



National Sclerotinia Initiative

NDSU/USDA-ARS
2010 Sclerotinia Initiative
Annual Meeting
Bloomington, MN
January 20-22, 2010



2010 National Sclerotinia Initiative Annual Meeting

January 20-22, 2010

Holiday Inn Select

Minneapolis (Bloomington), MN

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Sclerotinia Initiative 8th Annual Meeting
January 20-22, 2010
Holiday Inn Select Minneapolis/St. Paul International Airport

Agenda

Wednesday - January 20, 2010

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

Thursday - January 21, 2010

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

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

8:25 am Welcome from the Midwest Area – **Larry Chandler, USDA-Agricultural Research Service, Peoria, IL**

8:30 am Welcome from the Northern Plains Area – **Michael McGuire, USDA-Agricultural Research Service, Fort Collins, CO**

8:35 am ARS National Program Staff Update – **Roy Scott, USDA-Agricultural Research Service, Beltsville, MD**

Sclerotinia Research Activities – Session 1 (McIntosh)

Moderator – George Graef

9:00 am Introgressing white mold resistance from the secondary gene pool of common bean – **Shree Singh, University of Idaho, Kimberly, ID**

9:15 am Characterization of the genetic basis for partial resistance to *Sclerotinia sclerotiorum* in pea – **Kevin McPhee, North Dakota State University, Fargo, ND**

9:30 am (1) Identification of defense-response genes conferring partial resistance to white mold in dry bean & (2) Contribution of partial genetic resistance to white mold disease management in pinto and great northern beans – **Phil Miklas, USDA-ARS, Prosser, WA**

9:45 am Transfer and characterization of white mold resistance from *P. coccineus* into *P. vulgaris* – **James Myers, Oregon State University, Corvallis, OR**

- 10:00 am Discussion Break (**Ballroom Foyer**)
- Sclerotinia* Research Activities – Session 2 (McIntosh)**
Moderator – Kevin McPhee
- 10:30 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**
- 10:45 am The requirement for oxalate during pathogenesis on multiple crops – **Jeff Rollins, University of Florida, Gainesville, FL**
- 11:00 am Evaluation of wild *Helianthus* species for resistance to *Sclerotinia* stalk rot – **Charles Block, USDA-ARS, Ames, IA**
- 11:15 am Transferring *Sclerotinia* resistance genes from wild *Helianthus* species into cultivated sunflower – **Chao Chien Jan, USDA-ARS, Fargo, ND**
- 11:30 am Use of a transformation system in sunflower for *Sclerotinia* resistance studies – **John Finer, Ohio State University, Wooster, OH**
- 11:45 am Working Lunch (**Jonathan/Fireside**)
- Sclerotinia* Research Activities – Session 3 (McIntosh)**
Moderator – Martin Chilvers
- 1:30 pm 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**
- 1:45 pm New sources of *Sclerotinia* stalk rot in cultivated USDA plant introductions – **Tom Gulya, USDA-ARS, Fargo, ND**
- 2:00 pm Evaluation of sunflower hybrids and germplasm for resistance to *Sclerotinia* – **Blaine Schatz, North Dakota State University, Carrington, ND**
- 2:15 pm Candidate genes for fungal resistance: Mapping and SNP development for LysM-domain encoding genes in soybean – **George Graef, University of Nebraska, Lincoln, NE**
- 2:30 pm Identification and functional analysis of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and *Arabidopsis* – **Steve Clough, USDA-ARS, Urbana, IL**
- 2:45 pm Break & Poster Session (**Jonathan/Fireside**)
- Sclerotinia* Research Activities – Session 4 (McIntosh)**
Moderator – Jim Steadman

- 3:15 pm Enhancing soybean for resistance to *Sclerotinia* stem rot – **Dechun Wang, Michigan State University, East Lansing, MI**
- 3:30 pm Identification of novel loci for resistance to *Sclerotinia* stem rot in perennial soybean accessions – **Leslie Domier, USDA-ARS, Urbana, IL**
- 3:45 pm Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding – **Martin Chilvers, Michigan State University, East Lansing, MI**
- 4:00 pm Identifying molecular markers linked in lentil (*Lens culinaris* Medik.) to white mold resistance derived from the lentil cultivar Pennell – **George Vandemark (Weidong Chen presenting), USDA-ARS, Pullman, WA**
- 4:15 pm (1) Searching for resistance sources to *Sclerotinia* in wild relatives of cool season grain legumes & (2) Variation in pathogenicity and fungicide sensitivity in relation to variation of neutral markers of *Sclerotinia sclerotiorum* – **Weidong Chen, USDA-ARS, Pullman, WA**
- 4:30 pm Wrap-up & Adjourn (Dinner on your own)

Friday - January 22, 2009

- 7:00 am Steering Committee Breakfast Meeting (**Beacon Conference Room**)
- 7:15 am Continental Breakfast (**Jonathan/Fireside**)
- Sclerotinia* Research Activities – Session 5 (McIntosh)**
Moderator – Brent Hulke
- 8:30 am (1) Defining critical environmental and biological parameters needed to develop *Sclerotinia* stem rot on canola & (2) Evaluation of fungicide alternatives for control of *Sclerotinia* stem rot on canola – **Luis del Rio, North Dakota State University, Fargo, ND**
- 8:45 am Genetic resistance to white mold derived from multiple sources of common bean and scarlet runner bean – **Mark Brick, Colorado State University, Fort Collins, CO**
- 9:00 am Identification of QTL for white mold resistance in pinto bean – **James Kelly, Michigan State University, East Lansing, MI**
- 9:15 am On-farm validation of cultural practice adjustments to improve white mold management in dry bean irrigation systems – **Howard Schwartz, Colorado State University, Ft. Collins, CO**

- 9:30 am Improved resistance in common bean through multi-site screening and pathogen characterization throughout major production areas – **Jim Steadman, University of Nebraska, Lincoln, NE**
- 9:45 am Break (**Ballroom Foyer**)
- Sclerotinia Initiative Research: The next steps*** – (**McIntosh**)
Moderator – Bill Kemp
- 10:15 am ***Guest Speaker***
Strategic Planning & Reporting Progress – **Rich Wilson (USDA-Agricultural Research Service, National Program Staff – Retired, Raleigh, NC)**
- 10:45 am Strategic Plan Discussion, RFPP Process Improvement, Updating Website, On-line reprints & Fact sheets, Assignment of Additional Tasks & Wrap-up of Initiative Business
- 11:45am Working Lunch (**Jonathan/Fireside**)
- 1:30 pm Adjourn (Travel Safely!)

