an essential pathogenicity determinant for *S. sclerotiorum*. This determination was based on the isolation and characterization of UV-induced mutants that lacked oxalate production and the ability to elaborate symptoms of bean pods. The genetic nature of this mutant phenotype has never been established and it remains possible that traits other than oxalate production have been affected in these mutants. To clarify the role of oxalate in establishing *S. sclerotiorum* infections and elaborating symptom development, we have created a gene-specific deletion of the sequences encoding oxaloacetate acetyl hydrolase (Oah1), the enzyme predicted to catalyze the final step in the oxalate biosynthetic pathway. Two independent gene deletion mutants were isolated and characterized. Neither mutant was able to accumulate oxalate in culture or *in planta*. Despite the lack of oxalic acid production, these mutants were able to infect leaves of host plants including tomato, canola, sunflower, and bean. Symptom development on these hosts was greatly attenuated, but the ability to infect and produce symptoms indicates that factors other than oxalic acid may be responsible for establishing host-pathogen compatibility in this system.

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Cultivar, Plant Spacing and Fungicide Effects upon White Mold Management in Dry Bean

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Funded Plan of Work: Cultivar, Plant Spacing and Fungicide Effects upon White Mold Management in Dry Bean

ABSTRACT:
During 2006 to 2008, we investigated the roles of cultural practices (plant spacing), and timely application of a fungicide (Endura) to reduce damage from white mold for dry bean entries with varying degrees of resistance (plant architecture – disease avoidance, interspecific resistance). The 2006 trials provided preliminary data, which have been reported previously. Agronomic responses revealed that there was a noticeable increase in seed yield when plant population was increased by 50% from 1 to 2 lines for commercial pinto cultivars Montrose (type III growth habit) and Vision (type II growth habit). The % increase in yield in nurseries with light to moderate white mold pressure and no fungicide protection varied from -17 to +11% in 2007 to +3 to +31% in 2008 for Montrose; and varied from -11 to +13% in 2007 and +4% in 2008 for Vision. The addition of fungicide protection (with Endura) showed modest increases in yields that varied from +4 to +14% in 2007 to +3 to +19% in 2008 for Montrose; and varied from 0 to -2% in 2007 and -9 to +4% in 2008 for Vision. White mold disease incidences in control plots were 11 to 15% higher in 2007 and 5 to 19% higher in 2008 for Montrose, and 21 to 25% higher in 2007 and 3 to 45% higher in 2008 for Vision when compared to fungicide (Endura) protected plots in Idaho and Colorado.

Partially resistant CO23704 and CO54150 breeding lines exhibited similar degrees of white mold infection (28 to 85% and 32 to 95%, respectively) in both plant populations under moderate disease pressure in Idaho, but had up to 45% less white mold when protected with a fungicide. The resistant WM54 and WM55 interspecific breeding lines exhibited less infection (6 to 25% and 13 to 48%, respectively) with either density and/or fungicide program. Increased plant density did increase yields of CO23704 and CO54150, but decreased yield of WM54 and WM55 during testing at both locations in 2007 and 2008. Results from this project will help direct future efforts and access to funding critical to expand these multi-disciplinary and multi-state collaborations with improved white mold resistant germplasm, modified production practices, and an effective integrated pest management strategy to reduce the impacts of white mold upon bean production in the United States. This 3-year project has been completed, and its objectives supported the Sclerotinia Initiative areas of Germplasm Enhancement and Variety Development (25%) and Epidemiology & Disease Management (75%), and PM 4.0.7 of the Strategic Plan for the Sclerotinia Research Initiative.

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

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

ABSTRACT:
Partial white mold (WM) resistance is found in few large-seeded Andean and small-seeded Middle American common bean and in interspecific breeding lines (IBL) derived from *Phaseolus* species of the secondary gene pool (SGP). Resistance of individual genotypes is inadequate for combating WM in the USA and no effort has been made to pyramid and introgress high levels of resistance into cultivars. The goal of this research is to pyramid WM resistance (PWMR) from *Phaseolus* species and introgress into pinto bean, the largest market class in the USA. The specific objectives are to (1) determine complementation among the WM resistant large-seeded dry bean and among the WM resistant small-seeded IBL derived from *P. coccineus* of the SGP, (2) pyramid WM resistance from diverse genotypes, (3) introgress the PWMR into pinto bean, and (4) determine the effectiveness of PWMR across environments. These objectives support the Sclerotinia Initiative area of Crop Germplasm Resources and Genetics.

