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Meeting Strategic Milestones of the National Sclerotinia Research Initiative for 2009

Integrated Research for Disease Management in Sunflower, Canola, Dry Bean, Pea & Lentils and Soybean

, Canola,
Dry Bean, Pea & Lentils and Soybean

Executive Summary

Vision Statement: An integrated research approach will guide the effective development of diagnostic technologies, disease management systems, genomic resources, and crop germplasm exhibiting durable resistance to *Sclerotinia sclerotiorum*. Strategic deployment and use of these resources will help sustain the competitiveness of U.S. canola, pea, lentil, chickpea, common bean, soybean, and sunflower producers in domestic and global markets.

Introduction: The *National Strategic Plan for the Sclerotinia Initiative 2008-2012* provides programmatic transparency to all sectors of the agricultural value-chain and gives the research community a foundation for an integrated research approach for mitigating this devastating disease. The performance measures outlined in the strategic plan are relevant to the current needs of US agriculture. Each performance measure defines the actions that will be taken to solve the problem, describes what is promised or will be produced, and provides a mechanism for peer review and assessment of research progress. The current document, *Meeting Strategic Milestones of the National Sclerotinia Research Initiative for 2009*, provides an interim accounting of how the research community has addressed the goals and objectives the plan, and provides the basis for rating overall program performance on an annual basis. This document and information regarding the governance and activities of the National Sclerotinia Research Initiative may be accessed at: **http://www.whitemoldresearch.com/**

Rating Summary:

Sclerotinia Initiative Research Progress Evaluation

	2008	2009	2010	2011	2012
	number of accomplishment citations				
Total Accomlishments	76	61			
Milestones (multiple comps)	17	10			
Milestones (no comps)	30	30			
Milestones (not funded)	NA	14			
Milestones (accomplished)	0	1			
Total Milestones	76	76			
Achievement Rating (%)	56.7	62.2			
Total Projects	34	28			
Accomplishments / project	2.2	2.2			
Publications	47	53			
Germplasm lines released	4	5			
Varieties released	6	5			

Achievement Rating:
((all accomps x (milestones (multi accomps/(no accomps-not funded)))/milestones(all-not funded) x 100) + (milestones accomplished*0.75)

Milestone	2008	2009	2010	2011	2012
Crop Germplasm Resources & Genetics	number of accomplishment citations				
PM 1.1: New sources of resistant plant germplasm					
• *Exploration trips to obtain seeds of wild species*	0	3			
• *Improved germplasm screening methods*	3	3			
• *Increased availability of resistant germplasm*	1	3			
• *Doubled haploid lines for resistance, tested in multiple environments*	1	1			
PM 1.2: Transfer new resistance genes into plant germplasm					
• *Germplasm derived from interspecific crosses*	7	2			
• *Resistant selections of unadapted x agronomic traits*	0	1			
PM 1.3: Genetic analysis & QTL discovery					
• *Highly inbred mapping populations with validated QTLs*	2	4			
• *QTL analysis to generate a high density genetic maps*	3	2			
• *Integrated linkage maps*	4	1			
PM 1.4: Pyramid white mold resistance genes					
• *Improved conventional breeding methods for quantitative traits*	1	2			
• *Breeding populations segregating for multiple resistance alleles and other traits*	3	1			
PM 1.5: Marker-assisted selection					
• *Disease reaction of RILs in multiple field environments*	1	1			
• *Marker-assisted selection protocol for more efficient genotyping*	4	1			
• *Field verification of resistance*	3	1			
PM 1.6: GM improved resistance					
• *GM oxalate oxidase expression, inheritance and field evaluation*	1	1			
• *Catalog of candidate resistance genes, promoters, and constructs for transformation*	2	1			
• *Perlka-resistant soybean lines*	1				
• *Transgenic expression of antifungal peptides*	0	0			
PM 1.7: Plant germplasm/cultivars with improved resistance					
• *Enhanced adapted germplasm*	2	1			
• *Herbicide tolerance with resistance to Sclerotinia*	1	1			
• *Agronomic resistant varieties for commercial production.*	1	2			
• *Identifycrop germplasm with partial resistance to virulent isolates*	1	1			
Pathogen Biology & Mechanisms of Disease Resistance					
PM 2.1: Population structure & dynamics					
• *Standardized genotypic characterization on wild and cultivated crops*	1	1			
• *Defined environmental requirements for pathogen biotype germination & disease*	1	0			
• *Documented gene-flow or outcrossing contribution to population variability*	1	0			
• *Geographical inventory of US populations*	1	1			
PM 2.2: Durable host resistance					
• *Isolate virulence/aggressiveness across geographic areas and hosts*	2	0			
• *Pathogen population dynamics on partially resistant crops*	0	1			
• *Pathogen x environmental interaction*	0	1			
• *Knowledge of plant x pathogen x environmental interactions*	1	1			
• *Criteria for testing virulence/aggressiveness on specific hosts.*	1	1			
PM 2.3: Factors that mediate sclerotia germination					
• *Host factors that mediate myceliogenic germination*	2	1			
• *Defined envronmental requirements for pathogen biotype germination & disease*	0	0			
• *Effect of sclerotiasphere microbes on germination and dormancy*	0	0			
• *Effect of sclerotiasphere microbes on mycelial growth*	0	0			
PM 2.4: Genetic markers and molecular tools for pathogen biology					
• *Reporter gene constructs with inducible promoters, insertional mutant libraries*	0	0			
• *Standard molecular protocols to genotype isolates*	2	1			
• *Transformed isolates for host/pathogen & pathogen/microbe interactions*	1	2			
PM 2.5: EST libraries from pathogen stains					
• *Useful cDNA libraries from pathogen expressed genes*	1	1			
• *Useful genome sequence information*	1	0			
• *Full length, normalized cDNA libraries*	1	0			

