National Sclerotinia Initiative

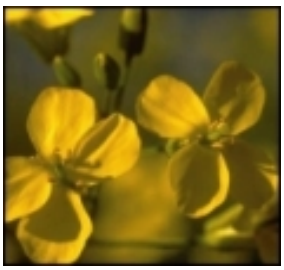
USDA-ARS

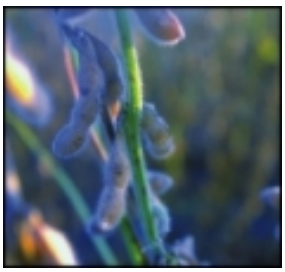
National Sclerotinia Initiative

2015 Annual Meeting

Bloomington, MN

January 21-23, 2015



13th Annual National Sclerotinia Initiative Meeting

January 21-23, 2015

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

AGENDA

National Sclerotinia Initiative
2015 Annual Meeting

January 21-23, 2015

Wednesday – January 21, 2015

6-8 pm	Poster Session/Reception *(posters are kept on display throughout the meeting)*	**Cortland**

Thursday – January 22, 2015

7:15 am	Registration/Continental Breakfast	**Cortland**
8:15 am	Welcome, Introductions & Meeting Charge – **Bill Kemp, USDA-ARS, Fargo, ND**	
8:25 am	Welcome & Update from the Plains Area – **John McMurtry, USDA-ARS, Fort Collins, CO**	
8:35 am	ARS Office of National Programs Update – **Roy Scott, USDA-ARS, Beltsville, MD**	
8:45 am	Genomic analysis of hybridization and introgression of wild disease-resistance traits into domesticated lineages - **Nolan Kane, Assistant Professor of Ecology & Evolutionary Biology, University of Colorado at Boulder, Boulder CO**	
10:00 am	Discussion Break	**Ballroom Foyer**
	Sclerotinia Research Activities – Session 1 **Fireside** **Moderator – John Finer, The Ohio State University, Wooster, OH**	
10:30 am	Sources of white mold resistance derived from wide crosses in common bean and progress in characterization of relevant pathogen isolates (Abstract p. 29; Poster #12) – **James Steadman, University of Nebraska, Lincoln, NE**	
10:45 am	White mold resistance-QTL: Identification, interactions, and fine mapping in common bean (Abstract p. 35; Poster #4) – **Phil Miklas, USDA-ARS, Prosser, WA; James Myers, Oregon State University, Corvallis, OR; & Phil McClean, North Dakota State University, Fargo, ND**	
11:15 am	Metabolomic profiles associated with resistance to *Sclerotinia sclerotiorum* in dry edible beans (Abstract p. 25; Poster #8) – **Adam Heuberger & Mark Brick, Colorado State University, Ft. Collins, CO**	

11:30 am	Pyramiding QTL for white mold resistance in Mesoamerican beans (Abstract P. 27; Poster #17) – **James Kelly, Michigan State University, East Lansing, MI**	
11:45 am	Working Lunch	**Cortland**
	Sclerotinia Research Activities – Session 2 **Moderator – James Steadman, University of Nebraska, Lincoln, NE**	**Fireside**
1:15 pm	Discovery and use of novel sources of resistance to head rot and stalk rot in sunflower and studies of Asteraceae genera stimulating myceliogenic germination (Abstract p. 32; Poster #25) **– Gerald Seiler, USDA-ARS, Fargo, ND**	
1:30 pm	Transferring Sclerotinia resistance genes from wild *Helianthus* species into cultivated sunflower (Abstract p. 26; Poster #22) – **Chao-Chien Jan, USDA-ARS, Fargo, ND**	
1:45 pm	Using genomic selection to optimize prediction of Sclerotinia and agronomic phenotypes for more efficient breeding (Abstract p. 34; Poster #27) – **Brent Hulke, USDA-ARS, Fargo, ND**	
2:00 pm	Identification of major genes-QTL for Sclerotinia resistance in cultivated sunflower and wild *Helianthus* (Abstract p. 21; Poster #19) – **Lili Qi, USDA-ARS, Fargo, ND**	
2:15 pm	Improved head rot resistance screening in sunflowers and impacts & implications of Sclerotinia infection timing in dry bean, soybean, and sunflower (Abstract p. 24; Poster # 3, 21) – **Michael Wunsch, North Dakota State University, Carrington, ND**	
2:30 pm	Use of a transformation system in sunflower for Sclerotinia resistance studies (Abstract p. 33; Poster #26) – **John Finer, The Ohio State University, Wooster, OH**	
2:45 pm	Break & Poster Session	**Cortland**
	Sclerotinia Research Activities – Session 3 **Moderator – Luis del Rio, North Dakota State University, Fargo, ND**	**Fireside**
3:15 pm	Strategies to identify functionally significant defense genes against *Sclerotinia sclerotiorum* (Abstract p. 30; Poster #9) – **Daina Simmonds, Agriculture and Agri-Food Canada, Ottawa, ONT**	
3:30 pm	Identifying and verifying genes for defense to *Sclerotinia* (Abstract p. 23; Poster #7) – **Steven Clough, USDA-ARS, Urbana, IL**	
3:45 pm	Complex genetics underlying natural variation in soybean resistance to white mold (Abstract p. 14; Poster #15) – **Dechun Wang, Michigan State University, East Lansing, MI**	

4:00 pm	Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial *Glycine latifolia* (Abstract p. 17; Poster #5) **– Leslie Domier, USDA-ARS, Urbana, IL**	
4:15 pm	Wrap-up & Adjourn (Dinner on your own)	

Friday – January 23, 2015

7:00 am	Steering Committee Breakfast Meeting	**Executive Conference**
7:15 am	Continental Breakfast	**Cortland**
	Sclerotinia Research Activities – Session 4 **Moderator – Berlin Nelson, North Dakota State University, Fargo, ND**	**Fireside**
8:15 am	Identification of candidate resistance genes to *Sclerotinia sclerotiorum* in canola using next generation sequencing (Abstract p. 18) – **Luis del Rio, North Dakota State University, Fargo, ND & Rubella Goswami, DuPont Crop Protection, Newark, DE**	
8:30 am	Identification of quantitative trait loci associated with resistance to Sclerotinia stem rot in canola (Abstract p. 22; Poster #20) – **Luis del Rio, North Dakota State University, Fargo, ND**	
8:45 am	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics assisted breeding (Abstract p. 16; Poster #11) – **Martin Chilvers, Michigan State University, East Lansing, MI**	
9:00 am	Characterization and validation of two distinct mechanisms for partial resistance to *Sclerotinia sclerotiorum* in pea (Abstract p. 13; Poster #14) – **Kevin McPhee, North Dakota State University, Fargo, ND & Lyndon Porter USDA-ARS, Prosser, WA**	
9:15 am	Applications of linkage disequilibrium decay to infer outcrossing in natural populations of the homothallic fungus *Sclerotinia sclerotiorum* (Abstract p. 11; Poster #10) – **Weidong Chen, USDA-ARS, Pullman, WA**	
9:30 am	Discussion Break	**Ballroom Foyer**
10:00 am	High density genotyping of a diverse population of *Sclerotinia sclerotiorum* (Abstract p. 19; Poster #6) – **Robert Brueggeman & Berlin Nelson, North Dakota State University, Fargo, ND**	
10:15 am	Evaluation of native and non-native phytoalexins in suppressing *in vitro*, *in vivo*, and *in planta* growth of *Sclerotinia sclerotiorum* (Abstract p. 15; Poster #16) – **Michelle Pawlowski, University of Illinois & Glen Hartman, USDA-ARS, Urbana, IL**	

10:30 am	Synergistic enhancement of resistance to *Sclerotinia sclerotiorum* (Abstract p. 20; Poster #18) – **Zhonglin Mou & Jeffrey Rollins, University of Florida, Gainesville, FL**	
	Sclerotinia Initiative Research: The next steps **Moderator – Bill Kemp, USDA-ARS, Fargo, ND**	**Fireside**
10:45 am	***Guest Speaker*** Strategic Planning & Reporting Progress – **Rich Wilson, USDA-ARS, Office of National Programs–Retired, Raleigh, NC**	
11:15 am	Strategic Plan Discussion – Writing Team Input/Revisions; Annual Reports	
11:30 am	Agreements Update	
11:45 am	Working Lunch	**Cortland**
1:00 pm	Assignment of Additional Tasks & Wrap-up of Initiative Business	
2:00 pm	Adjourn (Travel Safely!)	

