National Sclerotinia Initiative

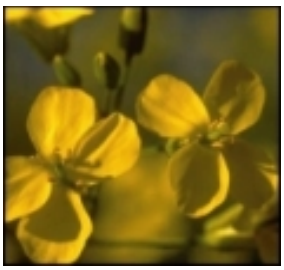
USDA-ARS

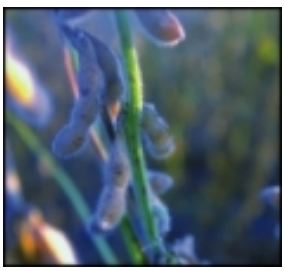
National Sclerotinia Initiative

2014 Annual Meeting

Bloomington, MN

January 22-24, 2014



12th Annual National Sclerotinia Initiative Meeting

January 22-24, 2014

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

AGENDA

National Sclerotinia Initiative – 2014 Annual Meeting

January 22-24, 2014

Wednesday – January 22, 2014

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

Thursday – January 23, 2014

7:15 am	Registration/Continental Breakfast	**McIntosh/Jonathan**
8:15 am	Welcome, Introductions & Meeting Charge – **Bill Kemp, USDA-ARS, Fargo, ND**	
8:25 am	Welcome and Update from the Northern Plains Area – **Mickey McGuire, USDA-ARS, Fort Collins, CO**	
8:35 am	ARS Office of National Programs Update – **Roy Scott, USDA-ARS, Beltsville, MD**	
8:45 am	***Featured Speaker*** Mycroviruses, hypovirulence, and exploration to control Sclerotinia diseases – **Daohong Jiang, College of Plant Science & Technology, Huazhong Agricultural University, Wuhan, People's Republic of China**	
10:00 am	Discussion Break	**Ballroom Foyer**
	Sclerotinia Research Activities – Session 1 **Moderator – Jim Meyers, Oregon State University, Corvallis, OR**	**Fireside**
10:30 am	*Sclerotinia trifoliorum* infecting chickpea: Epidemiology and population genetics (Abstract p. 23; Poster #11) – **Weidong Chen, USDA-ARS, Pullman, WA**	
10:45 am	Sources of white mold resistance derived from wide crosses in common bean and progress in characterization of relevant pathogen isolates (Abstract p. 31; Poster #14) – **James Steadman, University of Nebraska, Lincoln, NE**	
11:00 am	Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection using next generation sequencing (Abstract p. 26 ; Poster #23) – **Rubella Goswami, DuPont Crop Protection, Newark, DE and Luis del Rio, North Dakota State University, Fargo, ND**	

11:15 am	Strategies to identify functionally significant defense genes against *Sclerotinia sclerotiorum* (Abstract p. 32; Poster #10) – **Daina Simmonds, Agriculture and Agri-Food Canada, Ottawa, ONT**	
11:30 am	Identifying and verifying genes for defense to *Sclerotinia* (Abstract p. 20; Poster #6) – **Steven Clough, USDA-ARS, Urbana, IL**	
11:45 am	Working Lunch	**McIntosh/Jonathan**
	Sclerotinia Research Activities – Session 2 **Moderator – Michael Wunsch, North Dakota State University, Carrington, ND**	**Fireside**
1:15 pm	I) Most Brazilian isolates of *Sclerotinia sclerotiorum* did not require carpogenic vernalization, and II) The potential of elevated phytoalexins to suppress growth of *S. sclerotiorum* (Poster #13) – **Curtis Hill, University of Illinois, Urbana, IL and Glen Hartman, USDA-ARS, Urbana, IL**	
1:30 pm	Enhancing soybean for resistance to *Sclerotinia* stem rot (Abstract p. 18; Poster #20) – **Dechun Wang, Michigan State University, East Lansing, MI**	
1:45 pm	Fine mapping of loci for resistance to *Sclerotinia* stem rot in the perennial soybean *Glycine latifolia* (Abstracts p. 19, 27; Posters #5, 12) – **Leslie Domier, USDA-ARS, Urbana, IL**	
2:00 pm	Development and evaluation of canola breeding populations for resistance to *Sclerotinia sclerotiorum* (Abstract p. 14; Poster #18) – **Luis del Rio, North Dakota State University, Fargo, ND**	
2:15 pm	Identification of key regulators of host resistance and pathogen virulence in the *Sclerotinia sclerotiorum*-Arabidopsis pathosystem (Abstract p. 25; Poster #22) – **Zhonglin Mou and Jeffrey Rollins, University of Florida, Gainesville, FL**	
2:30 pm	Break & Poster Session	**McIntosh/Jonathan**
	Sclerotinia Research Activities – Session 3 **Moderator – Lili Qi, USDA-ARS, Fargo, ND**	**Fireside**
3:00 pm	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding (Abstract p. 16; Poster #4) – **Martin Chilvers, Michigan State University, East Lansing, MI**	
3:15 pm	Characterization and validation of two distinct mechanisms for partial resistance to *Sclerotinia sclerotiorum* in pea (Abstract p. 13; Poster #17) – **Kevin McPhee, North Dakota State University, Fargo, ND and Lyndon Porter USDA-ARS, Prosser, WA**	

3:30 pm	White mold resistance-QTL: Identification, interactions, and fine mapping in common bean (Abstract p. 12; Poster #3) – **Phil Miklas, USDA-ARS, Prosser, WA; James Myers, Oregon State University, Corvallis, OR; and Phil McClean, North Dakota State University, Fargo, ND**
4:00 pm	Pyramiding QTL for white mold resistance in Mesoamerican beans (Abstracts p. 21, 30) – **James Kelly, Michigan State University, East Lansing, MI**
4:15 pm	Metabolomic profiles associated with resistance to *Sclerotinia sclerotiorum* in dry edible beans (Abstract p. 28; Poster #8) – **Adam Heuberger and Mark Brick, Colorado State University, Ft. Collins, CO**
4:30 pm	Wrap-up & Adjourn (Dinner on your own)

Friday – January 24, 2014

7:00 am	Steering Committee Breakfast Meeting	**Apple Terrace**
7:15 am	Continental Breakfast	**McIntosh/Jonathan**

***Sclerotinia* Research Activities – Session 4** **Fireside**
Moderator – James Kelly, Michigan State University, East Lansing, MI

8:15 am	High density genotyping of a diverse population of *Sclerotinia sclerotiorum* (Abstract p. 24; Poster #7) – **Robert Brueggeman and Berlin Nelson, North Dakota State University, Fargo, ND**
8:30 am	A decade of progress in improving sunflower resistance to *Sclerotinia* head and stalk rot – a team effort (Abstract p. 10; Poster #15) – **Thomas Gulya, USDA-ARS, Fargo, ND**
8:45 am	Use of a transformation system in sunflower for *Sclerotinia* resistance studies (Abstract p. 33; Poster #24) – **Zhifen Zhang and John Finer, The Ohio State University, Wooster, OH**
9:00 am	Transferring *Sclerotinia* resistance genes from wild *Helianthus* species into cultivated sunflower (Abstract p. 11, Poster #16) – **Chao-Chien Jan, USDA-ARS, Fargo, ND**
9:15 am	Deployment of novel sources of *Sclerotinia* resistance in sunflower – 2013 progress (Abstract p. 15; Poster #19) – **Lili Qi, USDA-ARS, Fargo, ND**
9:30 am	Association mapping of *Sclerotinia* stalk rot resistance in domesticated sunflower plant introductions (Abstract p. 22) – **Brent Hulke, USDA-ARS, Fargo, ND**

9:45 am	Facilitating management of *Sclerotinia* head rot of sunflowers through screening hybrids for resistance and evaluating fungicides for efficacy (Abstract p. 17; Posters # 1, 2) – **Michael Wunsch, North Dakota State University, Carrington, ND**	
10:00 am	Discussion Break	**Ballroom Foyer**
	Sclerotinia Initiative Research: The next steps **Moderator – Bill Kemp, USDA-ARS, Fargo, ND**	**Fireside**
10:30 am	***Guest Speaker*** Strategic Planning & Reporting Progress – **Rich Wilson, USDA-ARS, Office of National Programs–Retired, Raleigh, NC**	
11:00 am	Strategic Plan Discussion – Writing Team Input/Revisions	
11:30 am	Agreements Update – **Marcie Currie-Gross, USDA-ARS, Fort Collins, CO**	
11:45 am	Working Lunch	**McIntosh/Jonathan**
1:00 pm	Assignment of Additional Tasks & Wrap-up of Initiative Business	
2:00 pm	Adjourn (Travel Safely!)	