Pathogen Biology & Mechanisms of Disease Resistance	2008	2009	2010	2011	2012
PM 2.6: Candidate genes for pathogenicity	number of accomplishment citations				
• *Large ATMT collections for phenotypic screens*	0	0			
• *Transcriptome profiles and high through put functional analyses*	0	0			
• *Promoters for RNAi constructs during infection*	0	0			
• *Catalog genes from ATMT random mutagenesis*	0	0			
• *Discovery of candidate gene function*	0	0			
Crop Genome Analysis and Genomic Tools					
PM 3.1: DNA markers for QTL identification and marker assisted selection					
• *Affordable high-throughput genotyping and phenotyping technology*	1	2			
• *High density genetic map of DNA markers for resistance*	2	1			
PM 3.2: Structure of resistance gene enriched genomic regions					
• *Extensive cDNA libraries from host tissues at different stages of infection*	1	1			
• *DNA markers from BAC-ends to anchor contigs to genetic maps*	1	1			
• *Physical map of genomic regions containing resistance genes*	0	0			
• *High through-put resequencing capacity and haplotype maps*	0	0			
PM 3.3: Function of candidate resistance genes					
• *Microarrays for high throughput gene screening*	2	1			
• *Sequenced cDNA libraries from infected host tissue*	0	0			
• *Discovery of candidate genes for Sclerotinia resistance*	1	1			
• *Function of candidate genes using gene silencing methods*	2	1			
PM 3.4: Mechanisms of Sclerotinia resistance					
• *Yeast screens for ecotypes and defense-related mutants for oxalate sensitivity*	0	0			
• *Efficacy of GM traits against Sclerotinia*	1	1			
• *Conventional analysis of genetic mechanisms*	1	1			
PM 3.5: Bioinformatic resources					
• *Web-based communication for the Sclerotinia Initiative*	1	1			
• *Interactive website for genetic, genomic & biotech resources*	1	1			
Disease Management & Pathogen Epidemiology					
PM 4.1: Optimized fungicide application programs					
• *S. sclerotiorum isolate collection to assess fungicide sensitivity*	0	1			
• *Efficacy of new chemistries*	2	1			
• *Updated management guides for disease management*	0	1			
• *Improved spraying technologies*	0	0			
PM 4.2: Bio-control alternatives for disease management					
• *Grower recommendations for commercial sclerotial antagonists*	0	1			
• *Catalog of commercial microbial biocontrol agents*	0	0			
• *Efficacy of Sporidesmium sclerotivorum as a biocontrol agent*	0	0			
• *Updated management guides for biofungicides in disease management*	0	0			
PM 4.3: Quantitative models for environmental and host-crop interactions					
• *Disease warning systems*	1	0			
• *Validated predictive models in other crops.*	0	0			
• *Yield loss models*	0	0			
• *Threshold levels for decision aids*	0	0			
PM 4.4: Optimized cultural practices for disease management					
• *Improved variety selection criteria*	0	0			
• *Management decision aids*	1	1			
• *Precision agriculture program*	0	0			