National Sclerotinia Initiative Poster Session

January 21-23, 2015
Cortland Room
Crowne Plaza Hotel & Suites

Epidemiology & Disease Management	
Poster No.	Title Authors
1	Carpogenic germination of Sclerotinia minor T. O'Malley, F. Hay, J. Scott, D. Gent, R. Shivas, S. Pethybridge
2	Development of enhanced strategies to monitor and manage Sclerotinia on lima bean K.L. Everts, R. Selleck
3	Impact and implications of timing of Sclerotinia disease onset in dry beans, soybeans, and sunflowers M. Wunsch, P. Gautam, M. Schaefer, B. Kraft

Genomics	
Poster No.	Title Authors
4	Association mapping of white mold resistance in the Middle American dry bean gene pool O.B. Oraguzie, S.M. Moghaddam, P.E. McClean, P.N. Miklas
5	Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial *Glycine latifolia* S. Chang, G.L. Hartman, L.L Domier
6	Identification of candidate Sclerotinia sclerotiorum virulence genes utilizing genotype-by-sequencing and association mapping R. Brueggeman, J. Richards, C. Qiu, L. Aldrich-Wolfe, S. Jain, J. LeBoldus, B.D. Nelson, Jr.
7	Identifying and verifying genes for defense to Sclerotinia L. Blahut-Beatty, L. Koziol, D. Simmonds, M. Belaffif, M. Hudson, S.J. Clough
8	Metabolic phenotypes associated with resistance to *Sclerotinia sclerotiorum* in common bean A.L. Heuberger, M.A. Brick, H.F. Schwartz, F.M. Robison, J.E. Prenni
9	Strategies to identify functionally significant defense genes against *Sclerotinia sclerotiorum* L. Blahut-Beatty, L. Koziol, A. Itaya, B. Calla, L. Buchwaldt, D.J. Neece, S.J. Clough, D. Simmonds

Pathogen Biology & Development	
Poster No.	Title Authors
10	Applications of linkage disequilibrium decay in inferring outcrossing natural populations of the homothallic fungus *Sclerotinia sclerotiorum* R.N. Attanayake, V. Tennekoon, D.A. Johnson, L.D. Porter, L. del Rio-Mendoza, D. Jian, W. Chen
11	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics assisted breeding : Delayed cell death mediates partial resistance to *Sclerotinia sclerotiorum* in *Pisum Sativum* (pea) P. Santos, X. Zhuang, C. Foster, J. Wang, M. Chilvers, K. McPhee, T. Coram
12	Sources of white mold resistance derived from wide crosses in common bean and progress in characterization of relevant pathogen isolates R. Jhala, R. Higgins, J.R. Steadman
13	Transfection of Sclerotinia sclerotiorum with cloned Sclerotinia sclerotiorum hypovirus 2 significantly reduces virulence of the fungus S. Marzano, H.A. Hobbs, B.D. Nelson, G.L. Hartman, D.M. Eastburn, N.K. McCoppin, L.L. Domier

Variety Development/Germplasm Enhancement	
Poster No.	Title Authors
14	Characterization and validation of two distinct mechanisms for partial resistance to *Sclerotinia sclerotiorum* in pea R. Ashtari Mahini, K. McPhee
15	Complex genetics underlying natural variation in soybean resistance to white mold Z. Wen, R. Tan, M. Chilvers, D. Wang
16	Evaluation of native and non-native phytoalexins in suppressing in vitro, in vivo, and planta growth of Sclerotinia sclerotiorum M.L. Pawlowski, C.B. Hill, G.L. Hartman
17	Identification and validation of QTL for white mold in pinto bean V. Hoyos-Villegas, W. Mkwaila, E. Wright, J.D. Kelly
18	Identification of key regulators of host resistance and pathogen virulence in the *Sclerotinia sclerotiorum*-Arabidopsis pathosystem C. Wang, J.A. Rollins, Z. Mou
19	Identification of major genes-QTL for Sclerotinia resistance in cultivated sunflower and wild *Helianthus* Z. Talukder, Y. Long, T. Gulya, C. Block, G. Seiler, L. Qi

Variety Development/Germplasm Enhancement	
Poster No.	Title Authors
20	Identification of QTL associated with resistance to Sclerotinia stem rot in canola K. Chittem, D. Horvath, J.V. Anderson, L.E. del Rio Mendoza
21	Optimizing head rot resistance assessment methods in sunflowers M. Wunsch, P. Gautam, L. Besemann, M. Schaefer, B. Kraft
22	Progress on transferring Sclerotinia resistance genes from wild perennial *Helianthus* species into cultivated sunflower Z. Liu, J. Zhang, X. Cai, G.J. Seiler, K.Y. Rashid, C.C. Jan
23	Sclerotia number and cuticle thickness as tools to evaluate white mold disease in common bean H. Arkwazee, J.R. Myers
24	Screening phaseolus iunatus for resistance to Sclertoina scerltiorum R. Selleck, E. Garver Ernest
25	Update on the discovery and use of novel sources of head and stalk rot resistance in sunflower and studies of asteraceae genera stimulating myceliogenic germination G.J. Seiler, T. Gulya, B. Hulke, M. Wunsch, R. Harveson, K. Olander, S. Markell, M. Gilley, B. Flett
26	Use of a transformation system in sunflower for *Sclerotinia* resistance studies Z. Zhang, J. Finer
27	Using genomic selection to optimize prediction of Sclerotinia and agronomic phenotypes for more efficient breeding B.S. Hulke, D. Horvath, N.C. Kane

Applications of linkage disequilibrium decay in inferring outcrossing in natural populations of the homothallic fungus *Sclerotinia sclerotiorum*

Renuka Attanayake, Dennis Johnson, Department of Plant Pathology, Washington State University, Pullman, WA; Vidhura Tennekoon, Department of Economics, University of Oklahoma, Norman, OK; Luis del Rio-Mendoza, Department of Plant Pathology, North Dakota State University, Fargo, ND; Daohong Jiang, Department of Plant Protection, Huazhong Agricultural University, Wuhan, Hubei, People's Republic of China; Lyndon D Porter and Weidong Chen, USDA-ARS, Grain Legume Genetics and Physiology Research Unit, WA

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

ABSTRACT:
Fungal species could be heterothallic or homothallic in sexual reproduction. Heterothallism promotes outcrossing, whereas homothallism is thought to favor selfing. The occurrence and frequency of outcrossing in homothallic fungal species in nature is an unresolved question. For the homohallic species *Sclerotinia sclerotiorum*, its populations have traditionally been considered as having clonal genetic structure. However, both clonal and recombining population structures have been reported in *Sclerotinia sclerotiorum* populations around the world using analyses of index of association on independent molecular markers. In using multilocus index of association to infer recombination among microsatellite alleles, high mutation rates confound the estimates of recombination. To distinguish high mutation rates from recombination to infer outcrossing, 8 population samples comprising 268 *S. sclerotiorum* isolates from widely distributed agricultural fields were genotyped for 12 microsatellite markers, resulting in multiple polymorphic markers on three chromosomes. Each isolate was homokaryotic for the 12 loci. Pairwise linkage disequilibrium (LD) was estimated using three methods: Fisher's exact test, index of association (I_A) and Hedrick's D**'**. For most of the populations, pairwise LD decayed with increasing physical distance between loci in two of the three chromosomes. Therefore, the observed recombination of alleles cannot be simply attributed to mutation alone. Different recombination rates in various DNA regions (recombination hot/ cold spots) and different evolutionary histories of the populations could explain the observed differences in rates of LD decay among the chromosomes and among populations. The majority of the isolates exhibited mycelial incompatibility, minimizing the possibility of heterokaryon formation and mitotic recombination. Thus, the observed high intrachromosomal recombination is due to meiotic recombination, suggesting frequent outcrossing in these populations, supporting the view that homothallism favors universal compatibility of gametes instead of traditionally believed selfing in *S. sclerotiorum*. Frequent outcrossing facilitates emergence and spread of new traits such as fungicide resistance, increasing difficulties in managing Sclerotinia diseases.