2010 Sclerotinia Initiative Poster Session

Crop Germplasm Resources & Genetics		
Poster No.	Title	Author
1	Evaluation of wild sunflower species for resistance to <i>Sclerotinia</i> stalk rot	C.C. Block, L.F. Marek, T.J. Gulya
2	Genetic resistance derived from multiple sources of common bean and scarlet runner bean	M.A. Brick, M.A. Newell, P.F. Byrne, H.F. Schwartz
3	Expression profiling of the pea- <i>Sclerotinia sclerotiorum</i> interaction for genomics assisted breeding	M.I. Chilvers, T.E. Coram, T.L. Peever, K. McPhee
4	Progress in pyramiding white mold resistance from across <i>Phaseolus</i> species in common bean	L. Crane, H. Terán, S.P. Singh, H.F. Schwartz, K. Otto
5	New sources of <i>Sclerotinia</i> stalk rot in cultivated USDA sunflower plant introductions	T.J. Gulya, B.S. Hulke, Z. Talukder, N. Balbyshev
6	Breeding and quantitative genetics advances in sunflower <i>Sclerotinia</i> research	B.S. Hulke, Z. Talukder, L. Qi, T.J. Gulya
7	Use of transformation system in sunflower for <i>Sclerotinia</i> resistance studies	J.J. Finer, Z. Zhang
8	Transferring <i>Sclerotinia</i> resistance genes from wild <i>Helianthus</i> species into cultivated sunflower	Z. Liu, X. Cai, G.J. Seiler, T. J. Gulya, K.Y. Rashid, C.C. Jan
9	Partial resistance in common bean and <i>Sclerotinia sclerotiorum</i> varies within and between test sites	S. McCoy, R. Higgins, J.R. Steadman
10	Characterization of the genetic basis for partial resistance to <i>Sclerotinia sclerotiorum</i> in pea	K. McPhee, L. Porter
11	Preliminary QTL analysis for yield and white mold resistance in pinto beans	W. Mkwaila, J.D. Kelly
12	Transfer and characterization of white mold resistance from <i>P. coccineus</i> into <i>P. vulgaris</i>	J.R. Myers, S.J. Zimmerman, M. Barrett, J. Davis, J.E. Haggard
13	Comprehensive linkage map of white mold resistance in QTL in common bean	M. Soule, P. Miklas
14	Production of double haploid <i>Brassica napus</i> lines with improved resistance to <i>Sclerotinia sclerotiorum</i>	Y. Su, W. Dai, A. Nepal, L.E. del Rio
15	Enhancing soybean for resistance to <i>Sclerotinia</i> stem rot	D. Wang, R. Kandel

2010 Sclerotinia Initiative Poster Session

Pathogen Biology & Development		
Poster No.	Title	Author
16	Genetic variation of <i>Sclerotinia sclerotiorum</i> on crops in the north central region	L. Aldrich-Wofle, B.D. Nelson
17	Variations between two field populations of <i>Sclerotinia sclerotiorum</i>	R.N. Attanayake, D.A. Johnson, L. Porter, W. Chen
18	Necrosis activities produced by <i>Sclerotinia sclerotiorum</i> independent of Oxalic acid	J.A. Rollins

Pathogen & Host Genomics		
Poster No.	Title	Author
19	Identification and functional analysis of candidate defense-related genes to <i>Sclerotinia sclerotiorum</i> in soybean and Arabidopsis	B. Calla, L. Blahut-Beatty, Y. Zhang, D. Simmonds, S.J. Clough
20	Screening accessions of wild relatives of <i>Pisum sativum</i> for resistance to <i>Sclerotinia sclerotiorum</i>	C. Coyne, W. Chen
21	Identification of novel loci for partial resistance <i>Sclerotinia</i> stem rot in perennial soybean accessions	L.L. Domier, G.L. Hartman

Pathogen Epidemiology & Disease Management		
Poster No.	Title	Author
22	Implementing the white mold management strategy	C. Kora, K.Y. Rashid
23	Contribution of partial genetic resistance and fungicide application to white mold disease management in pinto and great northern beans	P. Miklas, L. Porter
24	Influence of soil texture and moisture content on carpogenic germination of <i>Sclerotinia sclerotiorum</i>	A.Nepal, L.R. del Rio
25	Role of sclerotial moisture content on carpogenic germination of <i>Sclerotinia sclerotiorum</i>	A.Nepal, L.R. del Rio
26	On-farm validation of cultural practice adjustments to improve white mold management	H.F. Schwartz, M.A. Brick

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

Laura Aldrich-Wolfe and Berlin D. Nelson, Department of Plant Pathology, North Dakota
State University, Fargo, North Dakota, USA

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 commonly-grown crops in the north central United States, 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 2008 we collected 149 isolates of the pathogen from four crops (canola, dry bean, soybean, and sunflower) in twelve North Central states (North Dakota, South Dakota, Nebraska, Minnesota, Iowa, Missouri, Kansas, Wisconsin, Michigan, Indiana, Ohio and Illinois) and Wyoming, Montana and Colorado. We were unable to obtain isolates from pea and lentil. All isolates were evaluated for mycelial compatibility group (MCG) and 46 MCG's were identified. The most common was MCG 9 found in nine states and on all four crops. Six of the most common MCG's represented 58% of the isolates and were found across crops. Most of the other MCG's had only one or several isolates. Twelve microsatellite markers with fluorescently labeled tags were used to characterize the isolates. The PCR product lengths were measured on an automated sequencer. There was a strong association between MCG's and microsatellites. For example, within MCG 9 all microsatellites were the same, while in MCG 8 there were three microsatellite haplotypes, but they only differed at one locus. To date, mycelial compatibility groups do not appear to be restricted to particular geographic regions or host crops. Local areas may be as diverse genetically as broad geographic regions.

Contact Information: Dr. Berlin D. Nelson, Dept. Plant Pathology #7660, North Dakota State University, Fargo, ND 58108-6050; 701-231-7057; berlin.nelson@ndsu.edu

Variation in Fungicide Sensitivity and Mycelial Compatibility between Two Field Populations of *Sclerotinia sclerotiorum*

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

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

ABSTRACT:

Sclerotinia sclerotiorum is a ubiquitous necrotrophic pathogen. It causes white mold on more than 400 plant species including chickpea, lentil, pea and potato. In order to understand the effect of host plants, cropping history and cultural practices on evolutionary potential of *S. sclerotiorum*, a pathogen population from pea was compared with a population from potato in terms of mycelial compatibility grouping (MCG) and fungicide sensitivity. Fungicide application and irrigation are regular practices in potato production, whereas no fungicides or irrigation are used in dry pea production. A total of 57 isolates (31 from a commercial dry pea field and 26 from a commercial potato field) were used in the comparison. Twenty-three MCGs were found among 31 pea isolates (G:N ratio 0.74), and 17 MCGs in 26 isolates of the potato population (G:N ratio 0.65), suggesting that relatively higher genetic diversity exists in the pea population. Variation in sensitivity to two fungicides between the two populations was also compared. Colony diameters were determined 36 hrs after inoculation on PDA plates amended with Benomyl (0.2 µg a.i./ml) or Quadris (0.8 µg a.i./ml). The pea population showed greater variance than did the potato population for both the fungicides, suggesting that the pea population has higher diversity for loci controlling fungicide sensitivity and has more potential to adapt into new environments. No variation was found in sclerotial dry weight between the two populations suggesting that life history traits are less prone to selection pressure. These two populations are being assessed for variation at 12 microsatellite loci to estimate genetic differentiation at neutral markers between the two populations.

Contact Information: Dr. Weidong Chen, USDA- ARS, and WSU, Pullman, WA 99164; 509-335-9178; w-chen@wsu.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, Jr., USDA-ARS, Fargo, ND.

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

Research Area: Crop Germplasm Resources and Genetics.

ABSTRACT:

The objective of this project is to identify sources of resistance to Sclerotinia wilt (stalk and root rot) in wild *Helianthus* germplasm. The USDA sunflower collection contains both wild annuals (1,369 accessions) and perennials (799 accessions). The screening effort to date has concentrated mainly on the diploid annual species. Ninety percent of the accessions from all of the annual species, other than *H. annuus* itself, have now been screened. The accessions were initially tested with 60-plant populations under intensive disease pressure in the greenhouse. The goal was to identify accessions showing significantly better plant survival percentages and longer than average life spans than the most resistant checks. With this approach, much of the susceptible germplasm can be filtered out, making better use of the follow-up field screening trials.