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Germplasm lines	**4**	**5**
Varieties	**6**	**5**

Not funded

Overall Program Major Accomplishments 2008-2012

- A *Sclerotinia* risk map for **dry bean** producers and continued validation and expansion of risk maps for **canola**.
- Screening methods that enable identification of Sclerotinia resistance in wild & cultivated species of **sunflower**, **canola, pea, lentil, dry bean, soybean** in greenhouse and field settings.
- New sources of resistance in wild species of **pea, canola, common bean, sunflower**
- Transfer of resistance from wild species to cultivated types of **dry bean, sunflower**
- QTL discovery (gene locations) for resistance to white mold in **canola, pinto bean, pea, common bean** and **soybean**.
- Pyramiding QTL for resistance in **sunflower** and **soybean**
- Microarrays for expressed genes in soybean
- Marker Assisted Selection for resistance in **pinto bean, sunflower, common bean, soybean**
- A high density SNP marker map for Sclerotinia resistance in **soybean**
- Protocol for transformation of **sunflower** germplasm
- Standardized protocol for genotypic characterization of **kidney, pinto** and other cool season bean crops
- First characterized geographic inventory of Sclerotinia isolates from **canola, dry bean, filed pea, lentil, soybean and sunflower**
- Library of **pathogen** genes expressed during white mold infection of host crops
- Oxalate oxidase-minus pathogen mutants that showed oxalate was not necessary for pathogenicity, but did enhance virulence in **tomato, canola, sunflower, soybean**
- Over expression of oxalate oxidase genes in **soybean** enhanced resistance of white mold.
- Germplasm releases with improved tolerance to white mold in **pinto bean (1), pea (3), sunflower (3), common bean (2).**
- Variety releases with improved resistance to white mold in **pinto bean (3), soybean (3), common bean (2), lentil (1), great northern bean (2).**
- A comprehensive **National Sclerotinia Initiative website** to serve the needs of the agricultural community and provide educational information to the general public.
- Efficacy of *Coniothyrium minitans* as a **biological control agent** for white mold in various crops.
- Crop management decision aids for control of Sclerotinia in **dry beans**.
- Collaboration with the ***Sclerotinia sclerotiorum* whole genome sequencing project**, the **Soybean Genomic Research program**, the **Phaseolus CAP** grant, and the **Legume Information System.**

Meeting Strategic Milestones for Sclerotinia Research-2009

Crop Germplasm Resources & Genetics

PM 1.1: New sources of resistant plant germplasm

- *Exploration trips to obtain seeds of wild species*

ARS scientists at Pullman WA identified resistance sources in wild relatives of grain legumes. Wild pea species were collected during the first year of the project: *Pisum fulvum* (33 lines), *Pisum sativa subsp. abyssinicum* (27 lines), *Pisum sativum subsp. elatius* (45 lines), *Pisum sativum subsp. transcaucasicum* (4 lines). The thirty-three lines of the *Pisum fulvum* have been evaluated for resistance to *Sclerotinia sclerotiorum*. Sixteen seeds (four seeds/pot) of each line of Pisum fulvum were planted in the greenhouse. Four weeks after planting, an agar plug from actively expanding colony of S. sclerotiorum strain WM-A1 were place at the third internode with a piece of parafilm of each plant (12 plants per line) and an plain agar plug as control on four plants. Disease progress was monitored by measuring lesion expanding on the stem every other day for eight days. All *Pisum fulvum* lines tested were susceptible to *S. sclerotiorum*. However, there were two lines that showed significantly less lesion expansion. The results are being confirmed by repeating the experiment. The additional lines of wild legumes will be screened. This project directly impacts the Sclerotinia Initiative Goal of Crop Germplasm Resources and Genetics.