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Carpogenic germination of *Sclerotinia minor*

Tom O'Malley, Frank Hay, Jason Scott, Tasmanian Institute of Agriculture, University of Tasmania, Burnie, Tasmania, Australia; David Gent, USDA-ARS, Corvallis, OR; Roger Shivas, Department of Employment, Economic Development and Innovation Queensland, Brisbane, Queensland, Australia & Sarah Pethybridge, Cornell University, Geneva NY

ABSTRACT:
Pyrethrum (*Tanacetum cinerariifolium*) is grown as a broad-acre field crop in Tasmania, Australia for the production of pyrethrins, which are extracted from the seeds. White mold is one of the major diseases affecting productivity of plants through flower disease, and crown rot affecting green leaf area and regrowth following harvest. Sclerotinia crown rot can be caused by *Sclerotinia sclerotiorum* and *S. minor*. Sclerotinia flower blight has traditionally been associated with only *S. sclerotiorum*, as evidence for *S. minor* sclerotia germinating carpogenically and/or resultant disease following ascosporic infection is rare. However, during 2007 to 2009, isolates with morphological characteristics consistent with *S. minor* were obtained from surface-sterilized pyrethrum flowers collected from fields. The isolation frequency of *S. minor* from flowers in 2007, 2008, and 2009 was 15.8%, 5%, and 1.4%, respectively. Moreover, sclerotia with apothecia, consistent in size with the formal description of *S. minor*, were observed underneath the canopy on the soil in one field at early flowering. Colonies produced from individual ascospores from this isolate were identified as *S. minor* according to morphological characteristics. This identification was corroborated by phylogenetic analysis using the internal transcribed spacer (ITS) region and comparing previously published ITS sequences of *S. minor* and other *Sclerotinia* species with the pyrethrum isolates. Further confirmation of the identity of the pyrethrum isolates was obtained from using species-specific primers. Pathogenicity of *S. minor* to pyrethrum flowers was confirmed in the greenhouse using ascosporic inoculum. Symptoms of infection were withered, necrotic and/or abscised ray florets, and light-brown and pitted disk florets. These symptoms were indistinguishable from those caused by *S. sclerotiorum*. To the best of our knowledge, this is the first report of *S. minor* causing flower disease of pyrethrum. It is also one of only a handful of examples of carpogenic germination of *S. minor*. This finding adds to our knowledge of the biology of *S. minor* and has implications for the management of the white mold complex affecting pyrethrum and potentially other crops.

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Characterization and validation of two distinct mechanisms for partial resistance to *Sclerotinia sclerotiorum* in pea

Kevin McPhee and Rahil Ashtari Mahini, North Dakota State University, Fargo, ND

Funded Plan of Work: Characterization and validation of two distinct mechanisms for partial resistance to *Sclerotinia sclerotiorum* in pea

ABSTRACT:
Sclerotinia sclerotiorum (Lib.) de Bary, the causal agent of white mold, can cause severe yield losses in pea. Partially resistant pea accessions have been previously identified. Utilization of these accessions in breeding programs is challenging due to poor knowledge of genes involved in resistance. Previous research established a skeletal genetic map of the pea genome using a population of 190 F_2 plants developed from the cross 'Lifter'/PI240515. While phenoptyping the population it became clear that stem morphology was potentially introducing some bias into disease development. The recent research efforts were aimed at clarifying this impact and establishing a method to overcome this bias. A detached stem assay was established to more accurately assess the rate of lesion growth and the interference of the node on lesion transmission. Two recombinant inbred lines (RIL) have now been established and are available for phenotyping. Additional seed increase will be performed in 2015. In addition to the RIL populations, the Pisum single plant core collection was increased in 2014 and the seed is available for phenotyping using the whole plant and detached stem assay protocols.

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Complex genetics underlying natural variation in soybean resistance to white mold

Zixiang Wen, Ruijuan Tan, Martin Chilvers, and Dechun Wang
Department of Plant, Soil and Microbial Sciences
Michigan State University

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

ABSTRACT:
White mold caused by *Sclerotinia sclerotiorium* (Lib.) deBary is one of most devastating soybean disease worldwide. To dissect the genetic architecture of resistance to the disease in soybean, two independent association panels, consisting of 432 elite soybean cultivars and 408 soybean plant introductions (PIs), respectively, were evaluated for white mold resistance in disease nursery and greenhouse. The two association panels were genotyped with 52,041 single nucleotide polymorphisms (SNPs). Genome-wide association mapping was carried out using a mixed linear model that accounted for population structure and relatedness. A total of 15 and 12 loci underlying white mold resistance were identified from disease nursery test and greenhouse evaluations, respectively. Among these identified loci, only one locus was observed in both the disease nursery and the greenhouse evaluations. No matter which environment the two panels are tested in, most of loci identified from PI panel were different than those observed in elite cultivars panel. These results indicated that soybean has different genetic mechanism of white mold resistance under field and greenhouse environments. Additionally, soybean PIs could be important resources to expand the allelic pool for developing soybean cultivars with durable resistance against white mold.

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Evaluation of native and non-native phytoalexins in suppressing *in vitro*, *in vivo*, and *in planta* growth of *Sclerotinia sclerotiorum*

Michelle L. Pawlowski, Curtis B. Hill, and Dr. Glen L. Hartman
University of Illinois, Urbana, IL
USDA-ARS

Funded Plan of Work: Evaluation of native and non-native phytoalexins in suppressing *in vitro*, *in vivo*, and *in planta* growth of *Sclerotinia sclerotiorum*

ABSTRACT:
Sclerotinia stem rot has been a major threat to soybean production worldwide. Partial resistance found in crop germplasm has been only marginally effective in managing diseases caused by *Sclerotinia sclerotiorum*. Genetic manipulation of the innate defenses in plants may offer additional sources of resistance. Phytoalexins are important components of plant innate defenses and genetic manipulation resulting in increased or decreased levels of phytoalexin accumulation in plant tissues has been shown to significantly impact plant resistance. Resveratrol and pterostilbene are phytoalexins native to *Vitis vinifera* and other plant species that have been well-studied for their antimicrobial activity. Transgenic expression of these phytoalexins in foreign hosts has shown to significantly reduce disease caused by several generalist pathogens. One objective of this research was to evaluate resveratrol and pterostilbene to determine their effectiveness in suppressing growth of *S. sclerotiorum*. Mycelial growth on stilbene-amended agar was measured at different concentrations of resveratrol and pterostilbene to find the minimum inhibitory concentrations that would inhibit growth by 50% (MIC_{50}). Pterostilbene at concentrations of 25 µg/ml and 18 µg/ml reduced mycelial growth by 53% and 50%, respectively. A dual concentration of 25 µg/ml pterostilbene with 100 µg/ml resveratrol was found to be most effective, reducing mycelial growth by 69%. Results from this study showed pterostilbene and resveratrol suppressed growth of *S. sclerotiorum*. This indicated that an approach to transform soybean to produce these non-native phytoalexins has the potential to reduce Sclerotinia stem rot disease. The response of soybean plants transformed to synthesize resveratrol and pterostilbene to *S. sclerotiorum* will be tested during the next year of this project.