White mold reactions of known resistant large-seeded dry bean (A 195, G 122, MO 162, PC 50, VA 19), green bean (CORN 601), and small-seeded IBL (VCW 54, VCW 55, VRW 32, 92BG-7, I9365-25, 0785-220-1) genotypes were verified in two greenhouse environments in 2008. Three large-seeded dry bean (A 195, G 122, VA 19) and three small-seeded IBL (VCW 54, 92BG-7, 0785-220-1) genotypes with the lowest overall mean WM scores were selected for the complementation study. These six genotypes are being crossed within the group in all possible combinations excluding the reciprocals. Thus, three single-crosses within each of the two groups are being made.

In a parallel study, WM resistant pinto germplasm line USPT-WM-1, 'Chase', and 78 F_5 breeding lines developed from two double-cross (USPT-WM-1/CORNELL 601//USPT-CBB-1/92BG-7 and Chase/I9365-25//ABL 15/A 195) populations were evaluated for their disease reactions in the greenhouse. A randomized complete block design with three replicates was used. Three mycelial plugs from 48 hr old culture of *Sclerotinia sclerotiorum* were used in each of the two inoculations using the modified straw-test. White mold disease severity was recorded 28 days after the second inoculation and the resistance verified at maturity. Thus, 163 WM resistant plants out of 1440 screened were harvested individually. Progenies of these plants are being evaluated for WM resistance in the greenhouse in Colorado and Idaho. Pinto genotypes with the lowest WM scores will be used as recipient parents in crosses with the selected PWMR genotypes developed through each cycle of the gametic-recurrent selection.

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

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Funded Plan of Work: Introgressing White Mold Resistance from the Secondary Gene Pool of Common Bean

ABSTRACT:
Low levels of white mold (WM) resistance occur in the common bean whereas *Phaseolus* species of the secondary gene pool (SGP) possess higher levels of resistance. The objectives in the FY2007/2008 were to (1) complete the evaluation and selection of 433 interspecific breeding lines (IBL) derived from crosses of 'ICA Pijao' with the three *Phaseolus* species of the SGP, (2) compare the WM reaction of the IBL with known sources of WM resistance, and (3) continue screening of a new group of 482 IBL derived from crosses of pinto 'Othello' and 'UI 320' with highly WM resistant *P. coccineus* accessions PI 433246 and PI 439534. These objectives support the Sclerotinia Initiative area of Crop Germplasm Resources and Genetics.We completed screening of 433 inbred, inbred-recurrent backcross, and inbred-congruity backcross interspecific breeding lines (IBL) derived from 12 crosses of small-seeded black bean ICA Pijao with all three *Phaseolus* species of the SGP (*P. coccineus*, *P. costaricensis*, *P. polyanthus*). Two WM resistant IBL (VCW 54 and VCW 55) derived from ICA Pijao and *P. coccineus* accession G 35172 and one (VRW 32), the first of its kind, derived from ICA Pijao with *P. costaricensis* accession S 33720 were developed. Seed of VCW 54 and VCW 55 was increased and both released by the Idaho and Colorado Agricultural Experiment Stations on December 10, 2008. The three IBL, ICA Pijao, ICA Bunsi, G 122, A 195, VA 19, and 92BG-7 were evaluated again in the greenhouse in Colorado and Idaho in 2008. A randomized complete block design with three replicates was used. Plants in the greenhouse were inoculated three times using the cut-stem method or modified straw test and three mycelial plugs each time from a 48 hr old culture of *Sclerotinia sclerotiorum*. Thus, a more stringent and severe WM pressure was created. White mold reaction was recorded on a single-plant basis 28 days post-inoculation and resistance reaction verified at maturity. VCW 54 had the highest level of WM resistance, and VCW 55 and VRW 32 had a similar level of resistance as previously reported. Also, the three IBL possess unique combinations of flower and seed colors and have an upright growth habit; and will help determine optimum agronomic practices for an integrated management strategy to combat WM problems in the USA. Seed of VRW 32 is being increased for release and registration.
We also screened 81 IBL derived from an interspecific cross between the common bean and *P. coccineus* accession G 35172 obtained from University of Puerto Rico-Mayaguez. Twelve single plant selections with high levels of WM resistance were selected from six IBL that need to be progeny-tested, pure lined, and true-breeding IBL with high levels of WM resistance confirmed. The second round of screening of a new group of approximately 180 out of 482 IBL derived from crosses of pinto Othello and UI 320 with highly WM resistant *P. coccineus* PI 433246 and PI 439534 that had survived in 2007 is currently in progress in the greenhouse at Fort Collins (CO) and Kimberly (ID). Two manuscripts are under review for publication in refereed journals.