In 2009, the *H. petiolaris* and *H. niveus* collections were evaluated (144 accessions) along with re-tests of the top 40 accessions from previously tested species. Plants from 13 resistant accessions were saved and shipped from Ames to Fargo for greenhouse crossing with a susceptible inbred and potential genetic analysis of resistance. Twenty-five accessions were entered into field trials at Oakes, ND and Staples, MN. The five top-performing entries in the 2009 field trials were the perennial *H. resinosus* PI 650079 (96% and 100% survival at the two sites), and the annuals *H. annuus* PI 653604 (94% and 98% survival), *H. debilis* ssp. *cucumerifolius* PI 435654 (92% and 100% survival), *H. debilis* ssp. *silvestris* PI 468680 (96% and 98% survival) and *H. debilis* ssp. *silvestris* PI 468686 (95% and 96% survival). PI 649863 (*H. argophyllus*), one of the top accessions in previous greenhouse tests and in the 2008 field trial had 81% and 91% survival at the two locations, respectively. The field trials confirmed the concept that greenhouse testing was able to identify susceptible germplasm, making them more efficient by concentrating on more resistant germplasm.

There remains a wealth of untested perennial sunflower germplasm. The next phase of greenhouse testing will focus extensively on perennials and on a geographic cross-section of germplasm within wild *H. annuus*, to be followed up by field trials in 2010.

Contact Information – Dr. Charles C. Block, USDA-ARS, G-212 Agronomy Bldg, Iowa State University, Ames, IA 50011; 515-294-4379; charles.block@ars.usda.gov.

Genetic Resistance to White Mold Derived From Multiple Sources of Common Bean and Scarlet Runner Bean

Mark A. Brick, Mark A. Newell, Patrick F. Byrne &
Howard F. Schwartz, Colorado State University, Fort Collins, CO

Funded Plan of Work: Genetic Resistance to White Mold Derived From Multiple Sources of Common Bean and Scarlet Runner Bean

ABSTRACT:

Genetic resistance to white mold has been reported in both common (*Phaseolus vulgaris* L.) and scarlet runner (*P. coccineus* L.) beans. From previous USDA Sclerotinia Initiative funding we first developed a common bean RIL population derived from the cross G122/CO72548. In this RIL population, we identified 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 low Straw Test ratings. 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. Mean Straw Test ratings in the IBL population were lower for lines that had >25% scarlet runner alleles compared with lines that had <25% scarlet runner alleles (3.8 vs 4.8, respectively), indicating that lines with higher proportion of scarlet runner alleles also had higher levels of resistance to WM. In the final phase of this work we proposed to combine the genetic resistance found in the most resistant IBL with architectural avoidance found in the pinto breeding line USPT-WM 1 to validate the markers found in the IBL population. IBL lines 75A and 25B were crossed to USPT-WM1 to generate two random inbred populations with approximately 200 lines each. In 2009 we completed seed increase of the F₅ lines from each cross. In fall, 2009 we started screening the lines for reaction to WM using the straw test. Preliminary data suggest that mean ASI is much higher (less resistant) in these lines than in lines observed in the IBL population. Mean white mold scores ranged from 5.2 to 8.8 in one subset of the population. Our results are somewhat encouraging because we were able to recover lines that possess an intermediate level of resistance. Molecular marker data is being collected on a subset of resistant and susceptible lines to determine whether the markers previously linked to resistant QTL found will contribute to resistance. Our ultimate goal is to determine whether resistance QTL from scarlet runner bean can be introgressed into a commercial pinto breeding line.

Contact information – Dr. Mark Brick, Dept. of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523-1170; 970-481-6551; Mark.Brick@Colostate.edu.

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

Bernarda Calla, University of Illinois, Urbana, IL;
Laureen Blahut-Beatty, Yunfang Zhang and Daina Simmonds, AgCanada, Ottawa;
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:

Soybean is susceptible to *Sclerotinia sclerotiorum*. Different degrees of partial resistance have been documented (such as in PI194639), but no variety has been identified with true resistance. High-level resistance has been achieved in transgenic soybean carrying oxalate oxidase (OxO), a gene that degrades oxalic acid (OA), a major virulence determinant of *S. sclerotiorum*. We have been using PI194639 and transgenic OxO lines, as well as pure OA, to identify genes that may be involved in modifying responses to *S. sclerotiorum*, and to characterize the biology related to the effect of OA and *S. sclerotiorum* infection. We have completed two complex microarray experiments to determine differential gene expression, and a third is on-going. One experiment analyzed soybean response to natural infection of leaf tissue at two time points within 24 hours of inoculation. The other experiment looked at the response in soybean leaves 2 hours after infiltration of 5 mM OA, a concentration similar to that detected during *S. sclerotiorum* infection. Both of these experiments were successful at identifying genes that were significantly differentially expressed based on statistical analysis of three independent experimental replications. The experiment involving naturally infected leaves had 4526 genes across time and 1855 between varieties. The oxalic acid infiltration provoked differential expression of 5,093 genes out of approximately 38,000 screened. Our earlier published work on PI194639 identified 1375 differentially expressed genes (Plant Genome 2:149-166). Validation of the expression results is being conducted using quantitative real-time reverse-transcribed PCR. Expression data is being clustered across multiple experiments to identify putative defense-related genes. Candidate defense genes will be 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. Arabidopsis inoculation and disease assays are being perfected.

Contact Information – Steven Clough, US Department of Agriculture and the University of Illinois Department of Crop Sciences. Urbana, IL; 217-265-6452; sjclough@uiuc.edu

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

ABSTRACT:

A large expressed sequence tag (EST) data set was developed with massively parallel sequencing on a 454 Roche platform. Post-trimming the data set consisted of 145,049 reads with an average read length of >200 nucleotides. The sequence reads were assigned by the use of BLAST analysis and parsing script to one of four categories; *S. sclerotiorum*, *P. sativum*, *S. sclerotiorum* and *P. sativum*, and unassigned. BLAST alignment was performed against the *S. sclerotiorum* and soybean genome. As no *P. sativum* genome sequence is available the soybean genome was used as a proxy. A threshold for similarity of *P. sativum* to soybean was determined by blasting the c.a. 5,000 pea ESTs that are available on GenBank. Assignment of the reads demonstrated that most (71%) of the reads could be assigned to *P. sativum* and/or *S. sclerotiorum*. Twenty-nine percent of the reads were assigned to *S. sclerotiorum* and 46% of the reads assigned to *P. sativum*. Only 1% of the reads were assigned to both *P. sativum* and *S. sclerotiorum*, while 24% of the reads were unassigned. Unassigned reads may have resulted from ESTs that are not represented by genes in the soybean or *S. sclerotiorum* genomes, or did not provide significant match due to sequence variation. It is also possible that the unassigned reads were the result of PCR or sequencing artifact. Further analysis will aim to resolve the identity of these unassigned reads. Reads that were assigned to both *P. sativum* and *S. sclerotiorum* may be due to similarity between some regions of genes.

Future analysis of the data set will include assembly of contiguous sequences prior to sorting reads to species. This will enable assembly of consensus sequences in which we will have greater confidence in the base calls due to overlap of sequence reads. In addition, contiguous sequences should also be longer than initial reads which will provide more information on the genes being expressed at the time for which mRNA was sampled from this host-pathogen interaction.

The project is now focused on using an RNAseq approach to perform expression profiling of the *Sclerotinia*-pea host pathogen interaction. A susceptible and resistant pea line will be examined at multiple time points to identify genes involved in resistance response. The EST data set described above will assist in sorting the reads as currently there is no pea genome sequence and only ~5000 pea ESTs are publicly available in GenBank.