National Sclerotinia Initiative Poster Session

January 22-24, 2014
McIntosh/Jonathan Room
Crowne Plaza Hotel & Suites

Epidemiology & Disease Management		
Poster No.	Title	Author(s)
1	Prospects for managing Sclerotinia head rot of sunflowers with fungicides	M.J. Wunsch, R. Harveson, S. Halley, M.D. Schaefer, L. Besemann, A. Arens, B.J. Kraft
2	Susceptibility of sunflowers to Sclerotinia head rot during and after bloom and implications for screening sunflowers for resistance	M.J. Wunsch, S Halley, P. Gautam, M.D. Schaefer, A. Arens, B.J. Kraft

Genomics		
Poster No.	Title	Author(s)
3	Association mapping of white mold resistance and avoidance traits using the Bean CAP snap bean panel	J. Myers, J. Davis, P. Miklas, P. McClean
4	Expression profiling of the pea-*Sclerotinia sclerotiorum* interaction for genomics-assisted breeding	P. Santos, X. Zhuang, C. Foster, J. Wang, K. McPhee, T. Coram, M. Chilvers
5	Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial *Glycine latifolia*	L.L. Domier, G.L. Hartman
6	Gene expression studies provide insight on the Sclerotinia-soybean interaction	B. Calla, L. Blahut-Beatty, L. Koziol, D. Neece, Daina Simmonds, S. Clough
7	High density genotyping of a diverse population of *Sclerotinia sclerotiorum*	R. Brueggeman, C. Qiu, B.D. Nelson
8	Metabolomic profiles associated with resistance to *Sclerotinia sclerotiorum* in dry edible beans	A.L. Heuberger, M.A. Brick, H.F. Schwartz, F.M. Robison, J.E. Prenni
9	Prevalence of Mating Type (*MAT*) alleles in *Sclerotinia sclerotiorum* across the United States and first report of *MAT* heterokaryons	P. Chitrampalam, C. Qiu, L. Aldrich-Wolfe, B.D. Nelson, Jr.
10	Strategies to identify functionally significant defense genes against *Sclerotinia sclerotiorum*	L. Blahut-Beatty, L. Koziol, B. Calla, D. J. Neece, S.J. Clough, D. Simmonds

Pathogen Biology & Development		
Poster No.	Title	Author(s)
11	Heritability of some quantitative traits of *Sclerotinia sclerotiorum*	R.N. Attanayake, W. Chen
12	Identification of viruses infecting *Sclerotinia sclerotiorum* and their potential use as a biological fungicide	L.L. Domier, S.L. Marzano, D.M. Eastburn, G.L. Hartman, B.D. Nelson
13	I. Most Brazilian isolates of Sclerotinia sclerotiorum did not require carpogenic vernalization; and II. The potential of elevated Phytoalexins to suppress growth of S. sclerotiorum	C. Godoy, L. Koga, C.B. Hill, G.L. Hartman
14	Sources of white mold resistance derived from wide crosses in common bean and progress in characterization of relevant pathogen isolates	J. Jhala, R. Higgins, J.R. Steadman

Variety Development/Germplasm Enhancement		
Poster No.	Title	Author(s)
15	A decade of progress with discovering and incorporating novel genes for Sclerotinia head rot and stalk rot resistance in sunflower	T. Gulya, B. Hulke, Z. Talukder, M. Gilley, C. Misar, M. Boosalis, R. Schafer
16	Advancement on transferring Sclerotinia resistance genes from wild perennial *Helianthus* species into cultivated sunflower	Z. Liu, J. Zhang, X. Cai, G.J. Seiler, T.J. Gulya, K.Y. Rashid, C.C. Block, C.C. Jan
17	Characterization and validation of two distinct mechanisms for partial resistance to *Sclerotinia sclerotiorum* in pea	K. McPhee, R. AshtariMahini
18	Characterization of the reaction of a *Brassica napus* breeding population to *Sclerotinia sclerotiorum*	K. Chittem, J. Anderson, B. Bigger, L.E. del Rio Mendoza
19	Deployment of novel sources of Sclerotinia resistance in sunflower-2013 progress	Y. Long, T.J. Gulya, C.C Block, B.S. Hulke, L. Qi
20	Field and greenhouse evaluations of advanced soybean breeding lines for white mold resistance	Z. Wen, R. Tan, M. Chilvers, D. Wang
21	Genome-wide association analysis for reaction to white mold in the BeanCAP Mesoamerican panel	M. Dos Santos, V.H. Villegas, J.D. Kelly
22	Identification of key regulators of host resistance and pathogen virulence in the *Sclerotinia sclerotiorum*-Arabidopsis pathosystem	C. Wang, J.A. Rollins, Z. Mou
23	Identification of resistance and pathogenicity genes associated with *Sclerotinia sclerotiorum* infection in canola	K. Chittem, R. Goswami, L.E. del Rio Mendoza
24	Use of a transformation system in sunflower for *Sclerotinia* resistance studies	Z. Zhang, J. Finer

A decade of progress with discovering and incorporating novel genes for Sclerotinia head rot and stalk rot resistance in sunflower

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

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

ABSTRACT:
While the USDA-ARS effort to improve sunflower resistance to Sclerotinia head and stalk rot dates back to the 1970s when the Unit was founded, the most progress has been made in the last decade, during which time extra research funds became available through the USDA Sclerotinia Initiative. The first advance was to switch from relying upon natural infection in field nurseries to producing inoculum for both head and stalk rot. Now we annually produce between 200 and 400 kg of millet infested with Sclerotinia mycelium, and several hundred disks, each with ~ a half million ascospores. To effectively phenotype for head rot, we have worked with cooperators to establish two automated, mist irrigated sites. Our annual efforts have averaged 1000 rows for head rot (at two locations) and 3200 rows for stalk rot (at three to four locations). This is a coordinated research effort between six USDA scientists at Fargo, ND, and Ames, IA. The program has sought to identify resistance in cultivated material, primarily by phenotyping the NPGS collection, currently numbering 4087 accessions but also looking at accessions of the 52 annual and perennial *Helianthus* species, which are represented by 2201 accessions. As of 2013, ~ 95% of the cultivated sunflower accessions have been phenotyped for stalk rot resistance (many at multiple locations) while ~ half have been tested for head rot resistance. Of the 2201 accessions of wild *Helianthus*, ~ 90% have been screened in greenhouse tests for stalk rot by Dr. C. Block, in previously funded projects. After identification of new sources of resistance, four SYs in the Fargo Unit (Hulke, Seiler, Qi and Jan) have worked to transfer resistance genes into elite agronomic backgrounds. During the past decade, the Sunflower & Plant Biology Research Unit has made ~ 85 oilseed and confection germplasm releases, and ~ 30 of those were either specifically developed for Sclerotinia resistance, or have improved levels of resistance. The current major effort in our Unit is to move high levels of resistance from four annual *Helianthus* species and near immunity from a dozen perennial species. The later effort with perennial species is especially challenging due to the genome structure of the perennials and the diploid nature of cultivated sunflower. In addition to discovering new sources of resistance and the development of resistant germplasm, our Unit is identifying markers associated with both head and stalk rot resistance, which will make future breeding efforts more efficient, and lessen our reliance on field evaluations. Lastly, we now can start to examine biochemical and anatomical factors contributing to Sclerotinia resistance. Much progress has been made in the past decade, and the tools we have acquired should make our future research more productive. Genetic means to manage Sclerotinia head and stalk rot resistance are now a foreseeable goal, but this pathogen will continue to be one of the major efforts of public and private breeding programs.

Contact Information – Dr. Thomas Gulya, Sunflower Research Unit, USDA-ARS Northern Crop Science Laboratory, 1307 N. 18th Street, Fargo ND 58105-5677; 701-239-1316; Thomas.Gulya@ars.usda.gov

After 3 Feb., 2014: Dr. Gerald Seiler. Email: Gerald.Seiler@ars.usda.gov

Advancement on transferring Sclerotinia resistance genes from wild perennial *Helianthus* species into cultivated sunflower

Zhao Liu[1], Jichong Zhang[1], Xiwen Cai[1], Gerald J. Seiler[2], Thomas A. Gulya[2], Khalid Y. Rashid[3], Charles C. Block[4], and Chao-Chien Jan[2]
[1]North Dakota State University, Fargo, ND 58102
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58102
[3]Agriculture and Agri-Food Canada, Morden, Manitoba, Canada R6M 1Y5
[4]USDA–ARS, North Central Regional Plant Introduction Station, Ames, IA 50011

Funded Plan of Work: Transferring Sclerotinia resistance genes from wild *Helianthus* species into cultivated sunflower

ABSTRACT:
Sclerotinia, caused by *Sclerotinia sclerotiorum*, is one of the most damaging and difficult-to-control sunflower diseases. In the last nine years, this project has focused on the introgression of resistance genes from the wild perennial *Helianthus* species into cultivated sunflower due to the lack of sufficient tolerance to Sclerotinia in the crop. Field and greenhouse evaluations indicated excellent stalk and head rot resistance in six interspecific amphiploids and wild perennials. A five-year evaluation (2009-2013) for stalk and head rot has been conducted for the backcross progenies derived from amphiploids and wild perennial species, including *H. californicus, H. maximiliani, H. nuttallii*, *H. giganteus*, and *H. grosseserratus*. Follow-up replicated field tests of 53 families for head rot and greenhouse tests for stalk rot resistance for 51 families in 2013 indicated moderate to good resistance, further confirming successful gene introgression. Seed increase of 430 early generation families derived from the crosses of NMS HA 89 × (F_1s of *H. hirsutus*, *H. salicifolius*, or *H. occidentalis* crossed with HA 410 or HA 451) in 2013 will further diversify the pool of resistance genes and increase the probability of identifying useful major resistance QTLs. Seed was also increased for more than 160 families derived from the previous crosses in 2013. These families will provide new sources for replicated field tests in 2014. Backcross progenies with poor seed sets or high chromosome numbers derived from the *H. hirsutus*, *H. salicifolius*, *H. occidentalis*, *H. divaricatus*, and *H. resinosus* were planted in the greenhouse for further backcrossing. A new set of crosses using *H. strumosus*, *H. tuberosus*, and *H. decapetalus* were made in 2013 and are being backcrossed with HA 410. The BC_1F_1 or F_1 generations derived from the new crosses were established in greenhouse. 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 identify the chromosomes or segments of chromosomes introgressed into the cultivated background. We plan to release several Sclerotinia resistant germplasms derived from various perennial species, including amphiploids in the next year.