Contact Information – Michelle L. Pawlowski, Dept. of Crop Sciences, University of Illinois, 1101 W Peabody Drive, Urbana, IL 61801; 847-302-8050; mpawlow4@illinois.edu

Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics assisted breeding: Delayed cell death mediates partial resistance to *Sclerotinia sclerotiorum* in *Pisum sativum* (pea)

Patrícia Santos, Xiaofeng Zhuang, Cliff Foster[1], Jie Wang and Martin Chilvers, Department of Plant, Soil and Microbial Sciences and [1]Great Lakes Bioenergy Research Center , Michigan State University, East Lansing, MI & Kevin McPhee, Department of Plant Sciences, North Dakota State University, Fargo, ND & Tristan Coram, Dow AgroSciences, LLC, Indianapolis, IN.

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

ABSTRACT:
The molecular bases of the interaction between two *Pisum sativum* cultivars and *Sclerotinia sclerotiorum* were investigated using RNAseq. Lesion development was delayed at least 12 hours in the partially resistant PI240515 cultivar compared with the susceptible cultivar Lifter. At 24 hpi, most of Lifter plants were lodged at the point of inoculation, while PI240515 stems were all still up right with small water soaked lesions. Accordingly, the expression profiles between cultivars were most dissimilar at 24 hpi. Based on this result, some highly differential expressed genes in inoculated PI240515 were selected for further analysis. When compared with the expression in susceptible Lifter, the up-regulated genes from PI240515 pointed to a reinforcement of cell walls with lignin and possible production of flavonoids to fight off *S. sclerotiorum* infection. Lignin quantification confirmed the higher deposition of lignin in the vascular system and interfascicular cambium cells of inoculated PI240515 plants ($P < 0.05$). Additionally, representatives of specifically expressed genes related to cell death and signaling in defense, were evaluated by RT-qPCR in both cultivars, and found to be significantly up-regulated in inoculated Lifter ($P<0.05$), when compared to PI240515, during the time-course. These results suggest that the extreme susceptibility observed in Lifter is a consequence of the massive cell death occurring during the infection, while in PI240515, the delayed and weaker transcript levels over time are consistent with a strategy of disease avoidance. Moreover, cluster analysis indicated JA signaling pathway activation in the inoculated PI240515, a typical defense response to a necrotrophic fungus.

In parallel, we have started to unveil the molecular, biochemical and structural features of nodal resistance occurring in pea. Preliminary results showed the occurrence of phenolic depositions in the xylem vessels at the internode and node of inoculated plants but not in the non-inoculated ones. Differences were observed between the susceptible and partially resistant lines, and this result might explain, to some extent, the differences observed in phenotypes of infected plants, previously reported.

Contact Information – Dr. Martin Chilvers, Dept. of Plant Soil and Microbial Sciences, Michigan State University, 578 Wilson Rd, CIPS 104, East Lansing, MI 48823; 517-353-9967; chilvers@msu.edu

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

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

Funded Plan of Work: Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial *Glycien latifolia*

ABSTRACT:
Sclerotinia stem rot (SSR) causes significant losses in soybean (*Glycine max*), but like other crop species, effective resistance has not been identified in soybean germplasm. Chromosomal loci have been associated with reduced susceptibility of soybean to SSR, but have had low heritability, and have not been incorporated into soybean breeding programs. However, selected accessions of *Glycine latifolia*, a wild perennial relative of soybean, show high levels of resistance to SSR. To identify loci associated with resistance to SSR in *G. latifolia*, populations of F_2 and F_5 plants were developed, used to generate high-density linkage maps, and evaluated for their sensitivities to oxalic acid, a pathogenicity determinant for *Sclerotinia sclerotiorum*, the causal agent of SSR. In the F_2 population, the phenotypic data for sensitivity to oxalic acid were too variable to be useful for gene mapping. Using the population of F_5 recombinant inbred lines however, major loci for tolerance to oxalic acid were discovered on *G. latifolia* linkage groups (LGs) 14 and 19. The loci on LGs 14 and 19 explained 26% and 27% of the phenotypic variation for the trait, respectively. The locus on *G. latifolia* LG 19, but not the locus on LG14, overlapped a previously described locus in soybean for resistance to SSR. This finding suggested that the soybean locus might condition partial resistance to SSR by reducing sensitivity to oxalic acid. The sequences of *G. latifolia* chromosomes corresponding to the genetic intervals on LGs 14 and 19 contained 96 and 68 predicted genes, respectively. These experiments demonstrated that it is possible to identify loci for agronomically important traits in wild perennial relatives of soybean that are not present in the soybean primary gene pool. Identification of the genes underlying tolerance to oxalic acid in *G. latifolia* will increase our understanding of the mechanisms of resistance to this destructive disease.

Contact Information – Dr. Leslie Domier, USDA-ARS, Department of Crop Sciences, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801; 217-333-0510; leslie.domier@ars.usda.gov

Identification of candidate resistance genes to *S. sclerotiorum* in canola using next generation sequencing

Kishore Chittem, and Luis del Río Mendoza, North Dakota State University, Fargo, ND, and Rubella S. Goswami, DuPont Crop Protection, Newark, DE and North Dakota State University, Fargo, ND

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

ABSTRACT:
The objective of this project has been to identify genes associated with resistance to Sclerotinia stem rot in canola through transcriptome sequencing. RNASeq experiment was conducted to study canola – *S. sclerotiorum* interaction using two doubled haploid lines NEP32 (susceptible) and NEP63 (resistant), derived from a *Brassica napus* PI accession. Previously, differential expression analysis was conducted using a Brassica 95k EST assembly as reference to identify canola genes differentially expressed during infection that could be associated to resistance. With the *B. napus* genome being made available recently, the data was reanalyzed using the published genome as reference. From this analysis, we have identified several *B. napus* genes that were differentially expressed when inoculated with *S. sclerotiorum* compared to non-inoculated conditions. Further from this analysis, we have also identified ~ 9000 splice variants and ~ 3400 novel transcripts. From the initial analysis, *B. napus* genes involved in defense response, signal transduction and immune response were found to be differentially expressed during the interaction with the pathogen compared to non-inoculated controls. Functional categorization and gene enrichment analysis of these differentially expressed transcripts, and further analysis to confirm the novel loci are being conducted. Further analyses by combining data from this project, and QTL data obtained from another project funded by the Initiative, we have identified potential candidate resistance genes within the five QTL regions, including a novel gene, which was exclusively expressed in resistant parent. Functional characterization of these identified candidate resistance genes will be conducted.

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Identification of Candidate *Sclerotinia sclerotiorum* Virulence Genes Utilizing Genotype-By-Sequencing and Association Mapping

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

ABSTRACT:
Sclerotinia sclerotiorum, one of the most important broadleaf crop pathogens in the United States, had not previously been genetically characterized. Utilizing a restriction associated DNA genotype-by-sequencing (RAD-GBS) method, we genotyped a diverse natural population of 120 *S. sclerotiorum* isolates collected from 22 hosts and 25 states. A total of 4,544 SNP markers located on 2,788 "sequence tags" were dispersed throughout the 38 Mb genome. This placed a SNP marker every 13.6 kb on average. The genotypic and corresponding phenotypic data collected for lesion length on dry bean, soybean, canola and sunflower for 71 of the isolates was used for association mapping (AM) analysis utilizing JMP genomics software. The significant marker trait associations (MTAs) were located at seventeen loci within the *S. sclerotiorum* genome. The MTAs had remarkable correlations common to both soybean and dry bean at fourteen loci. Five of the loci had strong MTAs (-log10(p) >4) and the remaining twelve loci associated with virulence had weaker associations (-log10(p) >3). Many of the virulence loci identified could be delimited to relatively small genomic regions containing a limited number of candidate virulence genes. We are currently conducting an RNAseq analysis on infected soybean and dry bean and anticipate that some of the virulence genes underlying the loci identified by the AM analysis will be differentially expressed. The combination of the AM analyses and RNAseq data should identify strong candidate genes providing support for their putative roles in virulence. These candidate genes will be targeted for post-transcriptional gene silencing via stable transformation of siRNA constructs in the pathogen. We will also transform the model host *Arabidopsis* with siRNAs constructs in an attempt to silence the candidate pathogen virulence genes by host-encoded siRNAs via host induced gene silencing (HIGS). Utilizing this new genetic tool we will be able to elucidate some of the secrets of this necrotrophic generalist's virulence genes, which will help in the development of strategies to manage this important pathogen.