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Screening Accessions of *Pisum fulvum* and *P. sativum* subsp. *abyssinicum* for Resistance to *Sclerotinia sclerotiorum*

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

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

ABSTRACT:

Sclerotinia sclerotiorum causes the destructive disease of white mold of pea. Management of the disease is through cultural practices, fungicide applications, and resistance when available. Cultural practices are usually not adequate in managing the disease. Fungicide applications increase production costs and reduce the competitive edge of growers in the global market place. Employing resistance is the only viable long-term approach to manage white mold of pea. However, resistance to white mold on cultivated pea (*Pisum sativum*) is rare and when available, is at low to moderate levels in cultivars and germplasms of pea. New resistance sources are needed in order to improve resistance in elite pea cultivars.

The research is to search resistance to white mold in wild relatives of cultivated pea. Accessions of *Pisum fulvum* and *P. sativum* subsp. *abyssinicum* were obtained from the USDA Germplasm Collection. The seeds were stratified and germinated in seed germinator before transplanted into the greenhouse. Each accession was planted in four pots (four plants per pot) and maintained in the greenhouse. Plants were inoculated with a actively growing culture of *Sclerotinia sclerotiorum* when the plants were five weeks old by wrapping a colonized agar plug onto the third internode of the stem. Host resistance was monitored by measuring the lesion expansion on the stem every two days after inoculation for eight days. Significant differences in response to inoculation were observed among the accessions. In general, lesion sizes were smaller in *P. fulvum* than in *P. sativum* subsp. *abyssinicum*, although significant differences were observed with each taxon. Accession W6 15041 of *P. sativum* subsp. *abyssinicum* is the most resistant of the subspecies, whereas PI 595947 from Australia is the most resistant in *P. fulvum*. These resistant lines are promising to provide genetic sources for resistance to white mold. The resistant accessions are being tested further along with accessions of two other wild subspecies (subsp. *elatius* and *transcaucasicum*) of *Pisum sativum*.

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Progress in Pyramiding White Mold Resistance from Across *Phaseolus* Species in Common Bean

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

ABSTRACT:

White mold reaction of known contemporary resistant large-seeded Andean and small-seeded Middle American dry and green common bean and interspecific breeding lines was verified in the greenhouse in Idaho and Colorado. Five large-seeded dry bean genotypes (A 195, G 122, MO 162, PC 50, VA 19) and three small-seeded interspecific breeding lines (VCW 54, 92BG-7, 0785-220-1) derived from *P. coccineus* and were crossed within the group. Also, G 122 was crossed with VCW 54 and VCW 54 was crossed with 0785-220-1. Approximately 50 seeds of each cross were produced. The parents and a part of seed from the F₁ of eight single-crosses were evaluated for their reaction to white mold to determine complementation or lack thereof (preliminary test) and produce the F₂ seed. In a separate study, two double-cross populations: Pop I = USPT-WM-1 / CORN 601 // USPT-CBB-1 / 92BG-7 and Pop II = Chase / I9365-25 // ABL 15 / A 195 were developed. Gamete selection (GS) from F₁ to F₄ and two cycles of recurrent selection (RS) were practiced in both populations. Thirteen breeding lines selected from each method and each population were compared in two greenhouse environments in 2007-2008. All five large-seeded Andean dry beans and their four F₁ were resistant to white mold (score of 4 on a 1 to 9 scale, where 1= healthy with no white mold symptoms and 9= severely diseased or dead), indicating that they probably carried similar resistance genes/QTL. While the VCW 54/0785-220-1 F₁ also was resistant, in crosses with 92BG-7 both interspecific breeding lines exhibited a susceptible white mold reaction. Thus, very likely VCW 54 and 0785-220-1 had the same resistance genes/QTL, but both were different from 92BG-7. ICA Bunsu and Cornell 501 would need to be crossed with G 122 and VRW 32 would need to be crossed with VCW 54 and 92BG-7. Also, all parents, F₁, and F₂ will need to be evaluated in a replicated trial to verify and determine the complementation or lack thereof for white mold resistance. Both GS and RS were effective and the mean WM score of 13 selected families was significantly lower than the mean score for the four parents in both populations. Furthermore, 20.6% gain was realized in Pop I and 18.6% gain in Pop II from GS. The gain in WM resistance from RS for Pop I was 10.7% and for Pop II was 5.1%. But, the selected family with the lowest WM score had significantly lower WM score than the best WM resistant parent only from GS in Pop I.

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

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Funded Plan of Work: Development molecular markers linked to genes for partial resistance to *Sclerotinia* stem rot in *Glycine tabacina* and *G. tomentella*.

ABSTRACT:

Work was conducted in 2009 to confirm the resistant phenotypes of resistant and susceptible accessions within two perennial *Glycine* species (*G. tabacina* and *G. tomentella*) that will be used in mapping studies. The most resistant and susceptible accessions will be used to generate large populations (~500 lines each) of recombinant inbred lines (RILs) segregating genes for partial resistance to *Sclerotinia* stem rot. Individuals within the populations will be assayed for response to *Sclerotinia sclerotiorum* infection using a modified cut-stem assay. Since only a small fraction of the markers developed for *G. max* are informative in perennial *Glycine* species, two sets of new single nucleotide polymorphism (SNP) markers will be identified by massively parallel transcriptome sequencing. High-through-put GoldenGate SNP assays will be developed and used to genotype the populations. The phenotypic and SNP marker data will be combined and used to detect quantitative trait loci (QTL) for resistance to *S. sclerotiorum*. Because the SNP markers will be based on coding sequences, it will be possible to rapidly locate homologues on the soybean genome to determine whether or not the identified loci represent novel genes. Genetic intervals containing novel loci will be narrowed using additional SNP markers, synteny will be compared to the soybean genome. If syntenic, candidate genes from within regions will be selected for further analysis and eventual movement to *G. max*.

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

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

ABSTRACT:

Chitin is a normal component of fungal cell walls and is also known to induce a plant defense response similar to that seen upon fungal pathogen infection. The Stacey lab has demonstrated that members of the LysM-receptor kinase (LysM-RLK) family likely serve as the receptor for chitin. A mutation in LysM-RLK3 resulted in a constitutive chitin response and elevated response to fungal pathogen infection, suggesting that the wild-type receptor acts to repress a chitin response, which would then be de-repressed upon chitin recognition. This information suggests that the LysM genes represent promising candidate genes that could explain some of the resistance to white mold in selected genotypes. Analyses of soybean sequence databases indicated that soybean has 13 unique LysM-RLKs and at least another 17 LysM-domain containing proteins. The genes were cloned and sequenced. The objectives of this study are to (1) map LysM-domain encoding genes in soybean and correlate their map locations to known white mold QTLs, and (2) develop SNP markers for those LysM-domain genes mapping close to known white mold QTLs. Five soybean RIL populations used previously to identify QTLs for sclerotinia resistance will be used in this analysis. The common susceptible parent is the cultivar Williams 82. Out of 42 LysM-domain genes considered, we identified 37 that were located on Linkage Groups that contained significant QTL from our previous work. To date, we have determined that six of the LysM genes map to locations where we identified QTL on LGs D1b, E, F, G, K, and L. LysM genes on LG A1, B1, and D1a were not associated with our identified QTLs. We are in the process of identifying SNPs for the LysM genes in Williams 82 and the five susceptible parents of the RIL populations, and we will map selected LysM genes in those 5 populations. The SNP markers that are developed could be used for marker-assisted breeding to develop near-isogenic lines and potentially cultivars with improved resistance.