Contact Information – Chao-Chien Jan, Sunflower and Plant Biology Research Unit, USDA-ARS-NCSL, 1605 Albrecht Blvd N, Fargo, ND 58102-2765; 701-239-1319; chaochien.jan@ars.usda.gov

Association mapping of white mold resistance and avoidance traits using the Bean CAP snap bean panel

Jim Myers, Joel Davis, Oregon State University, Corvallis OR; Phil Miklas, USDA-ARS – Prosser, WA and Phil McClean, North Dakota State University, Fargo, ND

Funded Plan of Work: White mold resistance-QTL: Identification, interactions, and fine mapping in common bean

ABSTRACT:
The Bean Coordinated Agricultural Project (CAP) funded the development, genotypic and phenotypic characterization of a 150 accession panel of snap bean cultivars. These cultivars represent the range of diversity in snap beans. The panel has been genotyped using an Illumina 6,998 SNP GeneChip and this allows the evaluation for phenotypic traits beyond those of specific interest to the Bean CAP. In 2012, a subset of the snap bean panel with non-pole bean habit (134 lines) was grown in a white mold screening nursery at Corvallis, Oregon and evaluated for white mold incidence and severity and several phenological traits associated with disease avoidance. In 2013, a similar panel was grown and evaluated for disease. In addition, the panel was evaluated using the straw test in the winter 2013 greenhouse. GWAS was performed on the 2012 data to provide a preliminary examination of potential genotypic associations with disease resistance. We discovered three regions of interest on Pv02, Pv09 and Pv11. Pv02 had two peaks located at 3.2 Mb and approximately 34.8 Mb which may correspond to WM2.1 and WM2.3 based on the WM QTL compendium developed by Miklas and colleagues. GWAS on multi-trial data is ongoing. The wax bean 'Unidor' has consistently expressed high levels of resistance (as good as or better than resistant checks) in field evaluations. The cultivars in the Bean CAP snap bean panel represent novel sources of white mold resistance that in some cases appears unique from those observed in dry beans.

Contact Information – Jim Myers, Department of Horticulture, ALS 4017, Oregon State University, Corvallis, OR 97331; 541-737-3083; myersja@hort.oregonstate.edu

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

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

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.

Contact Information – Kevin McPhee, NDSU Dept. 7670, P.O. Box 6050, Fargo, ND 58108-6050; (701) 231-8156; kevin.mcphee@ndsu.edu

Characterization of the reaction of a *Brassica napus* breeding population to *Sclerotinia sclerotiorum*

Kishore Chittem[1], James Anderson[2], Brant Bigger[2], and Luis del Río Mendoza, [1] North Dakota State University, Fargo; [2]USDA-ARS, Sunflower and Plant Biology Research Unit, Biosciences Research Laboratory, Fargo.

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

ABSTRACT:
The F_2population of the cross between two doubled-haploid *Brassica napus* lines, NEP63 and NEP32, was evaluated in greenhouse conditions for their reaction to inoculation with mycelia of *Sclerotinia sclerotiorum.* NEP63 and NEP32 were obtained from a plant introduction material identified as resistant in an earlier project funded by the Sclerotinia Initiative. The former was identified as resistant while the latter was considered susceptible to *S. sclerotiorum.* A total of 182 lines, parental lines included, were grown in plastic pots and inoculated using the petiole inoculation technique when they were approaching the flowering stage. After inoculation the plants remained in the greenhouse room at approximately 20^{o} C and 16 hours light daily. Lesion size and plant mortality were recorded weekly. Within two weeks from inoculation approximately one half of the lines, including most plants from the susceptible parent were dead. The area under the disease progress curve was calculated for the surviving plants. While the surviving F_2 lines developed lesions of different sizes, most were able to set seeds. Several of these lines flowered within 60 days from planting, as many commercial spring canola cultivars do, but others took longer time. DNA samples were collected from each line. Molecular marker work is underway.

Contact Information – Dr. Luis del Rio, Dept. of Plant Pathology, North Dakota State University, Dept. 7660 P.O. Box 6050, Fargo ND 58108-6050; (701) 231-7073; luis.delrio-mendoza@ndsu.edu

Deployment of novel sources of Sclerotinia resistance in sunflower-2013 progress

Yunming Long[1], Thomas J. Gulya[2], Charles C Block[3], Brent S. Hulke[2], Lili Qi[2]
[1]Department of Plant Sciences, North Dakota State University, Fargo, ND
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND
[3]USDA-ARS, Plant Introduction Station, Ames, IA

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

ABSTRACT:
Stalk rot, caused by *Sclerotinia Sclerotiorum*, is a devastating disease of sunflower worldwide. Identification of wild resistant sources, introgression of resistance into cultivars and mapping of resistance genes could provide new insight into the genetic basis underlying the resistance and resistance breeding. The specific objectives of this study were to 1) pre-breed novel Sclerotinia resistance from wild annual species of *H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris* into cultivated sunflower, and develop an advanced backcross population for QTL mapping, 2) investigate inheritance of Sclerotinia resistance in introgressed lines. During the winter of 2012 and the spring of 2013, a total of 231 BC_2F_3 families (24 plants per family) derived from seven resistant accessions of *H. argophyllus*, *H. debilis*, and *H. praecox* were evaluated for stalk rot resistance in a greenhouse. A total of 47 resistant families were obtained. Out of the 47 resistant BC_2F_3 families, 27 with good seed set were selected to test their resistance to stalk rot with two to three replicates in a field trial at Crookston, MN and Grandin, ND, in the summer of 2013. In addition, 17 BC_2F_4 families selected from the best resistant lines of last year's field tests were further evaluated for their resistance to stalk rot with four to eight replicates at the same time and locations. Overall, out of 27 BC_2F_3 families tested, five from *H. argophyllus* had the lowest disease incidence (DI) ranging from 0 to 9.3%, suggesting that they have higher levels of resistance to Sclerotinia stalk rot compared to the resistant checks, Croplan 305 (DI 34.9%) and HA 441 (DI 28.6%). And six BC_2F_3 families had disease incidence from 10% to 20%. Among 17 BC_2F_4 families tested, 13 had disease incidence lower than 10% and four had disease incidence from 10% to 28%, further confirming stalk rot resistance in these lines. Out of the 13 most resistant BC_2F_4 families, the two resistant lines 12F-3405-2 and 12F-3406-5 were derived from accession PI435843 of *H. petiolaris*. Whole genome scans of the two lines with 508 sunflower SSR markers revealed the presence of one introgressed chromosome segment located on linkage group 15, indicating this chromosome probably associates with stalk rot resistance. We continued to develop an advanced backcross (AB) population to facilitate genetic characterization of novel QTL for stalk rot resistance derived from *H. argophyllus* PI 494573. A total of 150 plants derived from 14 BC_2F_1 plants were advanced to the BC_2F_5 generation by single-seed descent. We will grow BC_2F_5 families in the field in the summer of 2014 to increase the number of seeds for multi-environment testing.

Contact Information – Lili Qi, USDA-ARS Sunflower and Plant Biology Unit, 1605 Albrecht Blvd N, Fargo, ND 58102-2765, (701) 239-1351, lili.qi@ars.usda.gov

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

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

ABSTRACT:
Little information is available about the genetics of the resistance mechanism that prevails during the interaction between pea (*Pisum sativum* L.) plants and the white mold (*Sclerotinia sclerotiorum*) fungus. Therefore, we investigated the genetics of the interaction with RNA seq deep sequencing technology. Early time points (12, 24 and 48 hpi) of the infection of pea were examined. The expression pattern and phenotype of inoculated Lifter (susceptible) and PI240515 (partial resistant) pea lines showed that these patterns were most dissimilar at 24 hpi but similar at 12 and 48 hpi. Thus, we analyzed the most highly differential expressed genes (maximum of 20) in the *de novo* assembled transcriptome from PI240515 preferentially at 24 hpi, and compared them with Lifter at 24 hpi, as well as with the other time-points. Based on the biological functional homology to *Arabidospis thaliana*, five genes encoding two putative precursors of peroxidases (Psat_118093 and Psat_116532), a chalcone synthase (Psat_107301), a ferulate 5-hydroxylase (Psat_117663) and a β-1, 3-hydrolase (Psat_111657), previously linked to host defenses, were found to be preferentially up-regulated in the partial resistance PI240515 line. In order to find out to which extent the encoded proteins correlated with the biology of the partial resistance response in pea, transversal pea stem sections were stained with phloroglucinol-HCl and Maule reagents to indicate lignin and S monomer of lignin contents, respectively. Additionally, the total lignin content was also determined using the acetyl bromide method. Early indications suggest greater lignin deposition in inoculated PI240515, when compared with inoculated Lifter, at both 12 and 24 hpi.