Scientists at NDSU identified six *Brassica napus* accessions from the rapeseed plant introduction collection having high levels of resistance against *S. sclerotiorum* when inoculated using the petiole inoculation technique (PIT). Plants from these accessions were screened again and crossed (resistant x resistant). 230 F2 seeds from (PI458939 x Ames 26628) were inoculated in the greenhouse using the PIT; 23 days later, 69 plants survived the inoculation and were advanced for seed production.

Scientists at Idaho State University and Colorado State University verified White Mold Resistance of Dry and Green Common Bean and Interspecific Breeding Lines Derived from Phaseolus Species of the Secondary Gene Pool. Interspecific populations were created between P. coccineus (G35771, G35772, G35006, PI 439534, PI433246); P. costaricineus (S33720,); P. polipinthus (G35877); and cultivated common bean cultivars (ICA Pyao, Othello, V1320, 5593). Nine lines from these crosses demonstrated high levels of resistance to Sclerotinia. White mold reaction of known contemporary resistant large-seeded (A 195, G 122, MO 162, PC 50, VA 19) and small-seeded (ICA Bunsi) dry and green (CORN 501) common bean and interspecific breeding lines (IBL, VCW 54, VCW 55, VRW 32, 92BG-7, I9365-25, 0785-127-1, 0785-127-2, 0785-220-1) was verified in two greenhouse environments in Idaho and Colorado (June 2008 to May 2009). Five large-seeded dry bean genotypes (A 195, G 122, MO 162, PC 50, VA 19) and three small-seeded IBL (VCW 54, 92BG-7, 0785-220-1) derived from P. coccineus and one IBL (VRW 32) derived from P. costaricensis with the highest WM resistance across greenhouse environments were selected for the complementation study.

- *Improved germplasm screening methods*

Scientists at NDSU, University of Minnesota, Agric. AgriFood Canada, and ARS at Fargo ND improved the efficiency and efficacy of the Sclerotinia misting nursery. In 2009 an exploratory misting system protocol experiment was established to compare the two types of misting systems currently used at the Carrington Research Extension Center across a range of daily water application rates in order to quantify the microclimate created by the systems and to compare their ability to foster Sclerotinia in sunflowers. The experiment consists of five unreplicated treatments. These treatments are designed to compare the 10 ft and the 20 ft grid systems at their current standard application rates and at equivalent application rates by adjusting the frequency and duration of misting. Weather stations and data loggers are being used to monitor hourly air temperature, relative humidity, application water volume and frequency, leaf wetness, and soil moisture. The goal of this initial study is to decide which subset of treatments to include in a replicated study for the 2010 growing season. The overall goal of the project is to improve the efficiency and efficacy of the misting systems to foster Sclerotinia development for screening by better understand the environmental conditions necessary to foster the development of Sclerotinia and by providing data to help operators make management decisions for current misting system.

Scientists at the University of Nebraska, Oregon State University, Michigan State University, Colorado State University, Cornell University, NDSU, Idaho State University, Seminis Seeds, Harris Moran Seed Co., Syngenta, and ARS at Prosser WA identified resistance to *S. sclerotiorum* in adapted common bean lines. The hypothesis was that screening difficulties can be reduced by using multiple location screening sites and understanding the role of pathogen variation in the screening system. Preliminary results from greenhouse screening tests at three locations support the existence of variation between locations even with all participants using the same straw test and disease reaction rating scale. For example, a bayo seed type is more resistant, less resistant and no difference compared with a black seed type in the three tests. The resistance check also varied from susceptible to intermediate across the test locations. Different isolates were used in these three locations and this could explain the different reactions. Thus the value of using multiple tests is still evident. Aided by the multi-site nursery data over the past 3 years, a snap bean, two pinto lines, a bayo line and six kidney lines were released with WM resistance. New lines with WM resistance from wide crosses are now being sent for greenhouse screening. The multi-site field tests combined with greenhouse results have assisted identification of disease escape or avoidance in some lines, e.g. when a line is resistant or intermediate in the field but susceptible in the greenhouse test, there would be evidence that the field response is due to maturity or architecture disease avoidance.