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New sources of Sclerotinia stalk rot in cultivated USDA Sunflower Plant Introductions

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

ABSTRACT:

In a two-year study, 250 cultivated sunflower genotypes from the USDA sunflower collection (maintained and distributed by the North Central Regional Plant Introduction Station, Ames Iowa) were evaluated in multiple field trials for their reaction to stalk rot incited by *Sclerotinia sclerotiorum*. Three locations were planted in 2008 and again in 2009; one location each year flooded and thus only four datasets were usable. Due to the large experiment size, the study was designed as a randomized complete block design with sets in reps. Artificial inoculation was done using *Sclerotinia* grown on millet (mycelium without sclerotia) which was deposited in furrows mid-season using a granular chemical applicator mounted on a tractor. In addition to the 250 Plant Introductions (PIs), twelve USDA inbreds were also included for comparison with germplasm developed specifically for disease resistance. Averaged over four locations, stalk rot ratings ranged from 3.5% (USDA inbred HA 441) to 84% for PI 650710 (confection type from Spain). The entries which were rated in the top 10% of the study included six USDA inbred lines, plus a very diverse group of international germplasm originating from eleven countries (Argentina, Canada, Czech Republic, France, Hungary, Mexico, Paraguay, Poland, Russia, Spain, Zambia). This group of stalk rot resistant germplasm will form the basis of both improving our USDA breeding material and will help to diversify its overall genetic foundation. The data produced in this study will be used in association mapping by a companion project. Future plans call for testing either the elite stalk rot material, or the entire collection of 250 Plant Introductions in inoculated trials for head rot resistance, in an attempt to select for germplasm with high levels of resistance to both diseases.

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Breeding and quantitative genetics advances in sunflower *Sclerotinia* research

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Funded plan of work: Pyramiding *Sclerotinia* head rot and stalk rot resistances into elite sunflower breeding lines with the aid of DNA markers.

ABSTRACT:

In 2009, we continued the process of backcrossing the head rot QTL from the HA 441 x RHA 439 population into confectionery and elite oilseed backgrounds BC₁F₁ hybrids were produced and we are in the process of selecting progeny based on their marker profiles at 6 major QTL loci for advancement into another backcross.

Our efforts to perform association mapping with the 260 Plant Introductions (PIs) obtained from the North Central Regional Plant Introduction Station of USDA-ARS in Ames, IA, are moving forward. We have added two more locations of data with two replications each to the data set we formed last year with Tom Gulya's sister project. We have recently completed the analysis of both the 2008 and 2009 data. Based on the distribution of data, which is more broadly and normally distributed than we were expecting, we believe that the phenotypes will be adequate for mapping using the association mapping model. We are currently working on developing the marker set for our resistance candidate genes using *in silico* techniques and resequencing of ESTs, which will contribute the necessary genotypes with which we can complete our association model.

Our traditional breeding program had a successful season in 2009, with breeding lines in the F₄ to F₇ selfing generations being tested for *Sclerotinia* head and stalk rots, each at two locations. Our goal here is to continue introgression of new, minor resistance loci from many domesticated sources into elite germplasm.

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Selecting beans (*Phaseolus vulgaris*) for high yield under white mold pressure

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Funded Plan of Work: Crop Germplasm Resources and Genetics

ABSTRACT:

White mold is a disease of high productivity environments. Minimizing yield losses to white mold is critical for bean growers attempting to optimize yield potential of their crop. Selecting for resistance to white mold alone ignores the opportunity to improve yield. The objective of this study was to investigate the relationship between disease severity, yield and critical agronomic traits in 37 bean genotypes evaluated over three years under white mold pressure in Michigan. Canopy height exhibited a strong negative correlation with lodging (-0.53***) and lodging was positively correlated with white mold (0.64***). Although yield was negatively correlated with white mold (-0.3**), many white mold tolerant genotypes in the NSI trial were very low yielding, raising concerns as to their parental value in breeding programs. A few high yielding lines with moderate white mold resistance were identified and their performance was consistent over three years. Selection for yield should be practiced under severe white mold pressure if breeders are to develop high-yielding bean lines with tolerance to white mold.

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Transferring Sclerotinia Resistance Genes from 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:

Cultivated sunflower lacks a sufficient level of resistance to both Sclerotinia stalk and head rot, but abundant resistance in perennial *Helianthus* species has been confirmed. The objectives of this project were to transfer Sclerotinia head and stalk rot resistance from resistant wild perennial hexaploid and diploid *Helianthus* accessions and interspecific amphiploids into cultivated sunflower. Interspecific F₁ hybrids were produced between stalk rot resistant hexaploid *H. californicus* and *H. schweinitzii* and HA 410, and continued backcrossing of *H. californicus* crosses with HA 410 and selfing resulted in BC₄F₃ plants with improved pollen and seed fertility and with 2n chromosome numbers between 34 and 37 in 2008. Further backcrossing and selfing in 2008 increased seed for field evaluation in 2009. Five amphiploids highly resistant to stalk and head rot were crossed with HA 410 in 2006, and BC₂F₂/BC₃F₁ plants with chromosome numbers from 2n=34 to 36 were obtained in the greenhouse in 2008, and further backcrossed and selfed to produce seed for 2009 field evaluation. Crosses between NMS HA89 and head rot resistant *H. maximiliani* and *H. nuttallii* were backcrossed with HA 441 and advanced to the BC₁F₃ and BC₂F₃ generation in the field in 2008 and 2009 for seed increase. In 2007, stalk rot resistant diploid perennial *H. maximiliani*, *H. giganteus*, and *H. grosseserratus* were crossed with HA 410 and their BC₁F₂/BC₂F₁ progenies with 2n=34-35 chromosomes were obtained in 2008. Their selfed BC₁F₃ and BC₂F₂ progeny were grown in the field in 2009 for seed increase. Replicated field tests with 163 and 313 progeny families were screened for head and stalk-rot resistance in 2009, respectively. The results indicated moderate to good resistance, suggesting successful gene introgression. Molecular tracking studies using SSR markers indicated high polymorphism between wild resistant donors and the cultivated recurrent parents, and the retention of markers specific to resistant donors was higher for progenies from diploid perennials than from hexaploid or interspecific amphiploids, suggesting a higher frequency of gene introgression when perennial diploids species were used. Resistant lines identified in 2009 will be tested again in 2010 and the results will be used to select lines for germplasm release, providing new resistance genes to further enhance Sclerotinia resistance of the sunflower crop. Protocol of genomic *in situ* hybridization (GISH) distinguishing chromosomes of perennial *Helianthus* species and cultivated sunflower has been established, providing an additional tool for studying gene transfer.

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

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

ABSTRACT:

Bean cultivars with partial physiological resistance and/or architectural avoidance to white mold (WM) would reduce disease losses and require no input costs for growers. Thus, one goal was to identify sources of resistance in adapted and nonadapted common bean lines utilizing standardized greenhouse screening methods and field nurseries across major bean production regions. Multi-site testing has facilitated the release of a WM resistant snap bean and nine resistant dry beans since 2006. Collaborators at each location use the modified Petzoldt and Dickson rating scale for the straw test protocol for greenhouse screening, and the CIAT scale for rating field screening nurseries. Better sources of resistance, especially those derived from secondary gene pool sources, are available for testing due to the SI support. A second goal was to assess variation in common bean isolates of *Sclerotinia sclerotiorum*. To assess pathogen variation, we devised a unique study on pathogen variation across bean-production areas that tests the hypothesis that pathogen variation within and between test sites influence identification of WM resistance. Mycelial compatibility groupings (MCGs), aggressiveness (virulence), and microsatellites (SSRs) are being used to identify genetic and phenotypic isolate variation that can influence stability of identified WM resistance over time and location. Collecting isolates from specific bean host lines replicated at each resistance screening site permitted us to assess within and between location variations. High variation in aggressiveness and genetic variability (measured by MCGs or microsatellites) of pathogen isolates within and between field screening nursery locations and greenhouse test isolates was found. Another hypothesis we tested is that isolates collected from screening nursery sites and greenhouse tests show similar phenotypic and genotypic variability as isolates collected from growers' fields in the same region. In other words, that the isolates involved in screening reflect characteristics of those found in grower fields. When isolates from screening nurseries in three states were compared with grower field isolates, there were significant differences in aggressiveness.