In parallel, we also examined the unique expressed genes during pea-*S. sclerotiorum* interaction. To obtain these unique expressed genes, we compared EST data sets from fungal-inoculated samples with those ESTs from mock-treated samples. The results showed that 2,528 (77.3%) pea ESTs from PI240515 and 1,517 genes (62.7%) from Lifter were specifically expressed relatively to each other. In general, early in the interaction between pea and *S. sclerotiorum*, PI240515 plants seem to invest more of their genetic resources towards programmed cell death (PCD) related events, as a means to impede the spread of the disease; however, this will not be enough to stop the pathogen advances, since its necrotrophic life style preys on dead cells. These plants are also investing more in the expression of pathogenesis-related (PR) genes, reinforcement of cell walls and possibly production of phytoaleaxins. We posit that all of these other compounds of the plant defense arsenal might play a more important role in the partial resistance response of pea to *S. sclerotiorum*. From this vast transcriptome data set we also mined approximately 500 pea microsatellite markers for use in marker assisted pea breeding.

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

Facilitating management of Sclerotinia head rot of sunflowers through screening hybrids for resistance and evaluating fungicides for efficacy

Michael Wunsch, Michael Schaefer, and Billy Kraft, North Dakota State University Carrington Research Extension Center; Pravin Gautam, Amanda Arens, and Bryan Hanson, NDSU Langdon Research Extension Center; Leonard Besemann, NDSU Carrington Research Extension Center, Oakes Irrigation Research Site; and Robert Harveson, University of Nebraska Panhandle Research Extension Center, Scottsbluff

Funded Plan of Work: Facilitating management of Sclerotinia head rot of sunflowers through screening hybrids for resistance and evaluating fungicides for efficacy

ABSTRACT:
Sclerotinia head rot is a serious constraint on sunflower production, and management of the disease is currently limited to the use of partially resistant commercial hybrids. The objectives of this study were (1) to facilitate the development of partially resistant commercial hybrids by screening commercial breeding lines and hybrids for resistance; (2) to evaluate the susceptibility of sunflowers to head rot during and after bloom and utilize the susceptibility data to inform resistance screening techniques; and (3) to evaluate the potential use of fungicides for management of Sclerotinia head rot.

Screening nurseries were highly successful at differentiating the relative susceptibility of commercial sunflower hybrids and breeding lines to Sclerotinia head rot. In the large (nine-replicate) screening nursery conducted in Carrington, one commercial and four experimental confection lines and four experimental oilseed lines exhibited significantly lower head rot incidence and severity index relative to both susceptible checks ($P < 0.05$). Results were strongly correlated across the Carrington, Landon, and Oakes screening locations: Pearson correlation coefficients were 0.76 to 0.92 for head rot incidence (min. $P < 0.002$) and 0.79 to 0.92 for head rot severity index (min. $P < 0.0007$).

Results from inoculation timing studies suggest that obtaining unbiased results from disease screening nurseries requires that all inoculations be conducted at the same stage of bloom and that no inoculations be conducted after bloom. Sunflowers exhibited a sharp drop in susceptibility immediately after bloom ($P < 0.05$; Carrington and Langdon) and an increase in susceptibility as bloom progressed from the approx. 20% to approx. 80% of disk flowers blooming or completed bloom ($P < 0.05$; only assessed in Carrington).

Despite achieving an intermediate level of disease that is typically favorable for differentiating fungicide efficacy, fungicides did not show efficacy against Sclerotinia head rot at either the Carrington or Oakes locations. Several of the fungicides that were evaluated have excellent efficacy against Sclerotinia on other crops, and the poor disease control observed against Sclerotinia head rot is likely due to the difficulty of achieving satisfactory fungicide coverage on the front of sunflower heads using the available application technology.

Contact Information – Michael Wunsch; NDSU Carrington Research Extension Center, PO Box 219, Carrington, ND 58421; 701-652-2951; michael.wunsch@ndsu.edu

Field and greenhouse evaluations of advanced soybean breeding lines for white mold resistance

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 sclerotiorum* (Lib.) deBary can cause significant yield loss in soybean. To facilitate breeding for white mold resistance, 432 advanced soybean breeding lines were evaluated for resistance to white mold in the field and in the greenhouse. Disease severity index (DSI) was used to estimate the soybean response to white mold in the field. Mortality and number of surviving nodes were used to measure soybean response to the disease in the greenhouse. The heritability of mortality and the number of surviving node among advance breeding lines in the greenhouse was 81% and 78% respectively while the heritability of disease severity index (DSI) in the field test was 66%. The correlation between greenhouse rating scale (mortality) and field DSI was significant but low (r^2 =0.14, P =0.02). The DSI was highly significantly correlated with yield (r^2 = -0.34, P<0.01). Seven breeding lines were found resistant (DSI<15, mortality = 0%) to white mold in both field and greenhouse evaluations. The 432 lines were also genotyped with 52,041 SNP markers. Genome-wide association mapping was carried out with the marker and the resistance data. Seven loci underling white resistances were identified, and one locus was identified with both the field and the greenhouse data. Peak SNPs at the identified loci could explain ~31.5% of the phenotypic variance. The identification of seven new resistant lines and significant SNPs will facilitate white mold resistance breeding in soybean.

Contact Information – Dr. Dechun Wang, 1066 Bogue St., Rm. A384E, East Lansing, MI 48824-1325; 1-517-355-0271 Ext. 1188; wangdech@msu.edu

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

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

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

ABSTRACT:
To fine map loci for resistance to Sclerotinia stem rot in *Glycine latifolia*, high-density linkage maps were constructed using single nucleotide polymorphism (SNP) markers generated by genotyping by sequencing and evaluated in F_2 and F_5 populations. In each population, greater than 2,500 SNP markers were selected for analysis and segregated to form 20 large linkage groups. Marker orders were similar in the F_2 and F_5 populations and consensus linkage maps were constructed. To examine the relationships between *G. latifolia* linkage groups and *G. max* chromosomes, mapped SNP-containing sequences from *G. latifolia* were aligned to the genome sequence of *G. max*, which showed that 12 of 20 *G. latifolia* linkage groups were nearly collinear with sequences of 12 *G. max* chromosomes. The remaining eight linkage groups appeared to be products of multiple interchromosomal translocations relative to *G. max*. When combined with phenotypic data, the analysis identified loci for oxalic acid sensitivity on *G. latifolia* linkage groups 2, 10, and 17, which corresponded to soybean chromosomes 19, 10, and 17, respectively. Comparison of SNP data and the draft genome sequence of *G. latifolia* showed that the 3.4-cM genetic interval containing the locus on *G. latifolia* chromosome 2 represented a region of 2.7 Mbp that contained at least 68 predicted genes. To confirm the results, 400 additional F_2 plants were evaluated for sensitivity to oxalic acid, but the phenotypic data were too variable to be useful. The experiments will be repeated. Finally, an F_5 RIL population was analyzed for segregation of GBS markers and evaluations of the population for sensitivity to oxalic acid and inoculation with *S. sclerotiorum* were initiated.

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

Gene expression studies provide insight on the Sclerotinia-soybean interaction

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

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

ABSTRACT:
Oxalate oxidases catalyze the degradation of oxalic acid (OA). Highly resistant transgenic soybean carrying an oxalate oxidase (OxO) gene and its susceptible parent soybean line, AC Colibri (AC), were tested for genome-wide gene expression in response to the necrotrophic, OA producing pathogen *Sclerotinia sclerotiorum* using soybean cDNA microarrays. Although many genes and pathways were found similarly activated or repressed in both genotypes after inoculation with *S. sclerotiorum*, the OxO genotype displayed a measurably faster induction of basal defense responses as observed by the differential changes of defense related and secondary metabolite genes compared to its susceptible parent AC. Additionally, the experiment provides data that support the hypothesis that *S. sclerotiorum*, at least, partially elicits the hypersensitive response. Of the nine genes showing the most extreme opposite directions of expression between genotypes, eight of them were related to photosynthesis and/or oxidation, highlighting the importance of redox in control of this pathogen. A gene expression study was also conducted on RNA from infiltrated soybean leaves with 5 mM OA to examine gene expression changes 2 hours post infiltration. By comparing gene expression levels between leaves of a transgenic OxO soybean and its parent AC infiltrated with OA (pH 2.4) or water (pH 2.4 or 5.5), we were able to compare the effects of OA dependent or independent of its pH. Gene expression by microarray analysis identified 1054 genes that were significantly differentially expressed. Independent of pH, OA altered expression levels of 78 genes, with ferritin showing the strongest induction by OA. The combination of OA plus its low pH caused 1,045 genes (99% of all the significant genes) to be differentially expressed, with many of the up-regulated genes being related to basal defense, such as genes of the phenylpropanoid pathway and various cytochrome P450s. As expression of ferritin, a gene that encodes for an iron-storage protein, is induced by free iron, these results suggest that *S. sclerotiorum* is benefitting from the ability of OA to bind and release iron from plant proteins, as this would induce host cell death, and to also uptake and assimilate the iron for its own metabolic needs.