ARS scientists at Ames IA and Fargo ND developed a reliable protocol for greenhouse screening of wild sunflower species. This method enabled evaluation of 51 Helianthus species in 66 taxa, including 37 perennial and 14 annual accessions.

- *Doubled haploid lines for resistance, tested in multiple environments* ***(from 1.6)***

Scientists from NDSU bred F1 seeds from *S. sclerotiorum*-resistant plant introductions Ames 26628, PI458940 x Ames 26628 to produce double haploid lines. Of 105 inoculated plants, 9 were considered highly resistant to Sclerotinia while 10 others were considered moderately resistant. Only three plants produced self-pollinated seeds. An additional 125 Ames 26628 plants had their chromosome numbers doubled before being screened for their reaction to *S. sclerotiorum*.

- *Increased availability of resistant germplasm*

ARS Scientists at Fargo identified new sources of Sclerotinia stalk rot resistance in a large group (250) of USDA Plant Introductions, in comparison with 11 elite USDA released sunflower germplasm. The Plant Introductions were genetically very diverse, and originate from 30 countries, and are in various stages of development. 250 PIs and the 11 elite USDA lines were planted at six locations with artificial inoculation to induce Sclerotinia stalk rot. Four datasets were generated (two locations had high rainfall or flooded, which produced unusable data) over the 2-year period. In 2008, stalk rot severity across entries ranged from 1.6 to 100%, with an average of 54%, while in 2009, the same entries ranged from 2.3 to 79%, with a trial average of 28%. Lower disease in 2009 is probably due to cooler temperatures and more rainfall than in 2008. Of the top 25 entries identified in 2008, 9 were in the top 25 entries in 2009, with a Pearson correlation between the two years' data of 0.54. The top 25 entries, using both years' data, included 5 of the 11 elite USDA lines, and 20 Plant Introductions from ten different countries, ranging from Paraguay to Mexico to Russia to Zambia. This diversity of origin among the highly stalk rot resistant entries confirms that these sources will also enhance the genetic diversity when used as breeding material. This study has produced consistent, reliable data on the stalk rot resistance of 261 sunflower germplasm, using field evaluations of adult plants. By testing over multiple environments, we have confidence that these results accurately predict the resistance potential of this germplasm against Sclerotinia stalk rot. The material identified with high levels of resistance will form the basis of continued improvement of the unit's effort to develop both oilseed and confection sunflower germplasm with stalk rot resistance. Continued efforts to evaluate for head rot resistance will eventually help us identify those accessions with high levels of resistance to both forms of Sclerotinia attack. Additionally, these data sets will be used in association mapping, which is one more step to make our selection of Sclerotinia resistance material more efficient.

Scientists at Idaho State University and Colorado State University screened contemporary white Mold Resistant Pinto Bean Cultivars and Breeding Lines**. In a** parallel study, WM resistant pinto germplasm line USPT-WM-1, „Chase□, and 78 F5 families developed from two double-cross (USPT-WM-1/CORNELL 601//USPT-CBB-1/92BG-7 and Chase/I9365-25//ABL 15/A 195) populations were evaluated for their disease

reaction (June 2008– May 2009). A randomized complete block design with three replicates was used. Each plot consisted of six plants. Two mycelial plugs from 48 hr old culture of *S. sclerotiorum* were used in each of the two inoculations, using the modified straw-test. White mold disease severity was recorded 33 days post the second inoculations (DPI) on individual plant basis and the 6 resistance reaction verified at maturity. Pinto bean genotypes with the lowest WM scores will be used as the recipient parents in crosses with the selected PWMR genotypes to be developed through the gamete selection

Scientists at Michigan State University have identified new sources of resistance in soybean. 6,520 soybean plant introductions (PIs) were tested for resistance to *S. sclerotiorum* at many locations including Michigan State University and 68 PIs with partial resistance were identified. In the past few years, we made crosses to combine resistance from seven of the 68 new PI sources with resistance from old resistance sources such as S19-90. F4 derived lines from these crosses are at various stages of evaluation for agronomic traits and for resistance to Sclerotinia stem rot. The resistance genes in the five new resistant PIs are unknown. Populations that are segregating for genes derived from each of these PIs were developed and these populations will used for the study of the resistance genes in these PIs