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Integration of QTL for White Mold Resistance on the Bean Linkage Map

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Funded Plan of Work: Genetic characterization of scarlet-runner bean derived resistance to white mold in common bean

ABSTRACT:
Sources of resistance to white mold (caused by *Sclerotinia sclerotiorum* Lib. de Bary) in the common bean gene pool (*Phaseolus vulgaris* L.) are limited. Two dry bean germplasm lines, I9365-31 small black and VA19 light red kidney, putatively derived from interspecific crosses involving *P. coccineus* (scarlet runner bean) and *P. acutifolius* (tepary bean) as parents, respectively, possess partial resistance to white mold. Recombinant inbred line (RIL) populations (Raven/I9365-31,107 F_5 lines and Benton/VA19, 79 F_5 lines) combined with bulked-segregant analysis using SRAP, RAPD and SSR markers, were used for QTL analyses of the partial resistance in I9365-31 and VA19. Two major and three minor QTL were identified in the Raven/I9365-31 (R31) population and anchored to the core map using SSR markers assayed at CIAT. The major QTL on linkage group B2 explained 32% (R^2) of disease severity score (1 to 9) for field reaction to white mold, and maps to the same location on B2 as a QTL derived from Bunsi navy bean. The other major QTL on B7 explained 40% of the variation for disease reaction in the greenhouse straw test (scored 1 to 9). This QTL appears to be novel because of its position near SSR marker BM210, which is intermediate to previously mapped QTL on B7 that derive from G122 (near *Phs* locus) and Bunsi (near Bng047 locus). Three minor QTL in the R31 population were located on B5 (R^2=21% field, 7% straw test, 7% non-invasive greenhouse test), B6 (12% field), and B8 (8% non-invasive test, 5% field). Only the B8 QTL was located near a previously mapped QTL from pinto bean CO72548. All but the B6 QTL derive from I9365-31. In the Benton/VA19 (BV) population, one major- and one minor-effect QTL were identified. The major-effect QTL on B2 was detected by both straw (36%) and non-invasive (35%) greenhouse tests, and was also expressed in the field (13%). The minor-effect QTL on B8 conditioned partial field resistance to white mold (11%) and was not associated with disease avoidance traits. The SRAP markers most tightly linked with the QTL from VA19 were converted to SCAR markers and assayed in different RIL populations where they mapped within previously identified QTL derived from Bunsi and I9365-31 (B2) and NY6020-4 (B8). Co-location of QTL conditioning resistance to white mold from diverse sources validates the presence and importance of the major-effect QTL on linkage groups B2 and B8.