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Identification of Key Regulators of Host Resistance and Pathogen Virulence in the *Sclerotinia sclerotiorum*-Arabidopsis Pathosystem

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

ABSTRACT:
This project is investigating the potential to use a newly identified *Arabidopsis thaliana* gene hypersusceptible to *S. sclerotiorum* (*HSS1*) and the pathogen encoded oxalate decarboxylase2 (ODC2) protein for engineering high levels of disease resistance in canola. In the first two years of funding, we completed fine mapping of the *hss1* mutation and confirmed the identity of the *HSS1* gene by transforming candidate genes into the *A. thaliana hss1* mutant. One gene, which encodes a subunit of the Mediator complex, was found to genetically complement the *hss1* mutant phenotype. The Mediator complex is emerging as a master regulator of plant immunity against pathogens, especially necrotrophic fungal pathogens, which underlines our discovery of the critical role of *HSS1* in basal resistance against *S. sclerotiorum*. We compared *hss1* with another Mediator subunit mutant *med8*, which is susceptible to the necrotrophic fungal pathogen *Botrytis cinerea* but not to *S. sclerotiorum*, and analyzed their transcriptome changes after *S. sclerotiorum* infection by microarray assays. Results showed that the *hss1* mutation has a much broader impact than *med8* on *S. sclerotiorum*-induced transcriptome changes, confirming that HSS1 is a key Mediator subunit required for basal resistance against *S. sclerotiorum*. In addition to the progress on identifying the *HSS1* gene, we have cloned the *S. sclerotiorum ODC2* gene into the T-DNA vector pCAMBIA1300S and have transformed Arabidopsis plants with Agrobacteria carrying the T-DNA vector. Single insertion homozygous transgenic plants have been identified and will be tested for resistance to *S. sclerotiorum*. Moreover, phenotypic characterization of *odc2* gene deletion mutants of *S. sclerotiorum* revealed an oxalic acid over-accumulation phenotype, consistent with its function as an oxalate decarboxylase. The identification of the *HSS1* gene represents a major step forward in identifying regulators of host defense to *S. sclerotiorum*. The microarray expression data likewise contributes significantly to our understanding of the defense systems regulated by *HSS1* and could be helpful in the future as markers for identifying crop germplasm with endogenous high-levels of *HSS1*-mediated resistance. Together, our identification of key regulators of host resistance (*HSS1*) and pathogen virulence (*ODC2*) will facilitate the use of an overexpression strategy to enhance host resistance.

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

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Funded Plan of Work: Identification of major genes-QTL for Sclerotinia resistance in cultivated sunflower and wild *Helianthus*

ABSTRACT:
In 2014, we completed a multi-year and multi-location field screening trial of a sunflower recombinant inbred (RIL) population for Sclerotinia stalk rot resistance. All 106 RILs derived from the cross between a maintainer line, HA 441 and a restorer line, RHA 439 along with the parents were evaluated in inoculated fields at multiple locations in North Dakota and Minnesota from 2012-2014. An analysis of variance for disease incidence (DI) scores revealed a highly significant genetic variation (p <0.001) for stalk rot resistance in the RIL population. The phenotypic variation of stalk rot resistance in RIL population across all environments showed a near normal distribution, indicating polygenic control of the trait. Transgressive segregation was observed for the trait in the RIL population, where some of the progeny showed more extreme phenotypes than either of the parents, suggesting that both the parents of the RIL population are contributing to the stalk rot resistance. Genotype × environment (G×E) interaction effect was also highly significant (p <0.001) for the trait. However, the variance component due to genotypes was higher compared to their respective variance component for the G×E interaction, indicating that the variation in DI was mainly contributed by the genotypes. Genotype-by-sequencing (GBS) using the next generation sequencing (NGS) technology was used for simultaneous discovery and genotyping of large numbers of single nucleotide polymorphism (SNP) markers for the HA 441/RHA 439 RIL population. We recently received the GBS data from Cornell University, which we are processing for further analysis and QTL mapping of stalk rot resistance.

Our efforts to introgress Sclerotinia resistance from wild annual species into cultivated sunflower are moving forward. In 2014, 23 BC_2F_4 families derived from *H. argophyllus*, *H. petiolaris*, and *H. praecox* were evaluated for stalk rot resistance in replicated field trials at Carrington and Grandin, North Dakota. Across environments, all the introgressed BC_2F_4 families showed significantly higher Sclerotinia resistance than both the susceptible checks, Cargrill 270 and HA 89 (36% DI). The level of resistance in the introgressed families was similar to the resistance check, Croplan 305 (9.6% DI). However, ten BC_2F_4 families, five each derived from *H. argophyllus* and *H. praecox* had significantly higher stalk rot resistance (0 to 3.6% DI) than the other resistant check, HA 441 (18.3% DI).

The development of the advance backcross (AB) population derived from *H. argophyllus* (PI 494573) is in progress. The seeds of the 150 BC_2F_5 single-seed descent lines were increased in the field in the summer of 2014. The BC_2F_6 lines will be evaluated for Sclerotinia resistance in multi-environment field screening trials in 2015. A new AB population derived from HA 89/*H. praecox* was advanced to the BC_2F_3 generation.

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Identification of quantitative trait loci associated with resistance to Sclerotinia stem rot in canola

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

ABSTRACT:
Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum* is considered an economically important disease of canola (*Brassica napus*). Resistance to SSR is considered a quantitative trait. To identify quantitative trait loci (QTL) associated with resistance against this disease an F_2 population was developed from the cross between doubled haploid lines NEP32, susceptible parent, and NEP63, resistant parent. Projects funded by the Sclerotinia Initiative identified the plant introduction and the development of doubled haploids from which NEP32 and NEP63 were selected. DNA samples were taken from all F_2 individuals and the parental lines and sent for sequencing to Cornell University. Analyses of data from the genotyping-by-sequencing (GBS) identified >20,000 SNP markers that were polymorphic between the two parents. A subset of these markers filtered with high stringency for missing data was used for initial map construction. The resulting linkage map has 1313 marker loci covering 7820 cM, with an average marker density of 5.9 cM per marker, which corresponds to 0.62 Mb of the genome. Phenotypic response of the progeny was evaluated following PIT method and quantified as area under disease progress curve (AUDPC). Composite interval mapping was performed to identify five significant QTL associated with resistance to SSR. These QTL were detected on chromosomes A1, A3, and C8, and account for 10 to 19% of phenotypic variation.

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Identifying and verifying genes for defense to Sclerotinia

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

ABSTRACT:
Our recent studies on soybean transcriptome response to *Sclerotinia sclerotiorum* infection (Mol. Plant Pathol. 15:563-573) and pure oxalic acid (Mol. Plant Pathol. 15:576-588) identified several thousand genes with altered expression compared to controls. Some of the differential gene expression suggests that oxalic acid might be chelating iron away from plant host proteins, which could play a role in the death of host cells. As the infection study looked at gene expression in plant leaf tissue from 12-36 hours, this tissue would include many cells that have already died, or were dying. To investigate earlier responses to infection, we conducted a new transcriptome study analyzing leaf tissue 4 and 8 hours post inoculation. For this new study, we used infected flowers (3 days following addition of 10 µl ascospores in 0.0006% Triton X-100) as inoculum on V4 leaflets, and collected an approximately 8 mm diameter leaf disc of the leaf tissue directly underneath the infected flower. We collected 6 discs from 6 individual plants for each replication and each time point, for a total of 3 replications. The plant hosts were the Sclerotinia-resistant, oxalate oxidase transgenic 80(30)-1 and its susceptible parent, AC Colibri. The control was soybean leaf discs from plants treated with a clean flower (3 days following addition of 10 ul 0.0006% Triton X-100, no Sclerotinia spores). Leaf discs were immediately frozen in liquid nitrogen, and then stored at -80°C until RNA was extracted. Total RNA was treated with DNaseI to remove traces of DNA, and sent to the Roy J. Carver Biotechnology Center at the University of Illinois where the samples were further cleaned of ribosomal RNA and made into RNA-Seq libraries using the Illumina TruSeq Stranded RNAseq Sample Preparation kit. Sample libraries were converted to cDNA and sequenced on an Illumina HiSeq2500 with 8 libraries per lane. From 16.3 to 21.2 million 150-base, paired-end sequences were recovered per sample and are currently being processed to determine differential expression.