PM 1.2: Transfer new resistance genes into plant germplasm

- *Germplasm derived from interspecific crosses*

ARS scientists at Fargo ND have discovered resistance to both Sclerotinia stalk and head rot in perennial *Helianthus* species and have created populations of interspecific (hexaploid x cultivated; diploid x cultivated; amphiploid x cultivated BC populations, all 2n = 34. Field and greenhouse evaluations indicated excellent stalk rot and head rot resistance in six amphiploid parents. Amphiploids resistant to stalk and head rot were crossed with HA 410 in 2006, and BC2F2/BC3F1 plants with chromosome numbers ranging from 2n=34 to 36 were established in the greenhouse for further backcrosses or seed increase in 2008. Interspecific F1 progeny were produced between stalk rot resistant hexaploid *H. californicus* and *H. schweinitzii* and HA 410. Backcrossing of *H. californicus* crosses with HA 410 and selfing resulted in BC4F3 plants with 2n chromosome numbers between 34 and 37. Those progenies were further backcrossed or seeds increased for field evaluation.

Scientists at Idaho State University and Colorado State University conducted a complementation study of White Mold Resistance**.** Five large-seeded dry bean genotypes, namely A 195, G 122, MO 162, PC 50, VA 19 and three WM resistant IBL derived from *P. coccineus*, namely VCW 54, 92BG-7, 0785-220-1 were crossed within the group. Also, G 122/VCW 54 cross was made (October 2008 – January 2009). Approximately 50 seeds of each single-cross were produced. The parents and a part of seed from the eight single-cross F1 were evaluated for reaction to WM to determine complementation or lack thereof (preliminary test) and produce the F2 seed (February – May 2009). All five large-seeded Andean dry beans and their four F1 were resistant to WM (score of 4 on a 1 to 9 scale, where 1= healthy with no WM symptoms and 9= severely diseased or dead), indicating that they probably carried similar resistance genes/QTL. While the VCW 54/0785-220-1 F1 also was resistant, in crosses with 92BG-7 both IBL exhibited a susceptible WM reaction. Thus, very likely VCW 54 and 0785-220-1 had the same resistance genes/QTL, but both were different from 92BG-7. ICA Bunsi and Cornell 501 would need to be crossed with G 122 and VRW 32 would need to be crossed with VCW 54 and 92BG-7. Also, all parents, F1, and F2 will need to be evaluated in a replicated trial to verify and determine the complementation or lack thereof of WM resistance (June 2010– May 2011).

- *Resistant selections of unadapted x agronomic traits*

ARS scientists at Fargo ND have transferred resistance genes from interspecific amphiploids into a cultivated background, and studied the inheritance of resistance. Crosses between NMS HA89 and head rot resistant *H. maximiliani* and *H. nuttallii* were advanced to BC1F3 and BC2F3 in the field for seed increase. stalk rot resistant diploid perennial *H. maximiliani, H. giganteus,* and *H. grosseserratus* were crossed with HA 410 and their BC1F2/ BC2F1 progenies with 2n=34-35 chromosomes were obtained in 2008. Their selfed BC1F3 and BC2F2 progeny were grown in the field in 2009 for seed increase or for stalk rot resistance evaluation. Replicated field tests with 163 and 313 progeny families screened for head and stalk-rot resistance, respectively, indicated moderate to good resistance indicating successful gene introgression. A molecular tracking study indicated a higher frequency of gene introgression from diploid perennials than from hexpaloid or interspecific amphiploids.