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

Genome-wide association analysis for reaction to white mold in the BeanCAP Mesoamerican panel

Marilene Dos Santos, Valerio Hoyos Villegas, and James D. Kelly, Michigan State University, East Lansing, MI

Funded Plan of Work: Pyramiding QTL for white mold resistance in Mesoamerican beans

ABSTRACT:
The fungal disease white mold, caused by *Sclerotinia sclerotiorum* (Lib.) De Bary is significantly detrimental to common bean (*Phaseolus vulgaris* L.) production. To date, no major resistance genes have been reported for this disease, indicating that white mold is a complex, quantitatively inherited trait. In this study, we used a total of 96 common bean varieties from an assembled genotype panel as part of the Bean Cooperative Agricultural Project (BeanCAP) to perform genome-wide association study (GWAS) analysis to detect QTL associated with white mold resistance in Mesoamerican bean genotypes. The varieties were grown at the Montcalm Research and Extension Center located in Entrican, MI under irrigated conditions to promote disease pressure. Heavy disease symptoms were visible on the spreader rows around pod filling, and severity scores on a scale from one to nine were recorded on the varieties prior to harvest. The plots received a total of 176 mm from precipitation and 102 mm from irrigation from planting (6/18/2013) to harvest (09/20/2013). Other variables recorded were days to flowering, desirability score, days to maturity and lodging score. Significant differences (p<0.0001) were detected for white mold scores. The five genotypes with the highest (most susceptible) scores were Harold, Gloria, ABCP-8 and Common pinto; in contrast, the five genotypes with the lowest (most resistant) scores were A-55, Rosetta, I9365-31 and Black Rhino. The 96 varieties were screened with the BARCBEAN6K_3 Genechip consisting of 5,398 single nucleotide polymorphism (SNP) markers and data on the GWAS analysis will be presented.

Contact Information – James D. Kelly, Michigan State University, 370 Plant and Soil Sciences, Dept. Plant, Soil and Microbial Sciences, 1066 Bogue St., East Lansing, MI, 48824; (517) 355-0271 Ext. 1181; kellyj@msu.edu

Genome-wide association mapping of Sclerotinia stalk rot and head rot diseases in sunflower

Zahirul I. Talukder[1], Brent S. Hulke[2], Lili Qi[2] and Thomas J. Gulya[2]
[1]North Dakota State University, Fargo, ND 58102
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND

Funded Plan of Work: Discovery/use of novel sources of head and stalk rot resistance in sunflower

ABSTRACT:
Stalk rot and head rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary are the two most destructive diseases in sunflower (*Helianthus annuus* L.). Host resistance against *S. sclerotiorum* is controlled by several genes. Mapping resistance genes using diverse germplasm sources could provide new insight into the genetic basis underlying resistance and designing an efficient marker-assisted selection (MAS) breeding program in sunflower. The main objective of this study was to perform the genome-wide association mapping (GWAM) on a panel of sunflower lines comprised of plant introductions (PIs) and USDA-ARS released inbred lines and to identify significant DNA markers associated with Sclerotinia stalk rot and head rot disease resistance. GWAM was conducted using single nucleotide polymorphism (SNP) markers developed from both candidate genes known to function against Sclerotinia disease resistance and the ~8700 SNP markers developed by the National Sunflower Association (NSA) SNP consortium. Our preliminary analysis with stepwise regression models in SAS that account for population structure identified 31 markers significantly associated with stalk rot resistance and together explains a total of 60% of the phenotypic variation. Out of the 31 SNP markers, 22 were located in 13 linkage groups of sunflower while the map positions of the remaining 9 markers have yet to be identified. We are currently testing other recently developed GWAM mapping algorithms like, mixed linear model with Least Absolute Shrinkage and Selection Operator (MLM-LASSO) and Adaptive Mixed LASSO methods accounting for population structure, kinship and principal components either singly or in combination in order to identify the best fitting model for our analysis. The SNP markers associated with Sclerotinia disease resistance can together form a selection index model, which will simplify selection based on markers, thus improving the efficiency of MAS during Sclerotinia resistance cultivar development.

Contact Information – Dr. Brent S. Hulke, Sunflower and Plant Biology Research Unit, USDA-ARS Northern Crop Science Laboratory, 1307 18th Street N., Fargo, ND 58102-2765; (701) 239-1321, Brent.Hulke@ars.usda.gov

Heritability of Some Quantitative Traits of *Sclerotinia sclerotiorum*

Renuka N. Attanayake and Weidong Chen
Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA; and USDA-ARS, Washington State University, Pullman, WA 99164, USA

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

ABSTRACT:
Narrow sense heritability is the proportion of phenotypic variance that is due to genetic factors. It is a widely used concept in plant breeding in estimating the fraction of selection differential expected to be gained. Selection of a phenotypic trait over time reduces genetic variance and hence heritability. Conversely phenotypic traits with high heritability can readily respond to selection. Thus knowledge of heritability of agriculturally important traits of a pathogen can be exploited in studying pathogen biology and in developing management strategies. To our knowledge, heritability of phenotypic traits of *S. sclerotiorum* has not been studied before. The objective of the current study is to estimate heritability of agriculturally important traits such as oxalic acid production, growth rate, fungicide sensitivity and virulence.

Two hundred and seventy six isolates of *S. sclerotiorum* were collected from eight populations originated from alfalfa, canola, gourds, lentil, pea, and potato from United States and China. Phenotypic traits studied were total oxalate production, growth rate, sensitivity to fungicides benomyl, iprodione and fluazinam, and virulence in colonizing detached pea leaves. Heritability values are generally high among the populations, and ranged from 0.85 to 0.95 for total oxalate production and 0.61-0.9 for growth rate (mycelial dry weight). For sensitivity to the fungicides, heritability values ranged from 0.69 to 0.98 for benomyl, 0.78 for iprodione, and 0.6-0.9 for fluazinam. However, low heritability values were observed for virulence and ranged from 0.3 to 0.12. The data suggest that heritability values for a given trait is stable for the natural populations of *S. sclerotiorum*. Interestingly most of the measured traits have high heritability indicating that these traits have high potential in response to selection. For example, high heritability of fungicide sensitivity suggests a high likelihood of developing resistance to the fungicides in response to fungicide selection. On the other hand, the low heritability of virulence suggests large component of the virulence variation was due to environment. Thus closely controlled environment should be used in conducting virulence assays in screening for host resistance. Therefore, heritability can be used to predict evolutionary potential of the pathogen populations in response to control practices, and is useful in devising disease management strategies.

Contact Information – Dr. Weidong Chen, USDA- ARS, and WSU, Pullman, WA 99164; 509-335-9178; w-chen@wsu.edu

High-density genotyping of a diverse population of *Sclerotinia sclerotiorum*

Robert Brueggeman, Chengxiang Qiu, and Berlin D. Nelson Jr. Department of Plant Pathology, North Dakota State University, Fargo, ND.

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

ABSTRACT:
This is the first high-density genotype-by-sequencing (GBS) of a large population of *S. sclerotiorum* collected from 22 crops and 25 states in the United States. The goal is to develop a large number of single nucleotide polymorphism (SNP) markers at unique loci evenly distributed across the 38 Mb genome of *S. sclerotiorum.* To date we grew 120 isolates from our collection of *S. sclerotiorum* and extracted genomic (g) DNA from the mycelium. Genomic DNA from 120 isolates was utilized to construct dual enzyme GBS libraries. The isolated gDNA was restriction digested with *ApeKI*/*HhaI* and quantified to produce balanced GBS libraries with the goal of achieving 50,000 - 100,000 barcoded and sequestered sequence reads for each *S. sclerotiorum* isolate. The digested gDNA was size selected (~200 bp fragments) with the Pippin prep instrumentation and ligated to barcoded Ion Torrent sequencing adaptors specific to each isolate. The libraries were multiplexed (40 isolates per library) for parallel sequencing on three separate Ion Torrent 318 microprocessor chips. The sequencing reactions yielded 15.6 million sequences at an average of 160 bases per read for a total of ~2.5 billion bases. The sequence alignment identified 16,320 unique sequence tags/loci with ~30,000 SNPs present on ~60% of the sequence tags. Positioning and preliminary BLAST analysis of the 16,320 GBS tagson the *S. sclerotiorum* genome determined that they are randomly spread throughout the genome and that ~86% of the unique loci hit predicted genes suggesting that they represent over 50% of the predicted genes within the genome. This preliminary analysis predicts that we will be able to place a SNP marker approximately every 3.9 Kb throughout the *S. sclerotiorum genome*. Complete characterization and annotation of the SNP markers are currently underway and the genotyping data for all the isolates will be made freely available. The generation of a genetic database with free access to this genotyping data will provide a powerful tool to utilize the publicly available *S. sclerotiorum* genome sequence and facilitate high-resolution association mapping to identify genes underlying loci contributing to phenotypic variation.