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Identifying molecular markers linked in lentil (*Lens culinaris* Medik.) to white mold resistance derived from the lentil cultivar Pennell

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Funded Plan of Work: Identifying molecular markers linked in lentil (*Lens culinaris* Medik.) to white mold resistance derived from the lentil cultivar Pennell

ABSTRACT:
Lentils (*Lens culinaris* Medik.) are integral components of cereal-based cropping systems in the Pacific Northwest and North Central U.S. White mold disease, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a particularly destructive disease of lentils in the Pacific Northwest. Only limited efforts have been conducted to screen lentil materials for resistance to white mold, and the great majority of commercially successful lentil varieties are highly susceptible to the disease. It is critical that new lentil varieties with resistance to white mold be developed in order to maintain or expand the importance of lentils to American agriculture. Because of difficulties for variety improvement due to quantitative inheritance of resistance, efforts have been made in several crops to identify molecular markers linked to genes conferring resistance to *S. sclerotiorum*. Currently existing lentil genetic maps have only limited genome coverage, and no markers have been identified that are associated with resistance to white mold. The objective of this study is to identify molecular markers associated with resistance to white mold in a lentil population derived from a cross between the cultivars Pennell and Pardina. Markers linked to quantitative trait loci (QTL) conditioning resistance to white mold will be detected in a lentil population consisting of 229 F_8 recombinant inbred lines (RILs) derived by successive generations of single seed descent of progeny produced from a cross between the varieties Pennell x Pardina. To date we have developed a preliminary linkage map for this population based on 108 markers; 63 Sequence Related Amplified Polymorphisms (SRAPs), 20 Randomly Amplified Polymorphic DNAs (RAPDs), and 25 Simple Sequence Repeats (SSRs). F_9 seed will be produced from the 229 RILs in 2009 and screened in a mist chamber for resistance to white mold. Five plants of each RIL will be evaluated in replicated resistance screenings. Additional markers that are polymorphic between Pardina and Pennell will be identified and their linkage arrangements determined. DNA markers associated with disease resistance will be cloned and sequenced to develop sequence characterized amplified regions for marker assisted selection.

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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:
In a previous project funded by the National Sclerotinia Initiative, 1,147 lines derived from crosses in which either or both parents were partially resistant to Sclerotinia stem rot were evaluated for yield and other agronomic traits. Lines with acceptable yield and other agronomic traits were further evaluated for resistance to Sclerotinia stem rot. A cultivar Skylla (Wang et al., 2006) and a germplasm AxN-1-55 (Diers et al., 2006) with partial resistance to Sclerotinia stem rot were released. Skylla and AxN-1-55 were used as Sclerotinia stem rot resistant parents in our breeding program. Six progeny lines derived from these crosses were selected based on their yield and other agronomic traits. These six lines were evaluated in the Preliminary Test IIA in the Uniform Soybean Tests - Northern Region in 2008. Four lines were ranked among the top 10 highest yielding lines of the 34 entries at one or more locations in Michigan, Nebraska, Ohio, and Ontario. Three of these four lines showed similar resistance to Sclerotinia stem rot than our resistance checks S19-90 and AxN-1-55 in our greenhouse evaluations with the spray-mycelium method.

Three hundred ninety two lines from seven populations segregating for resistance from the five new PIs were evaluated in single 3-foot row plots with two replications for agronomic traits such as yield and lodging. The 392 lines were evaluated once for Sclerotinia stem rot resistance in the greenhouse using the spray-mycelium method we developed. Fifty four lines were selected and further evaluated for yield in 2008 at two locations in six-row 14 feet long plots with two replications at each location.

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Characterization of white mold resistance transferred into common bean from scarlet runner bean

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Funded Plan of Work: Transfer and characterization of white mold resistance from *Phaseolus coccineus* into *P. vulgaris*

ABSTRACT:
Two populations of interspecific crosses of *Phaseolus vulgaris* (common bean) and *Phaseolus coccineus* (scarlet runner bean) were developed by the backcross method with the aim to integrate the superior resistance of *P. coccineus* into the *P. vulgaris* background. The populations, 91G/PI 433251B and MO162/PI433251B, were characterized for white mold resistance in a greenhouse straw test for physiological resistance during the summer of at the BC_2F_5 generation on most lines, due to seed limitations. In the winter of 2008 a second straw test was performed on BC_2F_6 generation including all lines. In general, the M0162/PI 433251B population had a higher frequency of lines similar to or better than G122 (the resistant check), while the 91G/PI433251B population was skewed towards a greater number of susceptible lines.

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