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

Kevin McPhee, North Dakota State University, Fargo, ND and Lyndon Porter, USDA-ARS, Prosser, WA

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

ABSTRACT:

Sclerotinia sclerotiorum Lib. 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, 1) place the genetic factors (QTL) controlling partial resistance to white mold on the pea genetic map and 2) to pyramid the available mechanisms of resistance in an effort to develop durable resistance. Progress in the over the past twelve months has been to advance PRIL-17, 'Lifter'(PRIL-5)/PI240515 and PRIL-19, PI169603/'Medora'(PRIL-2) populations from the F₃ to F₅. DNA is being extracted for genetic analysis from leaf tissue collected from each F₂ plant. Phenotyping of the F₃ family seed from PRIL-17 and PRIL-19 populations has been completed. Approximately one half of the lines survived when assessed nine days after inoculation. The surviving plants were maintained in the greenhouse and seed harvested from them. These lines will be screened again to identify escapes and again allowed to produce seed. It is expected that this seed will be increased and evaluated in field trials. In addition those lines showing resistance based on nodal or reduced lesion expansion will be crossed in an effort to pyramid the resistance mechanisms.

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Contribution of partial genetic resistance and fungicide application 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:

Pinto and great northern bean market classes are susceptible to white mold. A few advanced lines and cultivars with partial resistance to white mold have been developed recently. Our objective has been to document what effect the partially resistant or less susceptible pinto and great northern breeding lines being developed have on overall white mold disease control. For each market class susceptible vine, susceptible upright, and partially resistant lines were chosen. 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. As in past years, the partially resistant lines did not require a fungicide application to achieve full yield potential. Conversely, the susceptible genotypes had a significant yield increase and decrease in disease severity score in response to one and/or two fungicide applications. The susceptible lines had greater yield potential than the partially resistant lines. So, although the partially resistant lines can be grown without fungicide the low yield potential for these lines is not commercially acceptable. The 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.

A preliminary comparison between fungicides in susceptible pink bean PK7-4 revealed a greater yield response with Endura (155%) versus Omega (127%) in comparison to the control with no fungicide (100%).

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QTL interactions and candidate gene analysis in common bean

Phillip Miklas and Marilyn Soule, Vegetable and Forage Crop Research Unit, Prosser, WA; Phil McClean, Dept. of Plant Sciences, North Dakota State University

Funded plan of work: Identification of defense-response genes conferring partial resistance to white mold in dry bean

ABSTRACT:

More robust markers and a better understanding of the nature of quantitative resistance to white mold will assist development of resistant cultivars. Objectives of this research are to examine phenotypic interactions among the major QTL, and to identify genes differentially expressed in response to white mold infection in populations segregating for major resistance QTL. Different recombinant inbred populations with combined QTL from linkage groups 2 and 8 ,and 7 and 8, respectively, were screened for white mold reaction in replicated field and/or greenhouse environments. Generally one QTL had greater effect than the other, and lines with both QTL were slightly more resistant than lines with only one QTL. We investigated LsyM (chitin receptor) as a candidate gene for WM resistance in common bean. LsyM sequence was located in the Soybean genome on Gm02 near to a soybean WM resistance QTL. A putative LsyM gene for common bean aligns perfectly with the LysM motif located on Gm02. This Gm02 region in soybean has synteny with the Pv8 WM resistance QTL region in common bean. To date we have not detected polymorphism for specific mapping of LsyM in common bean. Work is ongoing. By leveraging common bean-soybean synteny six additional gene-based EST markers were developed at the terminal end of Pv8 linkage group to increase marker density of the region for fine mapping the QTL. Gene transcript profiling between resistant and susceptible lines for a particular QTL is ongoing.

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Preliminary QTL analysis for yield and white mold resistance in pinto bean

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Funded Plan of Work: Crop Germplasm Resources and Genetics

ABSTRACT:

Pinto beans are the most important market class grown in the U.S. but they are among the most susceptible to white mold. Given that resistance to white mold is quantitative, results of a preliminary analysis of quantitative trait loci associated with white mold resistance and yield in pinto bean are reported. The parents (AN-37 and P02630) were initially screened with 150 SSR markers 46 of which were found to be polymorphic. To date 26 of these markers were assayed across the population of 94 F4:8 lines and mapped. A QTL for resistance to white mold was identified in the field in 2007 near markers IAC 67, BMd42 and Bm157. This QTL was not observed in 2008 and underscores the major role that environment plays on the expression of the disease in the field. A QTL for yield located between markers Bmd17 and Pvat011 on linkage group 1 was present in both 2007 and 2008. The analysis emphasizes the importance of measuring yield when selecting for resistance to white mold in pinto beans.

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

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

ABSTRACT:

An advanced backcross QTL analysis has been used to introgress the white mold resistance found in *P. coccineus* into *P. vulgaris* with the focus on three populations: 91G/PI 255956, 91G/PI 433251B, and MO162/PI433251B. In 2005-07, we advanced and characterized the 91G/PI 255956 population (consisting of 115 lines) with three straw tests, one oxalate test, and one field trial. Seventy-seven of 98 polymorphic SSRs were scorable in the progeny, and 2 revealed single introgressions. A single pair of AFLP primers amplified 59 scorable segregating fragments. The linkage map consisted of 11 linkage groups that corresponded to 9 of the 11 core map linkage groups. Composite Interval Mapping revealed QTL on Pv2, Pv6 and Pv9 that explained 34.7% of the phenotypic variation. QTL on Pv2 and Pv6 were also associated with straw test resistance, explaining 18.6% of phenotypic variation. Pv1, Pv5, and Pv8 were missing and high levels of segregation distortion were observed. All but three SSR loci had significantly significant residual heterozygosity. Furthermore, a number of polymorphic SSRs were unlinked, and based on their position on the bean consensus map, we were able to infer that large regions of the mapped linkage groups had reverted to common bean. The Pv2 and Pv9 QTL are located near markers that are in chromosome regions associated with white mold resistance QTL in common bean. We identified eight lines that have white mold resistance levels statistically similar to partially resistant common bean checks G122, NY6020, and Ex Rico over three field seasons. One line was submitted for straw testing in the National WM Nursery in 2009. In 2007-2008, the OR 91G/PI 433251B and MO162/PI 433251B populations lines (263 and 120 respectively) were advanced with DNA being extracted in the BC₂F₄, and straw and field tests performed in the BC₂F₅ and BC₂F₆. In general, the MO162/PI 433251B population had a higher frequency of lines similar to or better than G122, while the OR 91G/PI 433251B population was skewed towards a greater number of susceptible lines. In 2009-2010, we augmented field and straw test evaluations of lines in the OR 91G/PI 433251B and MO162/PI 433251B populations. Populations have been now been subjected to four and five straw tests, respectively, and have undergone two field trials. Ninety-two and 57 SSRs, respectively have been screened in the two populations. Preliminary mapping efforts revealed similar patterns for missing chromosomes as well as markers associated with potential resistance QTL on some of the same linkage groups as the OR 91G/PI255956 map. Results to date provide insight into why prior efforts to introgress resistance from *P. coccineus* have had limited success.