Contact Information – Dr. Robert Brueggeman, Dept. Plant Pathology #7660, North Dakota State University, P. O. Box 6050, Fargo, ND 58108

Identification of key regulators of host resistance and pathogen virulence in the *Sclerotinia sclerotiorum*-Arabidopsis pathosystem

Jeffrey A. Rollins & Zhonglin Mou, University of Florida, Gainesville, FL

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 decarboxylase1 (ODC1) protein for engineering high levels of disease resistance in canola. In this first year of funding, we have been working on positional cloning of the *HSS1* gene in *A. thaliana*. We crossed homozygous *hss1*, which is in the Columbia genetic background, to the polymorphic ecotype Landsberg *erecta*. Linkage analysis of 130 F_2 plants with *hss1* morphology located the *HSS1* gene between the molecular markers Ciw5 and Ciw6 on Chromosome 4. Further recombination analysis of 1479 F_2 mutant plants placed the *HSS1* gene between the markers m602 and m268. We have sequenced the candidate genes in this region and found several mutations. We are currently confirming the *hss1* mutation by genetic complementation. We have cloned the candidate wild-type genes in this region in plant expression vectors and expect to unequivocally identify the *HSS1* gene early in year two. Microarray experiments to monitor *S. sclerotiorum*-induced transcriptome changes in *hss1* and wild-type plants indicate that the *hss1* mutation significantly affects *S. sclerotiorum*-induced transcriptome profiles in the host. The most interesting among the observed changes is the down regulation of genes involved in ethylene signaling and glucosinolate biosynthesis in the *hss1* mutant following challenge with *S. sclerotiorum*. In addition to the progress on identifying the *HSS1* gene, we have cloned the *S. sclerotiorum ODC1* gene into the T-DNA vector pCAMBIA1300S and have transformed Arabidopsis plants with Agrobacteria carrying the T-DNA vector. T_1 transgenic plants are growing and we will identify single insertion homozygous plants in the next two generations. Moreover, continued phenotypic characterization of *odc1* gene deletion mutants of *S. sclerotiorum* demonstrates an oxalic acid over-accumulation phenotype, consistent with its function as an oxalate decarboxylase. The identification of the *HSS1* locus 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 (*ODC1*) will facilitate the use of an over expression strategy to enhance host resistance.

Contact Information – Dr. Jeffrey A. Rollins, Department of Plant Pathology, University of Florida, 1453 Fifield Hall, Gainesville, FL 32611-0680; 352-273-4620; rollinsj@ufl.edu

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

Kishore Chittem[1] 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 goal of this study has been to identify white mold resistance genes in canola and pathogenicity genes in *Sclerotinia sclerotiorum* associated with infection of this host through transcriptome analysis. cDNA libraries were created from *S. sclerotiorum* inoculated canola doubled haploid resistant (NEP63) and susceptible (NEP32) lines derived from a PI line of *Brassica napus,* in a time course experiment and the pathogen grown in culture. Approximately 95 million, 76bp reads were obtained from these libraries using Illumina sequencing. Analysis of the RNA-Seq data following alignment of libraries to the *S. sclerotiorum* whole genome and Brassica 95kEST assembly identified several differentially expressed fungal and pathogen genes during infection that could be associated with pathogenicity in *S. sclerotiorum* and resistance in canola to Sclerotinia stem rot respectively. Functional categorization of the differentially expressed ESTs of both plant and fungal origin was conducted and expression of significant number of genes involved in defense response, signal transduction and immune response were detected among others. qRT-PCR of selected differentially expressed plant and fungal genes was performed using gene specific primers to validate the RNA-Seq differential expression analysis. Similar expression profiles were observed for selected genes by qRT-PCR and RNA-Seq. Putative gene knock-out mutants were generated for six candidate pathogenicity genes; however, PCR assays conducted to verify the target gene had been replaced indicated all mutants were ectopic (target gene had not been affected). Generation of new mutants is under way.

Contact Information – Dr. Luis del Rio, Dept. of Plant Pathology, North Dakota State University, Dept. 7660 P.O. Box 6050, Fargo ND 58108-6050; (701) 231-7073; luis.delrio-mendoza@ndsu.edu

Identification of viruses infecting *Sclerotinia sclerotiorum* and their potential use as a biological fungicide

Domier, L.L.[1,2], Marzano, S.L.[2], Eastburn, D.M.[2], Hartman, G.L.[1,2], and Nelson, B.D.[3]; [1] USDA-ARS, Urbana, IL; [2] Department of Crop Sci., University of Illinois, Urbana, IL; [3] Department of Plant Pathology, North Dakota State University, Fargo, ND.

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

ABSTRACT:
To identify viruses infecting *Sclerotinia sclerotiorum*, total RNA was extracted from pure cultures of 138 *S. sclerotiorum* field isolates and analyzed by high-throughput sequencing, which identified 20 novel *S. sclerotiorum* viruses with RNA genomes, more than doubling the number of viruses known to infect *S. sclerotiorum*. No viruses with DNA genomes were detected. Four viruses were selected for further analysis including a negative-sense single-stranded RNA [ssRNA(-)] virus that is related to the plant pathogen *Maize mosaic virus* and other arthropod-transmitted viruses that infect plants and animals. The *S. sclerotiorum* isolate infected with the ssRNA(-) virus grew more slowly and produced fewer sclerotia in culture than isolates not infected with the virus. The nucleotide sequence of the virus genome suggested that it is capable of forming virus particles, and hence may be transmitted extracellularly, which would mean that its transmission may not be limited by vegetative incompatibility. The analysis also identified a large double-stranded RNA virus infecting *S. sclerotiorum* related to *Cryphonectriahypovirus* 1, which has been used to reduce the virulence of the chestnut blight fungus in Europe. The *S. sclerotiorum* isolate infected with the new hypovirus produced dense mycelial mats with extensive hyphal branching in culture. Finally, two viruses with relatively small positive-sense ssRNA [ssRNA(+)] genomes were identified whose sequences were related to a group of soil-borne plant-infecting viruses in the family *Tombusviridae*, at least one of which is transmitted without a vector, i.e., extracellularly. The *S. sclerotiorum* isolates infected with the viruses did not show obvious phenotypes. However, because of their ssRNA(+) genomes and relationship to a soil-borne plant virus that can be transmitted extracellularly, the two viruses may be more suitable than non-encapsidated dsRNA viruses for development of persistent biological fungicides.

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

Metabolomic profiles associated with resistance to *Sclerotinia sclerotiorum* in dry edible beans

Adam L. Heuberger, Mark A. Brick, Howard F. Schwartz, Faith M. Robison, and Jessica E. Prenni, Colorado State University, Fort Collins, CO

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. For dry bean (*Phaseolus vulgaris* L.), however, the molecular and metabolic processes that mediate resistance to white mold (*Sclerotinia sclerotiorum*) are largely unknown. Identifying dry bean metabolites associated with *Sclerotinia* infection may provide novel targets to breed for enhanced resistance. The metabolic changes that occur during *Sclerotinia* infection of a detached leaf were characterized using a non-targeted metabolomics workflow spanning primary and secondary metabolism. A resistant (A195) and susceptible (Sacramento LRK) Andean bean line were inoculated with *Sclerotinia sclerotiorum* isolate S20 for metabolite profiling at 16, 24, and 48 hours post inoculation (hpi). Metabolites from healthy tissue directly adjacent to the necrotic lesion were extracted with methanol: water (80:20) and detected using non-targeted UPLC-MS and GC-MS workflows. The analysis detected 144 metabolites that varied between A195 and Sacramento, with the greatest metabolite variation occurring at 16 hpi. The metabolites that varied included amines, amino acids, saccharides, organic acids, phytoalexins, hormones, ureides, and molecules involved in cell wall and membrane composition. Some of the observed metabolic phenotypes were additionally observed in a stem-inoculation assay. The diversity in metabolic changes observed in the resistant line point towards a multi-faceted mechanism for resistance to *Sclerotinia* in dry bean. The integration of metabolomic and genomic data can be used to discover functional markers of metabolic resistance to white mold.