PM 1.3: Genetic analysis & QTL discovery

- *Highly inbred mapping populations with validated QTLs*

Scientists at Michigan State University developed pinto beans with enhanced levels of resistance to white mold and identified QTL associated with white mold resistance. The AP630 population has variation for disease incidence. Mean values for yield ranged from 29 to 46 cwt/acre. Most of the RILs also exhibited mid season (90 days) to late maturity (>100 days) in relation to the AN37 parent which has late maturity. Significant differences in height ($p<0.05$) were also recorded ranging from 42-52 cm in 2008 and 46 to 59 cm in 2009. Disease scores ranged from10 to 90% in all three seasons with P07863 consistently having the desirable combination of high yield (> 40cwt/acre) and low disease incidence (<20%). Lodging and height were associated with disease incidence ($r=-0.40$; $p<0.1$) in this population and this finding confirms that these agronomic traits contribute towards disease avoidance in the field. Large differences were also observed in the disease scores across the three years which is expected as white mold is highly influenced by the environment. The 2009 season characterized by predominantly cooler temperatures was particularly conducive for disease development.

Scientists at NDSU and Washington State University discovered effective QTLs conferring resistance to white mold in peas in the segregating populations of PRIL-17 and PRIL-19. Both parents were developed from resistant pea germplasm recently identified in the *Pisum* core collection. F2 seed from PRIL-17 and PRIL-19 populations were grown in greenhouses to advance generations and produce F3 family seed for initial screening for reaction to Sclerotinia infection. Several morphological data were collected on the individual F2 plants to contribute to mapping the resistant phenotype based on F3 family data. Leaf tissue was also collected from each plant for genotyping and genetic map development. A single F3 seed was planted in the greenhouse in the fall of 2008 to advance to the F4 and toward development of recombinant inbred lines. The remaining F3 seed was provided to Dr. Porter and remnant F4 seed from the Fall 2008 increase will be provided to Dr. Porter in Prosser, WA for screening against Sclerotinia infection. F4 seed of PRIL-17 was planted in the greenhouse in the spring of 2009 at Fargo and in the fall of 2009 F4 seed was planted in the greenhouse at Minot for PRIL-19. F5 seed of PRIL-17 was planted in the greenhouse at Fargo in the fall of 2009. F3 family lines from the mapping population PRIL-19 and PRIL-17 were screened twice in replicated growth chamber trials for resistance to *Sclerotinia sclerotiorum* at 21.1ºC and 100% RH. A maximum of four plants were screened per line per screening trial by inoculating the plants at the fourth node. Lesions that developed on individual plants were measured 48 hours after inoculation. Plants were then placed in a growth chamber at 21.1ºC with a RH of 70% for one week and the advancement of the lesion was classified according to the following severity values: 0 = plant did not survive, 1 = lesion expanded from the 4th to the 1st node, 2 = lesion expanded from the 4th to the 2nd node, 3 = lesion expanded from the 4th to the 3rd node, and 4 = lesions did not expand beyond the initial inoculation point at the 4th node. Approximately fifty percent of the lines from the PRIL-19 population survived when assessed nine days after inoculation per screening trial. This may indicate that there is a major resistance gene associated with the survival of plants inoculated with *S. sclerotiorum*. Individual lines of plants that survived were transferred to 6 inch pots and propagated in a greenhouse to generate seed. These lines will be further screened for resistance in an effort to identify the most resistant pea lines from the PRIL-19 mapping population with resistance to *S. sclerotiorum*. The F3 PRIL-17 population has been screened but the data has not be processed regarding this line. Resistant information gathered from each pea line from each population will be compared to DNA extractions from each line to identify genes and QTLs associated with the resistance.

Scientists at Oregon State University developed 2 interspecific populations of P. coccineus x P. vulgaris (91G/PI433251B) and (MO162/PI433251B). Three QTL representing 35% of variations were discovered in the BC2F4:6 91G population. Six SSR markers were verified in BC2F4:5 lines from the MO162 population.

Scientists at the University of Nebraska and the University of Missouri mapped LysM domain genes in soybean to QTL associated with WM resistance. LysM genes encode receptor like kinases which are involved in chitin degradation. Three SSR markers (Satt 172C, Satt 458, Satt 271) flanked a QTL on LG D1a. SSR markets (Satt182, Satt143, Satt134, Satt401) flanked a WM resistance QTL on LG L.