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Influence of soil texture and moisture content on carpogenic germination of *Sclerotinia sclerotiorum*

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Funded Plan of Work: Defining critical environmental and biological parameters required for development of *Sclerotinia* stem rot in canola

ABSTRACT:

Sclerotinia stem rot epidemics in canola and many other broadleaf crops start when ascospores, produced by apothecia on overwintered sclerotia, land on floral tissues. Most studies that evaluated the role of moisture, as a factor influencing production of apothecia by overwintered sclerotia, carpogenic germination, have used sand as substrate and kept moisture contents at constant levels. The role of fluctuating moisture conditions on carpogenic germination was evaluated using sand as substrate in a recent manuscript. While there are a few sandy soils dedicated to agriculture in North Dakota, none of them could be considered to be pure sand. The texture of most North Dakota soils range from heavy clays to sandy loams. To evaluate the role of soil texture and fluctuating moisture conditions on carpogenic germination, a controlled environment study was conducted at North Dakota State University. Two soils, a Fargo Silty clay (44% clay content) and an Aylmer-Bantry fine sand (92% sand content), were mixed in proportions of 1:0, 2:1, 1:1, 1:2, and 0:1 v/v to create different textures. After the soil matric potential was calculated for each soil mixture, samples of each texture were set at constant 100%, 75%, 50% or 25% saturation; or to conditions fluctuating between these levels and 0 % saturation, at 25 unit intervals. Samples were incubated at 14 to 18° C for 82 days. The amount of carpogenic germination was recorded at five days interval starting at the time of first apothecium production. Moisture fluctuations reduced carpogenic germination compared to constant moisture conditions ($\alpha=0.05$). Under constant moisture, germination decreased with increased moisture; sclerotia incubated at constant 25% moisture saturation yielded highest and earliest (22 days) carpogenic germination. No carpogenic germination was observed when sclerotia were incubated at constant 100% saturation and at the end of the study all of them were dead. Under fluctuating conditions, highest carpogenic germination was observed when moisture remained between 75 and 50% saturation. Under fluctuating soil moisture conditions, carpogenic germination is significantly affected by wider range of fluctuation. This information will be used to develop a predictive model for carpogenic germination.

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Role of sclerotial moisture content on carpogenic germination of *Sclerotinia sclerotiorum*

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Funded Plan of Work: Defining critical environmental and biological parameters required for development of *Sclerotinia* stem rot in canola

ABSTRACT:

A previous study determined that, when kept at constant levels, the optimum soil moisture content for carpogenic germination of *S. sclerotiorum* sclerotia was at 25% of saturation for most soil textures evaluated. Since previous literature on the topic, suggesting that high soil moisture content was required for germination, were in apparent contradiction with our findings, a study was set to evaluate the relationship between sclerotial moisture content and its ability to germinate carpogenically, as well as to study the dynamics of water absorption by sclerotia in soil. The study was conducted under controlled conditions at North Dakota State University using laboratory-produced sclerotia from isolate WM031. Sclerotia were classified as large, medium and small by sieving. The amount and rate of water absorption by sclerotia was calculated after recording sclerotial weight at different time periods on sclerotia immersed in pure water or buried in soil kept at different levels of moisture saturation. In this study water absorption by sclerotia was measured in Fargo Silty clay, Sandy loam and Aylmer-Bantry fine sand set at 100, 75, 50 and 25% of soil saturation. The role of sclerotial moisture content was evaluated on sclerotia kept at 100%, 70-80%, 40-50%, and 20-30% of their maximum hydration level. Sclerotial hydration levels were kept constant using cool mist humidifiers. Smaller sclerotia absorbed water at a significantly faster rate ($\alpha=0.05$) than larger sclerotia in all moisture treatments. When buried in soil, small sclerotia reached their water saturation point within 5 hours irrespectively of the soil texture and its moisture content. Medium-sized and large sclerotia took three and five times longer, respectively, to reach it. A significant interaction ($\alpha=0.05$) between sclerotial moisture content and sclerotial size was observed for both, carpogenic germination and the average number of apothecia produced per sclerotia. Optimum sclerotial moisture content for carpogenic germination was at 100% hydration. At 70 to 80% hydration, <15% germination was observed among medium and small sclerotia, but large sclerotia failed to germinate. No germination was observed when sclerotia were kept at less than 50% hydration. The effect of wider fluctuations on sclerotial moisture content is being investigated.

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Resistance to sclerotinia head rot in sunflower hybrids

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ABSTRACT:

A total of 100 sunflower hybrids have been evaluated for reaction to sclerotinia head rot infections under field conditions with misting system at the Agriculture and Agri-Food Canada research Station, Morden Manitoba in 2008 and 2009. All hybrids were artificially inoculated twice, at early and late flowering with 10 days apart, using ascospores suspended in water. A wide range of reaction was observed among sunflower hybrids from the 20% infected plants (resistant hybrids) to the 60% infected plants (susceptible hybrids). Several hybrids were consistently showing resistant reactions over the two year study and are considered moderately resistant. Such hybrids can be recommended for production in areas where epidemics of sclerotinia head rot are expected in the Northern Central Plains of USA and Canada.

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Necrosis Activities Produced by *Sclerotinia sclerotiorum* Independent of Oxalic Acid

Jeffrey A. Rollins, University of Florida, Gainesville, FL

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

ABSTRACT:

The specific objective of this project is to determine the roles and requirements for oxalic acid and other factors in the disease process on sunflower, dry bean, canola, and pulse crops. Oxalic acid has been implicated as an essential pathogenicity factor or as an important virulence factor in a number of independent reports. These studies have ranged from physiological studies correlating exogenous oxalic acid treatment with symptom development to inoculation studies using natural isolates or mutants of *S. sclerotiorum* that produce little or no oxalic acid. If we are to control Sclerotinia diseases we must understand the specific roles of various pathogen-produced factors using systematic, genetically defined and robust methods. As such, this study use a genetically-defined mutant in the oxalate biosynthetic pathway to determine if there are other biochemical compounds produced by *S. sclerotiorum* that can condition host susceptibility. We recently created this mutant and found that it produces no detectable oxalic acid under any tested condition *in vitro* or *in planta*. Despite the complete lack of oxalic acid production, this mutant is still capable of infecting plants and causing disease symptoms. We are currently working to identify the biochemical nature of the activity that allows *S. sclerotioum* to produce symptoms on hosts despite the lack of oxalic acid production. To date we have used culture filtrates from the wild type and the oxalate minus mutant relative to oxalic acid solutions and media controls to demonstrate that the observed necrosis activity is distinct from the effects of oxalic acid and can be produced in the absence of oxalic acid. We are continuing studies to determine activity range of these filtrates using a variety of hosts and the biochemical nature of the activity through biochemical purification and analysis methods.