Contact Information – Adam Heuberger, Proteomics and Metabolomics Facility, Colorado State University, 2021 Campus Delivery, Fort Collins, CO, 80523; 970-491-2273; Adam.Heuberger@colostate.edu

Prevalence of Mating Type (*MAT*) alleles in *Sclerotinia sclerotiorum a*cross the United States and first report of *MAT* heterokaryons

Periasamy Chitrampalam, Chengxiang Qiu, Laura Aldrich-Wolfe, and Berlin D. Nelson, Jr.;

First, second and fourth author, Department of Plant Pathology, North Dakota State University; third author Biology Department, Concordia College, Moorhead, MN

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

ABSTRACT:
Sclerotinia sclerotiorum is a yield limiting pathogen of several economically important crops, and it reproduces sexually by self-fertilization. Based on the presence of an inversion in the mating type locus, *S. sclerotiorum* was recently classified into inversion negative (*MAT* (Inv-)) and inversion positive (*MAT* (Inv+)) isolates. In this study, the distribution of Inv- and Inv+ *MATS sclerotiorum* isolates across the United States was determined. In total, 179 isolates from 23 States and from 17 hosts were evaluated of which 77 isolates were from North Dakota and 52 isolates were from soybean. PCR screening was performed separately for Inv- and Inv+ *MAT* alleles with allelic specific primers. Both Inv- and Inv+ *MATS sclerotiorum* isolates were commonly prevalent across the United States and across the susceptible hosts. Both Inv- and Inv+ *MATS sclerotiorum* isolates were identified from 15 states and in 14 hosts. There was only a single isolate from the other six states. However, Inv- *MAT* isolates were more predominant than Inv+ *MAT* isolates with 51% and 27% of the isolates Inv- and Inv+ *MAT*, respectively. Additionally *MAT* heterokaryons were also identified for the first time in *S. sclerotiorum* with 22% of the isolates *MAT* heterokaryons. In ND which had the largest number of isolates tested, 52, 30, and 18% of isolates were Inv-, Inv+ and *MAT* heterokaryons, respectively. Among the hosts tested, sunflower had a higher percent of Inv- *MAT* isolates (86%).

Contact Information – Periasamy Chitrampalam, North Dakota State University, Walser Hall 306, Fargo, ND 58102; 520-370-8885; perasamy.chitrampal@ndsu.edu

Quantitative trait loci analysis of resistance to white mold in pinto bean

Wezi Mkwaila, Evan Wright and James D. Kelly,
Michigan State University, East Lansing, MI

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 two pinto bean recombinant inbred line (RIL) populations (AP630 and AP647). The two populations were screened with the BARCBEAN6K_3 Genechip consisting of 5,398 single nucleotide polymorphism (SNP) markers. A total of 1024 SNP markers were polymorphic in AP630 and 1016 markers were polymorphic in the AP647 population. The final map assembly resulted in 11 linkage groups corresponding to the 11 chromosomes of *P. vulgaris* in both populations. The two pinto bean RIL populations genotyped with SNP markers resulted in two linkage maps of 1183 and 953 cM and a total of fifty 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, lodging and the greenhouse straw test. These QTL were located across all 11 chromosomes with LOD scores ranged from the threshold of 2.5 to 10.5. Six new QTL for yield under white mold pressure were identified on Pv01, Pv02, Pv03, Pv05 and Pv09. Seven new QTL for white mold were identified on Pv01, Pv04, Pv07 and Pv08 and two others were validated on Pv02 and Pv03.

This study suggests that moderate levels of white mold resistance have been transferred from navy bean into upright type II pinto beans. Agronomic traits exhibiting moderate heritability estimates suggests that advances in breeding simultaneously for multiple traits can be made. However correlations between greenhouse straw test and field disease scores were very weak similar to data observed in other mapping populations. 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 Pv02, Pv03 and Pv07 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.

Contact Information – James D. Kelly, Michigan State University, 370 Plant and Soil Sciences, Dept. Plant, Soil and Microbial Sciences, 1066 Bogue St., East Lansing, MI, 48824; (517) 355-0271 Ext. 1181; kellyj@msu.edu

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

R. Jhala, R. Higgins and J.R. Steadman (University of Nebraska-Lincoln)
Collaborators: J. Kelly (MI), P. Griffiths (NY), M Wunsch (ND), J. Myers (OR), P. Miklas (WA), H. Schwartz (CO), S. Singh (ID), and K. Kmiecik (WI)

Funded Plan of Work: Improved 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 2012-13 greenhouse tests provide evidence for 11 bean lines of various seed types with intermediate levels of white mold (WM) resistance. In the field nursery, all nine 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 and haplotypes. There were no significant differences in aggressiveness between isolates collected from the three control hosts with different levels of resistance to *S. sclerotiorum* in the screening nurseries in any year of collection. However, there were significant differences in aggressiveness between many screening nurseries. There also were examples of significant variation in aggressiveness found between grower field isolates collected in the same state or between states. The development of a large dendrogram using 16 polymorphic microsatellites and UPGMA cluster analysis has enabled us to define 21 clusters in the 366 isolates. The 21 clusters were similar for the three control hosts in the screening nurseries. However, state or country origin isolates exhibited variability in total clusters and distribution between the 21 clusters. Grower field isolates were also variable when compared by state origin. The next step in characterization of isolates is comparison of isolate sensitivity to common fungicides. Expected Outcome: isolates with higher or lower levels of aggressiveness and widely or locally distributed will be available for use in WM resistance screening.

Contact Information – Dr. James R. Steadman, Department of Plant Pathology, University of Nebraska-Lincoln, 406 Plant Sciences Hall, Lincoln, NE 68583-0722, 402/472-3163, jsteadman1@unl.edu.

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

Laureen Blahut-Beatty[1], Lisa Koziol[1], Bernarda Calla[2], Lone Buchwaldt[3], David J. Neece[4], Steven J. Clough[4], and Daina Simmonds[1]

[1]Agriculture and Agri-Food Canada, Ottawa, ON Canada
[2]University of Illinois, Urbana, IL
[3]Agriculture and Agri-Food Canada, Saskatoon, SK Canada
[4]USDA-ARS and University of Illinois, Urbana

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

ABSTRACT:
Effective resistance to Sclerotinia sclerotiorum (Ss) has been obtained with a transformation that expresses an oxalate oxidase (OxO) enzyme in soybean. OxO catalyzes oxalic acid (OA), the major Ss virulence factor. Microarrays and RNAseq were used to examine differential expression in response to Ss infection and OA infiltration in OxO (transgenic resistant line) and its susceptible isogenic parent AC Colibri. Hundreds of genes changed expression significantly, and it is necessary to determine which ones have major roles in defense. The ultimate functional verification requires rating of Ss infection in soybean carrying an over expressed or silenced candidate transgene. Several genes have been selected for functional analysis in soybean, including the G-protein coupled receptor (GPCR),matrix metalloproteinase (MMP), and 14-3-3. Both GPCR and 14-3-3 are more susceptible to Ss when these genes are silenced, indicating a role in defense. The MMP transgenics have not, as yet, been rated for Ss response.

Hundreds more genes require functional verification, a task that cannot, realistically, be accomplished with the labor- and resource-intensive functional evaluation in soybean. Therefore we are investigating several approaches to narrow our list of candidate defense genes to attain feasible numbers for final soybean verification. These include: a) transformation of soybean with oxalate decarboxylase (ODC) to generate an Ss resistant line that does not generate H_2O_2, as is the case for the OxO lines. This will offer novel material for gene expression studies to narrow down the candidate defense gene list, and b) development of a high-throughput screen to eliminate insignificant candidate genes. The model systems under investigation are, i) Arabidopsis using T-DNA knockout lines and over expression, ii) Nicotiana benthamiana using transient expression of infiltrated gene constructs and iii) the viral expression system (Bean Pod Mottle Virus Viral Vector) to screen candidate genes directly in soybean.

Contact Information – Daina Simmonds, Agriculture and Agri-Food Canada, Ottawa, ON, K1A0C6; 613-759-1320; daina.simmonds@agr.gc.ca

Use of a transformation system in sunflower for *Sclerotinia* resistance studies

Zhifen Zhang and John J. Finer
Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH

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

ABSTRACT:
A reliable transformation system in sunflower will provide a useful tool to study and characterize *Sclerotinia* resistance in sunflower. A sunflower confection line RHA280 was previously identified as having a high response to shoot induction. Although large numbers of adventitious shoots have been consistently obtained from cotyledonary explants of this line, the shoot-forming tissue has not been very responsive to transformation. To increase the susceptibility of the dry cotyledonary tissue to wounding for *Agrobacterium* infection, seeds were imbibedfor 1-2 d, resulting in an apparent softening of the target tissue. Different wounding methods, including Sonication Assisted *Agrobacterium*-mediated Transformation (SAAT), vortex, vortex with different types of particles, and particle bombardment, were evaluated and the percentage of explants producing transgenic shoots were 1.6-10%, 0-27.7% 3.3-11%, and 0%, respectively. A large number of non-transgenic adventitious shoots were obtained when 7.5 mg/L hygromycin was used for selection, five days after the end of co-culture. Use of 15 mg/L hygromycin directly following co-culture without a 5 d resting period increased the frequency of transgenic shoot primordia production 3-5x. The percentage of explants having GFP-expressing shoot primordia was almost 30%, with about one transgenic shoot per regenerative explant on average. In the absence of early selection, it seems that the non-transgenic shoot primordia were able to "outcompete" the transgenic shoots and that the timing and level of selection are quite important. From this point forward, we hope that standard optimizations can be performed for more consistent recovery of transgenic sunflower. In addition, efforts are underway to recover transgenic plants from the shoots using micrografting. Oxalate oxidase and other pathogen response genes will be evaluated after some further optimization of the transformation system.