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

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

ABSTRACT:
This project assessed the impact of timing of Sclerotinia disease onset on disease severity and seed yield and quality in sunflowers, dry beans and soybeans, and it facilitated the development of sunflower hybrids with reduced susceptibility to Sclerotinia head rot.

The timing of Sclerotinia disease onset affected disease levels and seed yields differently in dry beans, soybeans, and sunflowers. In dry beans, Sclerotinia was most severe and yield loss conferred by each percentage-point increase in Sclerotinia was highest when environmental conditions favorable for disease occurred at bloom initiation. In soybeans, Sclerotinia was most severe when environmental conditions favorable for Sclerotinia occurred at early bloom, but yield loss conferred by each percentage-point increase in Sclerotinia incidence was constant irrespective of the timing of disease development. In sunflowers, overall disease levels were highest when infection occurred at either at early or late bloom, depending on the length of time that the sunflowers remained at that growth stage, and yield loss conferred by each percentage-point increase in Sclerotinia incidence was highest when Sclerotinia developed at late bloom.

Delays in the timing of Sclerotinia disease onset may impact optimal fungicide usage differently in dry beans and soybeans. In dry beans, the yield gains conferred by controlling Sclerotinia with fungicides decreased sharply with delays in the timing of disease onset. In soybeans, the yield gains conferred by controlling Sclerotinia with fungicides showed little change with delays in the timing of disease onset and remained high even when conditions favorable for Sclerotinia did not occur until late bloom.

Sunflower heads with Sclerotinia head rot often shatter, and sunflower hybrids can differ sharply in their susceptibility to shattering. Results from a Sclerotinia head rot resistance screening nursery conducted in Carrington, ND in 2014 suggest that including susceptibility to shattering in disease assessments may confer modest gains in the ability to use disease assessments collected in screening nurseries to predict yield potential of sunflower hybrids under high Sclerotinia head rot pressure.

The project improved our understanding of the returns to fungicide applications that can be expected in dry beans and soybeans when environmental conditions favoring Sclerotinia do not develop until late bloom. The project also provided the first assessment of the seed yield and quality implications of resistance to shattering when sunflowers develop Sclerotinia head rot, and it provided a preliminary assessment of the gains that might be associated when susceptibility to shattering is included in Sclerotinia head rot disease assessments collected in resistance screening nurseries.

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Metabolic phenotypes associated with resistance to *Sclerotinia sclerotiorum* in common bean

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Funded Plan of Work: Metabolomic profiles associated with resistance to Sclerotinia sclerotiorum in dry edible beans

ABSTRACT:
Plant metabolic processes are being increasingly recognized as central to disease resistance. Metabolomic studies are complementary to standard genomic analyses and can help identify new mechanisms and targets that explain complex phenotypes. The Andean bean line A195 (resistant) and Sacramento (susceptible) were inoculated with *Sclerotinia sclerotiorum* isolate Ss20 for metabolite profiling of leaves at 16, 24, and 48 hours post inoculation (hpi). The resistant line A195 had noticeably less mycelia growth on detached leaves as early as 16 hpi. Metabolites from healthy tissue directly adjacent to the necrotic lesion were extracted with methanol:water (80:20) and detected using UPLC-MS and GC-MS non-targeted metabolomics workflows. Out of 14,500 detected compounds, 144 metabolites varied between A195 and Sacramento in response to inoculation with Sclerotinia (i.e. compared to mock inoculation controls). The metabolite variation indicated a multi-faceted molecular resistance response to *Sclerotinia* characterized by shifts in amino acids, ureides and phytoalexins. Some of the observed metabolic phenotypes were additionally observed in a stem-inoculation assay. The metabolic variation may explain physiological defense mechanisms that occur in A195 including changes to stomatal conductance and leaf surface pH. These data support that metabolite variation among resistant and susceptible lines may be directly associated with the rate of pathogen growth on bean leaves and stems. Future studies will identify gene sequences associated with the metabolic resistance response to *Sclerotinia* to facilitate marker-assisted selection in breeding.

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

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

ABSTRACT:
Cultivated sunflower lacks sufficient tolerance to Sclerotinia stalk and head rot. Field and greenhouse evaluations have indicated excellent stalk and head rot resistance in six interspecific amphiploids and wild perennials. The backcross progenies with 2n=34 chromosomes derived from amphiploids, hexaploid, and diploid perennials crossed with HA 410, HA 441 or HA 451 were evaluated in replicated trials in 2009-2014. In 2014, 559 families were tested for stalk rot at Carrington ND and Grandin, ND, and 163 families for head rot at Carrington, ND and Staples, MN. Families with better resistance than the recurrent parents were identified from the first retest, second retest and new selections in the different trials to be retested again or released as a germplasm. The results for the two retest groups derived from amphiploids and wild perennial species, including *H. californicus*, *H. maximiliani*, *H. nuttallii*, *H. giganteus*, and *H. grosseserratus*, indicated moderate to excellent resistance, further confirming successful gene introgression. More than 400 new early generation families of *H. hirsutus*, *H. salicifolius*, and *H. occidentalis* tested in replicated stalk rot field trials in 2014 suggested excellent stalk rot resistance. Seed was increased in the field for nearly 200 progeny families derived from different crosses in 2014. Progenies with high chromosome numbers derived from these new crosses were backcrossed with HA 410 or HA 451. These materials will further diversify the potential pool of resistance genes and increase the probability of identifying useful major resistance QTLs. New crosses using *H. strumosus*, *H. tuberosus*, *H. decapetalus*, and *H. simulans* were completed in 2013 and are in the process of being backcrossed with HA 410 at BC_1F_1. Further backcrossing will be needed to reduce the chromosome numbers, and to improve the pollen fertility and seed set. The genomic *in situ* hybridization technique (GISH) will be used to study meiotic chromosome pairing between chromosomes of wild perennial species and the cultivated line, and to characterize the alien chromosomes or segments of chromosomes in chromosome addition lines and selected resistant 2n=34 families. Sclerotinia head and stalk rot resistant germplasms derived from various perennial species will be released.

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Quantitative Trait Loci Analysis of Resistance to White Mold in Pinto Bean

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

ABSTRACT:
Pinto beans (*Phaseolus vulgaris*) are the most widely grown commercial class of dry beans in the U.S. and are among the most susceptible to white mold (*Sclerotinia sclerotiorum).* The goal of this project was to identify quantitative trait loci (QTL) associated with resistance to white mold and to other agronomic traits associated with disease avoidance in a pinto bean recombinant inbred line (RIL) population (AP630). The population was screened with the BARCBEAN6K_3 Genechip consisting of 5,398 single nucleotide polymorphism (SNP) markers. A total of 449 SNP markers were polymorphic in the population. The final map assembly resulted in 11 linkage groups corresponding to the 11 chromosomes of *P. vulgaris*. The two pinto bean RIL populations genotyped with SNP markers resulted in a linkage map of 1499 cM and a total of twenty-three QTL were identified in different years for the following traits; white mold disease incidence, seed yield, days to flowering, days to maturity, 100 seed weight, canopy height, and lodging. These QTL were located across all 11 chromosomes with LOD scores ranged from the threshold of 2.5 to 10.5. Four new QTL for yield under white mold pressure were identified on Pv03, Pv08, Pv07 and Pv11 and a major QTL for white mold was validated on Pv03.