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

Howard F. Schwartz and Mark A. Brick, Colorado State University, Fort Collins, CO

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

ABSTRACT:

This project during 2009 – 2012 will investigate the roles of cultural practices and timely application of a fungicide in reducing damage from *Sclerotinia sclerotiorum* to *Phaseolus vulgaris* cultivars when grown under different irrigation systems in Colorado. During 2009, this project conducted a replicated field trial in a white mold-infested nursery and grower fields to investigate the role and value of cultural practice modification within an Integrated Pest Management context that compared the effects of fertilizer rates and fungicides when promising varieties were grown under varying irrigation systems on grower fields and an experiment station. We encountered low white mold pressure in fields with a history of the disease apparently due to delayed plantings (early-spring rains) which delayed flowering until late July when weather conditions were warm and dry. Yields of the 4 entries averaged 2015 lb/acre (37.9 g/100 seed) at the research station and 3299 lb/acre (36.7 g/seed) at the better commercial field in the absence of white mold and with trace presence of bacterial diseases. When combined over locations, yield ($P < 0.01$) and seed size ($P < 0.001$) differences between entries were significant. The study will be repeated during 2010 at an experiment station in Colorado (furrow irrigated at CSU-Fort Collins), and under grower conditions (sprinkler and/or furrow irrigated) in Colorado. Results will be shared with colleagues and growers via progress reports, refereed publications, extension releases, web sites, field days, and meetings. Agronomic and chemical (fertilizer, fungicide) implications from this IPM approach will be applicable to other host cropping systems affected by foliar phases of white mold.

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

Shree P. Singh, Henry Terán & Laura Crane, University of Idaho, Kimberly, ID & Howard F. Schwartz & Kristen Otto, Colorado State University, Fort Collins, CO

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

ABSTRACT:

Between 2002 and 2010 we completed sequential screening, in the greenhouse and field for resistance to white mold (WM), 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 secondary gene pool (*P. coccineus*, *P. costaricensis*, and *P. polyanthus*). Two highly WM resistant IBL (VCW 54 and VCW 55) derived from the congruity-backcross between ICA Pijao and *P. coccineus* accession G 35172 were identified. Seed of VCW 54 and VCW 55 increased and both were released by the Idaho and Colorado Agricultural Experiment Stations and registered in the Journal of Plant Registrations. VCW 54 possesses the highest level of WM resistance ever reported while VCW 55 has a similar level of resistance as previously reported in *P. coccineus* derived IBL. We developed one WM resistant IBL (VRW 32), the first of its kind, derived from the recurrent-backcross of ICA Pijao with *P. costaricensis* accession S 33720. Seed of VRW 32 is being increased for release and registration. We introduced and screened 81 IBL derived from *P. coccineus* accession G 35006 from University of Puerto Rico-Mayaguez. One IBL with high level of WM resistance was identified. We also developed and screened 216 IBL between pinto UI 320 and *P. coccineus* accession PI 433246 and 266 IBL between pinto Othello and *P. coccineus* accession PI 439534. Five IBL with high levels of resistance derived from PI 433246 were identified. The nine IBL with WM introgressed from the secondary gene pool species need to be compared with other IBL and WM resistant germplasm. Also, complementation or lack thereof among the IBL and other WM resistant germplasm needs to be determined and resistance from across *Phaseolus* species need to be pyramided and introgressed in common bean cultivars.

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Comprehensive linkage map of white mold resistance QTL in common bean

Marilyn Soule and Phillip Miklas
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Funded Plan(s) of Work: Genetic characterization of scarlet-runner bean derived resistance to white mold in common bean and previous QTL studies funded by SI.

ABSTRACT:

Inheritance of resistance to white mold is being characterized for different sources but associations among all the QTL identified has not been fully illustrated. Our objectives in the past have been to characterize partial resistance from different sources and then integrate the identified resistance-QTL on the *Phaseolus* genetic linkage map. The current objective is to provide a comprehensive linkage map for all the QTL for white mold resistance in bean reported to date. We integrated 37 QTL for partial resistance onto a single linkage map. These 37 QTL coalesced into 16 regions across nine linkage groups. Seven QTL, one each on LG 2, 3, and 9, and two each on 7 and 8, were identified in more than one population and detected by more than one test. Four of these QTL on LG 2, 7 (2) and 8 were further validated in marker-assisted selection studies. This map provides a blueprint of resistance genes available to breeders, will facilitate marker-assisted breeding, and provides a framework for integrating and interpreting future QTL.

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Production of double haploid *Brassica napus* L. line with improved resistance 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:

A microspore culture technique was used to attempt production of double haploid lines from *Brassica napus* plant introduction materials Ames 26628, PI458939, PI458940; and F₁ hybrids from PI458939 x Ames 26628, PI458940 x Ames 26628, Westar x PI169075, and Westar x PI169080. These materials had been identified, in a project previously funded by the Sclerotinia Initiative, as having high levels of resistance against Sclerotinia stem rot. The procedure was conducted in the facilities of the Plant Science department at North Dakota State University. The quality and quantity of embryos obtained at the start of the experiments varied significantly among genotypes and therefore the number of haploid plantlets produced was also very variable. Ames 26628 was the accession that produced the highest number of normal embryos and haploid plantlets, and therefore was the accession that was first screened for resistance. A total of 154 Ames 26628 haploid plants were transplanted into larger pots and eventually moved to greenhouse rooms. At flowering, 104 plants were inoculated with isolate WM031 of *S. sclerotiorum* using the petiole inoculation technique. Lesion development and the number of dead plants were estimated daily starting three days after inoculation. Fourteen days after inoculation, 26 plants were alive and had either small lesions (<14 mm in size) or essentially no symptoms, while susceptible plants had died within 5 to 7 days from inoculation. A standardized Area Under Disease Progress Curve was calculated using lesion development for each plant and the reactions were categorized as resistant, moderately resistant or susceptible. Chromosome doubling was obtained by immersing roots of the most resistant plants and some moderately resistant and susceptible haploids in 8.5 µM of colchicine. Plants immersed for 1.5 hours had a – 62% success rate, whereas those immersed for three hours had a 76% success rate. True seeds are being collected from some of the most resistant plants. Stem cutting and micropropagation techniques are being used to reproduce the escaped haploids and resistant double haploids that failed to produce true seeds. The resistant materials will be used to develop breeding populations.

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Enhancing soybean for resistance to Sclerotinia stem rot

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

ABSTRACT:

Sclerotinia stem rot is an economically important soybean disease in the North Central states of US. Using resistant cultivars is the most cost-effective method to control the disease. The objectives of this research are to further evaluate advanced breeding lines for yield and disease resistance and release the best lines as germplasm or varieties, to evaluate seven populations of F₄ derived lines with resistance from five new resistant plant introductions (PIs) for resistance to Sclerotinia stem rot and agronomic traits, and to determine if reported quantitative trait loci (QTLs) associated with resistance to Sclerotinia stem rot are also associated with the resistance in the five new resistance sources.

In 2009, 59 lines selected from over 600 lines derived from crosses with Skylla and AxN-1-55 as the resistant parents were tested for yield in our preliminary yield trials at two locations. Twenty two of the 59 lines were among the top 20% high yielding lines of the 120 entries in the test. All 22 lines were higher yielding than the high-yield check IA2021 in the test.

The seven populations with 392 F_{4:6} lines derived from five new resistant PIs were evaluated for resistance to Sclerotinia stem rot with the drop-mycelium method in 2009. Significant ($p < 0.05$) differences were found among lines within populations and between populations. The 392 lines are being genotyped using simple sequence repeat (SSR) markers. SSR markers that flank the 33 reported resistance QTLs were selected from the integrated soybean linkage map. Markers that showed polymorphisms across the parental combinations are being used to genotype the respective populations. The marker data and disease resistance data will allow us to determine if and which reported QTLs confer resistance in the new resistant PIs.

Six lines selected from the 392 lines were tested in our advanced yield trials at six locations throughout Michigan in 2009. Three of the six lines were ranked the top three high yielding lines among the 84 entries in the test. All three lines were significantly higher yielding than the high-yield check IA2021 in the trial.

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