Contact Information – John J. Finer, Department of Horticulture and Crop Science, OARDC/The Ohio State University, 1680 Madison Ave., Wooster, OH 44691; 330-263-3880; finer.1@osu.edu

2014 Sclerotinia Initiative Meeting Participants								
Last Name	First Name	Company	Address	City	State	Zip	Phone	Email
Anderson	James	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1263	james.anderson@ars.usda.gov
AshtariMahini	Rahil	NDSU	166 Loftsgard Hall	Fargo	ND	58108		rahil.ashtarimahini@my.ndsu.edu
Berghauer	Erika	Seminis Vegetable Seed	7202 Portage Rd.	DeForest	WI	53532	608-842-1435	emberg@monsanto.com
Brueggeman	Robert	North Dakota State University	Dept. Plant Pathology, 306 Walster	Fargo	ND	58102	701-231-7078	robert.brueggeman@ndsu.edu
Chao	Wun	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102	701-239-1256	wun.chao@ars.usda.gov
Chen	Weidong	USDA-ARS	303 Johnson Hall, WSU	Pullman	WA	99164	509-335-9178	w-chen@wsu.edu
Chilvers	Martin	Michigan State University	578 Wilson Rd., 104 CIPS	East Lansing	MI	48823	517-353-9967	chilvers@msu.edu
Chitrampalam	Periasamy	North Dakota State University	306 Walster Hall	Fargo	ND	58102	701-231-5134	periasamy.chitrampal@ndsu.edu
Chittem	Kishore	North Dakota State University	306 Walster Hall	Fargo	ND	58102	701-429-8381	kishore.chittem@ndsu.edu
Clough	Steve	USDA-ARS	1101 W. Peabody Dr.	Urbana	IL	61801	217-265-6452	steven.clough@ars.usda.gov
Currie-Gross	Marcie	USDA-ARS	2150 Centre Ave, Bldg D, Ste 310	Fort Collins	CO	80526-8119	970-492-7022	marcie.currie-gross@ars.usda.gov
del Rio	Luis	North Dakota State University	Dept. 7660, P.O. Box 6050	Fargo	ND	58108-6050	701-231-7073	luis.delrio-mendoza@ndsu.edu
Domier	Leslie	USDA-ARS	1102 S. Goodwin Avenue	Urbana	IL	61801	217-333-0510	ldomier@illinois.edu
Elrod	Michelle	Monsanto	1907 Forest Ave	St. Louis	MO	63139	636-737-2456	michelle.a.elrod@monsanto.com
Finer	John J.	The Ohio State University	1680 Madison Avenue	Wooster	OH	44691	330-466-4735	finer.1@osu.edu
Foley	Michael	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1322	michael.foley@ars.usda.gov
Gilley	Michelle	USDA-ARS Sunflower and Plant Bio.	1605 Albrecht Boulevard N	Fargo	ND	58102	701-239-1315	michelle.gilley@ndsu.edu
Gulya	Tom	USDA-ARS NCSL	1307 18th St N, USDA-NCSL	Fargo	ND	58102	701-239-1310	Thomas.Gulya@ars.usda.gov
Heuberger	Adam	Colorado State University	2021 Campus Delivery	Fort Collins	CO	80523	970-491-2273	adam.heuberger@colostate.edu
Hill	Curt	University of Illinois		Urbana	IL	61801	217-244-3257	curthill@illinois.edu
Horvath	David	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102	701-239-1255	david.horvath@ars.usda.gov
Hulke	Brent	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1321	brent.hulke@ars.usda.gov
Jan	Chao Chien	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1319	chaochien.jan@ars.usda.gov
Jiang	Daohong	Huazhong Agricultural University	No. 1#, Shizishan Street	Wuhan	P R China	430070	86 27 87280487	daohongjiang@mail.hzau.edu.cn
Kelly	James D.	Michigan State University	1066 Bogue St.	East Lansing	MI	48824	517-355-0271 x1181	kellyj@msu.edu
Kemp	William P.	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1371	william.kemp@ars.usda.gov
Kmiecik	Ken	Monsanto	7202 Potage Rd	De Forest	WI	53711	608-698-5198	ken.kmiecik@monsanto.com
Lackermann	Karen	Dupont Pioneer	7230 NW 70th Ave. PO Box 177	Johnston	IA	50023	515-535-8089	karen.lackermann@pioneer.com
Liu	Jean	Dupont Pioneer	7230 NW 70th Ave.	Johnston	IA	50131-0177	515-535-2491	jean.liu@pioneer.com
Liu	Zhao	NDSU	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1324	zhao.liu@ars.usda.gov
McClean	Phillip	North Dakota State University	Loftsgard Hall	Fargo	ND	58102	701-231-8443	phillip.mcclean@ndsu.edu
McGuire	Michael	USDA-ARS	2150 Centre Ave., Bldg D, Suite 300	Fort Collins	CO	80526-8119	970-492-7058	michael.mcguire@ars.usda.gov
McPhee	Kevin	North Dakota State University	370G Loftsgard Hall	Fargo	ND	58108	701-231-8156	kevin.McPhee@ndsu.edu
Misar	Christopher	USDA-ARS	819 10th Ave. N. Apt # Basement	Fargo	ND	58102	605-661-2530	christopher.misar@ars.usda.gov
Mou	Zhonglin	University of Florida	Microbiology and Cell Science	Gainesville	FL	32611-0770	352-392-0285	zhlmou@ufl.edu
Myers	James	Oregon State University	Department of Horticulture, ALS 4017	Corvallis	OR	97331	541-737-3083	myersja@hort.oregonstate.edu
Nelson, Jr.	Berlin D.	North Dakota State University	Dept. of Plant Pathology	Fargo	ND	58102	701-231-7057	berlin.nelson@ndsu.edu
Qi	Lili	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1351	lili.qi@ars.usda.gov
Qiu	Chengxiang	North Dakota State University	Dept. of Plant Pathology #7660	Fargo	ND	58102	701-231-5134	chengxiang.qiu@ndsu.edu
Rashid	Khalid Y.	Agriculture and Agri-Food Canada	Research Station, 101 Route 100	Morden	Manitoba, Canada	R6M 1Y5	204-822-7520	khalid.rashid@agr.gc.ca
Rollins	Jeffery	University of Florida	Dept. of Plant Pathology , 1453	Gainsville	FL	32611-0680	352-2734620	rollinsj@ufl.edu
Sandbakken	John	National Sunflower Association	2401 46th Avenue SE	Mandan	ND	58554	701-328-5100	johns@sunflowernsa.com
Santos	Patricia	Michigan State University	578 Wilson,Rd. Room 101 (CIPS)	East Lansing	MI	48824	517-290-4832	psantos@msu.edu
Schafer	Michael	NDSU - Carrington Research Ext. Ctr	PO Box 219	Carrington	ND	58421	701-652-2951	michael.schaefer@ndsu.edu
Scholz	Todd	USA Dry Pea & Lentil Council	2780 W Pullman Road	Moscow	ID	83843	208-882-3023	scholz@pea-lentil.com
Scott	Roy	USDA-ARS	5601 Sunnyside Avenue	Beltsville	MD	20705	301-504-4670	roy.scott@ars.usda.gov
Seiler	Gerald	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1380	gerald.seiler@ars.usda.gov
Simmonds	Daina	Agriculture and Agri-Food Canada	960 Carling Avenue	Ottawa	Ontario,Canada	K1A0C6	613-759-1320	Daina.Simmonds@agr.gc.ca
Steadman	James R.	University of Nebraska	406 PSH - UN-L East Campus	Lincoln	NE	68583-0722	402-472-3163	jsteadman1@unl.edu
Swanson	Kim	USDA-ARS	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1370	kimberly.swanson@ars.usda.gov
Talukder	Zahirul	North Dakota State University	1605 Albrecht Boulevard N	Fargo	ND	58102-2765	701-239-1324	zahirul.talukder@ars.usda.gov
Tentinger	Krystal	Dupont Pioneer	7230 NW 70th Avenue	Johnston	IA	50131	515-535-4087	krystal.tentinger@pioneer.com
Varner	Greg	Michigan Dry Bean Research Bd.	8439 North Blair Road	Breckenridge	MI	48615-9725	989-751-8415	varnerbean@hotmail.com
Wang	Dechun	Michigan State University	1066 Bogue St.,Rm. A384E	East Lansing	MI	48824-1325	517-355-0271 x 1188	wangdech@msu.edu
Wen	Zixiang	Michigan State University	1066 Bogue St., Dept of PSMS	East Lansing	MI	48824-1325	517-355-0271 x 1188	wangdech@msu.edu
Whiting	Kelly	United Soybean Board (SmithBucklin)	16305 Swingley Ridge Road	Chesterfield	MO	63017	314-579-1598	kwhiting@smithbucklin.com
Wilson	Richard		5517 Hickory Leaf Drive	Raleigh	NC	27606-9502	919-906-6937	rfwilson@mindspring.com
Wunsch	Michael	NDSU - Carrington Research Ext. Ctr	PO Box 219	Carrington	ND	58421-0219	701-652-2951	michael.wunsch@ndsu.edu
Zhang	Jichong	USDA-ARS	1307 18th St N	Fargo	ND	58102	701-239-1378	jichong.zhang@ars.usda.gov
Zhang	Zhifen	Ohio State University/OARDC	1680 Madison Avenue	Wooster	OH	44691	330-263-3979	zhang.653@buckeyemail.osu.edu

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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