The QTL identified on Pv03 appears to the same one identified by Miklas et al. (2007) for disease avoidance traits such as canopy porosity, and stay green trait. One of the parents AN-37 used to develop the AP630 population was identified as a resistant RIL in the previous study which suggests that moderate levels of white mold resistance have been transferred from navy bean ND88-106-04 into upright type II pinto beans. This is the first high density mapping study of white mold resistance with SNP markers in common bean. The markers associated with QTL for white mold resistance on Pv03 could be used for selection or as indicators of regions that warrant future genomic analysis to identify biological factors involved in conferring resistance to white mold in pinto bean.

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Sclerotia number and cuticle thickness as tools to evaluate white mold disease in common bean

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ABSTRACT:
Counting sclerotia and measuring cuticle thickness are two experiments that were conducted independently in summer 2013 and 2014 respectively, to find new tools to evaluate white mold *Sclerotinia sclerotiorum* Lib. de Bary in common bean *Phaseolus vulgaris* L. Under severe white mold disease, 60 common bean lines with varying degrees of resistance were screened. Counting sclerotia under bean canopy within limited area showed a clear association between numbers of sclerotia and disease severity or variety status (resistance and susceptible). Less sclerotia were produced under canopy under resistant varieties while high aggregation of sclerotia was noted under canopy of susceptible varieties. In a separate experiment, 14 lines of common beans were examined to measure the force that was required to penetrate their epidermal layers. Cuticle layer is considered one of the first defenses against many diseases that infect plants. Our result showed that resistant varieties required more force to penetrate the cuticle and epidermis of stems then for susceptible varieties. This result does not establish cause and effect but suggest a morphological trait that should be further explored.

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

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Collaborators: J. Kelly (MI), E. Withofs (Bel), M. Wunsch (ND), J. Myers (OR), P. Miklas (WA), H. Schwartz (CO), S. Singh (ID), and E. Berghauer (WI)

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

ABSTRACT:
One goal of our project is to facilitate identification of partial resistance to *Sclerotinia sclerotiorum* in secondary gene pool derived as well as in *Phaseolus vulgaris* adapted dry and snap bean lines. The results from 2014 greenhouse tests provide evidence for 7 bean lines of various commercial seed types with intermediate levels of white mold (WM) resistance. In limited field nursery data, six entries had significant levels of WM resistance. This data illustrates the progress that the NSI support has made in identifying functional WM resistance. The second goal is to characterize *S. sclerotiorum* isolates. The 366 isolates of *S. sclerotiorum* collected over the past six years from nine bean production regions in the U.S. as well as regions in Mexico and France have been characterized for aggressiveness, haplotypes and fungicide sensitivity. *Sclerotinia sclerotiorum* was assayed for sensitivity to thiophanate methyl, prothioconazole, pyraclostrobin, iprodione and metconazole in pure cultures on agar medium. To evaluate the inhibitory effect of five fungicides on growth of *S. sclerotiorum* in vitro, potato dextrose agar (PDA) was amended with the fungicides at six concentrations. Based on measurement of fungal radial growth, pyraclostrobin, iprodione and metconazole were more effective than thiophanate methyl and prothioconazole in inhibiting *S. sclerotiorum* mycelial growth at 1.0 µg a.i./ml of PDA. At field rates, prothioconazole, metconazole and iprodione were more effective than thiophanate methyl and pyraclostrobin in inhibiting *S. sclerotiorum* mycelial growth. Ranges of reduction of radial growth of 64 *S. sclerotiorum* isolates on PDA amended with the field rate of thiophanate methyl, prothioconazole, pyraclostrobin, iprodione and metconazole were 1 to 22%, 63 to 93%, 42 to 93%, and 35 to 93% respectively. Isolates from MI in group (cluster) 1 were variable for aggressiveness and fungicide sensitivity but there was no correlation. This pattern was also observed for group 2 isolates all from ND and group 3 isolates from grower fields in five states from the northwest to lake states.
Expected Outcome: isolates with higher or lower levels of aggressiveness and widely or locally distributed will be available for use in WM resistance screening.

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

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

ABSTRACT:
Oxalate oxidase (OxO) expressing soybean germplasm, known to resist *Sclerotinia sclerotiorum*, and its susceptible parent, have been used to investigate changes in genome-wide gene expression in response to the pathogen. Analysis of soybean cDNA microarrays revealed that hundreds of genes were changing expression significantly over a 36-hour time course. Likewise, significant changes in gene expression were noted following vacuum infiltration of the host plants with oxalic acid, the major virulence factor of *S. sclerotiorum*. The vast amount of expression data have been annotated and sorted by patterns of expression and physiological function to focus on genes that contribute to defense against the pathogen. In order to identify key defense response genes, functional verification is conducted in soybean by overexpression or silencing of candidate genes. Several genes have been selected for functional analysis in soybean, including a G-protein coupled receptor (GPCR), a matrix metalloproteinase (MMP), and a 14-3-3. Both the GPCR and 14-3-3 appear to have a role in defense; transgenic soybean plants, with silenced MPP, are currently undergoing infection screening.
Dozens more genes require functional verification, a task that cannot be accomplished by soybean transformation alone, due to its labour-intensive and high resource requirements. Therefore we are investigating other methods to narrow our list of candidate defense genes to attain feasible numbers for ultimate soybean functional verification. High-throughput screening approaches under consideration, include, 1) candidate gene overexpression or T-DNA knockout in *Arabidopsis thaliana*, 2) transient expression of genes by infiltration of *Nicotiana benthamiana*, and iii) viral expression in soybean using the Bean Pod Mottle Virus Vector system. Progress on the high-throughput systems will be presented.

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Transfection of *Sclerotinia sclerotiorum* with *in vitro* transcripts of a naturally occurring interspecific recombinant of Sclerotinia sclerotiorum hypovirus 2 significantly reduces virulence of the fungus

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

ABSTRACT:
A recombinant strain of Sclerotinia sclerotiorum hypovirus 2 (SsHV2) was identified from a North American *Sclerotinia sclerotiorum* isolate (#328) from lettuce (*Lactuca sativa* L.) by high-throughput sequencing of total RNA. The 5' and 3' terminal regions of the genome were determined by rapid amplification of cDNA ends. The assembled nucleotide sequence was up to 92% identical to two recently reported SsHV2 strains, but contained a deletion near its 5' terminus of more than 1.2 kb relative to the other SsHV2 strains and an insertion of 524 nt that was distantly related to Valsa ceratosperma hypovirus 1. This suggests that the new isolate is a heterologous recombinant of SsHV2 with a yet uncharacterized hypovirus. We named the new strain Sclerotinia sclerotiorum hypovirus 2 Lactuca (SsHV2L) and deposited the sequence in Genbank with accession number KF898354. *Sclerotinia sclerotiorum* isolate #328 was coinfected with a strain of Sclerotinia sclerotiorum endornavirus 1 and debilitated compared to cultures of the same isolate that had been cured of virus infection by cycloheximide treatment and hyphal tipping. To confirm that SsHV2L was the causal agent of hypovirulence, a full-length cDNA of the 14,538-ntviral genome was cloned. Transcripts corresponding to the viral RNA were synthesized *in vitro* and transfected into a virus-free isolate of *S. sclerotiorum*, DK3. Isolate DK3 transfected with SsHV2L was hypovirulent on soybean and lettuce and exhibited delayed maturation of sclerotia relative to virus-free DK3, completing Koch's postulates for the association of hypovirulence with SsHV2L.

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

Gerald J. Seiler, Thomas Gulya (Retired), and Brent Hulke, USDA-ARS, Sunflower and Plant Biology Research Unit, Fargo ND, Michael Wunsch, NDSU, Carrington Research Extension Center, North Dakota State University, Robert Harveson, University of Nebraska, Scottsbluff, Keith Olander, Central Lakes College, Staples, MN, Sam Markell and Michelle Gilley, NDSU, Department of Plant Pathology, Fargo, ND, and Bradley Flett, Agricultural Research Council, Grain Crops Institute, Potchefstroom, South Africa

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

ABSTRACT:
In 2014, we had two successful inoculated stalk rot nurseries (Carrington, ND and Grandin, ND) totaling 4,200 rows, and two successful inoculated, misted head rot nurseries (Carrington, ND and Staples,MN) totaling 500 rows evaluating germpalsm in various stages of breeding for four USDA scientists' Sclerotinia projects developing head and stalk rot resistance. In addition to testing of the USDA materials, germplasm from the Grain Crops Institute, Potchefstroom, South Africa was evaluated for stalk rot resistance. A multi-year and multi-location field screening trials of a sunflower recombinant inbred (RIL) population for Sclerotinia stalk rot resistance was completed in 2014. Genotype-by-sequencing (GBS) using the next generation sequencing (NGS) technology was used for simultaneous discovery and genotyping of large numbers of single nucleotide polymorphism (SNP) markers for the HA 441/RHA 439 RIL population. Twenty-three BC_2F_4 families derived from annual *H. argophyllus*, *H. petiolaris*, and *H. praecox* were evaluated for stalk rot resistance in replicated field trials at Carrington and Grandin, ND. Across environments, the introgressed BC_2F_4 families showed significantly higher Sclerotinia resistance than both of the susceptible checks. Additionally, 559 families from amphiploids, hexaploid, and diploid perennials crosses were tested for stalk rot at Carrington ND and Grandin, ND, and 163 families for head rot at Carrington, ND and Staples, MN. Families with better resistance than the recurrent parents were identified from the first retest, second retest, and new selections in the different trials to be retested again or released as a germplasm. More than 400 new early generation families of perennial *H. hirsutus*, *H. salicifolius*, and *H. occidentalis* tested in replicated stalk rot field trials in 2014 suggested excellent stalk rot resistance, further confirming successful gene introgression. While sunflower is the only crop plant documented to develop root infection by *Sclerotinia*, there are reports of ornamental plants within the Asteraceae family dying from root rot. Preliminary results showed many related genera of Asteraceae were susceptible to Sclerotinia infection, but this was using mycelial inoculum rather than sclerotia. If other genera are susceptible to root infection, this implies their root systems have exudates that stimulate myceliogenic germination of sclerotia, or, conversely, may inhibit carpogenic germination. Currently, a cross section of Asteraceae genera is being tested to determine which ones effectively induce myceliogenic and carpogenic germination of sclerotia.

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Use of a transformation system in sunflower for Sclerotinia resistance studies

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

ABSTRACT:
An efficient and reliable sunflower transformation system will be useful for characterizing sunflower resistance to Sclerotinia. Generation of transgenic sunflower has been challenging due to the difficulty in integrating transformation and regeneration. In our previous studies, we have increased the frequency of production of transgenic shoot primordia using early selection with hygromycin after *Agrobacterium*-mediated transformation, but shoot elongation and transgenic plant recovery was still very inefficient. The regeneration protocol was modified to improve the efficiency of shoot elongation and plant recovery. Instead of placing cotyledon explants on shoot induction medium (SIM) for 16 d, the incubation time on SIM was reduced, including 2, 4, 8 and 12 d of incubation on SIM followed with elongation medium containing 0.1 mg/L GA. A 4-d pulse treatment on SIM led to a large increase in production of elongated shoots: over 20 times more elongation shoots than 16-d SIM incubation followed with the same elongation medium. Addition of 0.1 mg/L BA or alternative cytokinins (zeatin riboside, kinetin or 2-iP) to the elongation medium resulted in a further increase in elongated shoot number by 1-2 X. Rooting efficiency was slightly improved but remained low. For transformation, a novel inoculation approach successfully increased the production of transgenic shoot primordia by 10 fold. Using the alternative inoculation approach with the 4-d pulse followed with elongation medium containing 1 mg/L zeatin riboside and 0.1 mg/L GA, about 0.5 transgenic elongated shoots were obtained per explant. Although problems still exist in transgenic plant recovery in sunflower, our new inoculation approach shows much promise for transformation of sunflower as well as other crops.

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

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

ABSTRACT:
Sunflower breeding has made huge gains in disease resistance and quality traits that are simply inherited, but lacks efficiency to adequately deal with Sclerotinia resistance, because of the complex genetic architecture. Many genes of small effect must work in concert to facilitate partial resistance. Lines exist with high levels of resistance in the field, as demonstrated by recent germplasm surveys. They do not, however, bring the most favorable yield and agronomic characteristics to sunflower hybrids. Now that we understand the genetic architecture of Sclerotinia resistance in sunflower, we must develop methods to deal with the complexity together with other important traits such as yield, oil content, insect and disease resistance, and resistance to lodging. Genomic Selection (GS) is a new statistical technology we would like to investigate for this purpose. We are interested in Genotyping By Sequencing (GBS) technology as the source of genotypic information because of the data density and low cost. The first question we need to address is how best GBS data can be used in GS, given the genome structure and size of the sunflower genome. Having whole genome sequence of parental lines will help, allowing us to impute missing data with high accuracy, and a collaborator has already conducted the resequencing on most of our released inbred lines to provide this resource. We have also had success in using GBS for other types of analysis in sunflower and other species, so there is good justification for this approach, and we now know a great deal about the structure and content of the sunflower genome. At this time, we are in the process of isolating DNA from breeding program lines developed since 2009, as discussed in the FY-14 project proposal. We are within our expected timeline of completing this work.

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

Phillip Miklas , USDA-ARS, Prosser, WA; James Myers, Oregon State University, Corvallis, OR; and Juan Osorno & Phillip McClean, North Dakota State University, Fargo, ND

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

ABSTRACT:
The identification of major genes (QTL) conferring physiological resistance to white mold (WM) from diverse sources such as *Phaseolus coccineus* and common beans (dry and snap), and linking them with molecular markers to facilitate marker-assisted breeding for development of cultivars with partial resistance to WM, is the long term goal of this project. Populations are under development for verification and fine mapping of QTL derived from *P. coccineus* accession PI 255295. Genome wide association studies have been conducted on the Mesoamerican (MDP) and Snap Bean Diversity (SBDP) Panels to identify SNPs associated with resistance in the field and straw test to white mold. QTL have been mapped on Pv 02, 03, 06, 07, 08, 09, 10 and 11 based on field data and on Pv03, 07, and 11 based on straw test data for the SBDP, and on Pv08 for field and Pv07 and Pv09 for straw test for the MDP. The MDP and SBDP GWAS peaks do not overlap. In the SBDP, the wax bean 'Unidor' was identified as highly resistant and has been used in crosses to create populations to characterize resistance. The best line for white mold resistance in the MDP was the check pinto bean USPT-WM-12, an output of the NSI. The use of a RIL population to characterize the QTL conferring WM resistance in USPT-WM-12 nears completion. Fine structure mapping of the WM7.1 and WM8.3 QTL using RNA-seq and DNA-seq analysis was continued with the coordinates for WM7.1 set as 1.0-9.1 Mb and WM8.3 as 41.8-59.7 Mb. Using data generated from the genome sequencing projects, the corresponding genetic distance for the two QTL are 1.6cM (WM7.1) and 3.8 cM (WM8.3). These are the narrowest intervals determined to date for these QTL. Among the genes differentially expressed in these QTL were Ring/U-box family protein family, senescence-associated E3 ubiquitin ligase 1, NB-ARC domain containing proteins (RPM1/RPS3 orthologs), and multidrug resistance-associated MRP3 protein in the WM7.1 QTL. For WM8.3, Ring/U-box family protein family and NB-ARC and LRR domain containing proteins were differentially expressed. These results provide a framework for additional research to address the location of the genes underlying these two important QTL. The WM-MAGIC population continues to be in development, with both single and double crosses finished. Final 4-way cross and RIL development will be conducted next year with the finished population expected in the pipeline for genetic studies in early 2016.

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Meeting Room Specifications

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

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