- *QTL analysis to generate a high density genetic maps*

ARS scientists at Prosser WA and NDSU identified genes differentially expressed in response to WM infection in common bean populations segregating for major resistance QTL. Fine mapping and gene

expression was characterized for Pv8 QTL. 1) Total RNA was extracted from GH-straw test-inoculated R and S NILs for the Pv8 QTL as a trial run. Five R lines and five S lines were sampled 48h post inoculation and pooled to form separate R and S pools. mRNA and cDNA for both pooled samples await advanced generation Solexa sequencing which will replace suppression subtractive hybridization as a means to detect differentially expressed genes in the resistant lines This change improves the project because its increased sensitivity to discovering all of the genes expressed in response to the pathogen and the opportunity to define expression patterns of many more genes than by subtractive hybridization. 2) LsyM (chitin receptor) was investigated as a candidate gene for WM resistance in common bean. LsyM sequence was located in the Soybean genome on Gm02 near to a soybean WM resistance QTL. A putative LsyM gene for common bean aligns perfectly with the LysM motif located on Gm02. This Gm02 region in soybean has synteny with the Pv8 WM resistance QTL region in common bean. To date no polymorphism for specific mapping of LsyM has been detected in common bean. 3) The common bean-soybean syntenty approach (see Project Description this proposal) was used to add six additional gene-based EST markers at the terminal end of Pv8 linage group to increase marker density of the region for fine mapping the QTL. 20 more markers were added to this region.

- *Integrated linkage maps*

ARS scientists at Prosser WA and NDSU developed a comprehensive integrated linkage map of WM QTL in common bean. 37 QTL were discovered on 22 regions across 9 linkage groups. A high degree of synteny was found with soybean. Putative LysM gene on the PV8 LG for common bean aligned with GM02 near soybean WM QTL.

PM 1.4: Pyramid white mold resistance genes

- *Improved conventional breeding methods for quantitative traits*

ARS scientists at Fargo ND have pyramided head and stalk rot resistances into elite sunflower lines. We are continuing to backcross head rot QTL from the HA 441 x RHA 439 population into confectionery and elite oilseed backgrounds. BC1F1 hybrids were produced and selected progeny based on marker profiles at 6 major QTL loci for advancement into another backcross.

ARS scientists at Prosser WA developed three F2:4 populations that segregate for two QTL in pinto x GN breeding lines.

- *Breeding populations segregating for multiple resistance alleles and other traits*

Scientists at Michigan State University to date, a total of 34 QTLs are associated with this trait. Three QTLs were identified in the soybean variety S19-90. Thirty-one QTLs were identified in soybean varieties Corsoy 79, Dassel, DSR173, IA2053, Kottman, PI 391589A, PI 391589B, S19-90, Vinton 81, and Williams 82. Of the 34 QTLs, only 9 QTLs were confirmed in at least two populations. Results from QTL studies clearly demonstrate that resistance to Sclerotinia stem rot in soybean is controlled by many genes and each gene has a relatively small effect. Therefore, pyramiding resistant genes from different sources may increase the level of resistance. The resistant germplasm AxN-1-55 is a good example of increasing the resistance level by combining resistance from different sources. AxN-1-55 was developed by combining the resistance from two partially resistant varieties, S19-90 and A2506, and its resistance level to the disease is higher than both parents.

PM 1.5: Marker-assisted selection

- *Disease reaction of RILs in multiple field environments*

Scientists at Michigan State University evaluated pinto bean RILs for resistance to white mold in the greenhouse through the straw test (2008) and the oxalate test (2009). In general both tests were able to separate the two parents (AN 37 = 3.0 and P02630 = 5.0 $p<0.05$) and the highly resistant from the susceptible RILs however the oxalate test was not found to be very suitable to distinguish the intermediate reaction observed in the field in some of the RILs for this population. Parents were screened with 150 SSR markers 46 of which were found to be polymorphic. 26 of these markers were assayed across the population and mapped using Joinmap 4.0 and single marker analysis. Nine of the 26 polymorphic markers evaluated in the population showed segregation distortion of the expected 1:1 ratio of the parental alleles. 13 of the markers were significant with the chi-square goodness of fit. The partial linkage map was generated from these markers and

the association of markers and field disease score and yield was determined by composite interval mapping performed by QTL Cartographer. A QTL for resistance to white mold in the field was identified near markers IAC 67, BMd42 and Bm157. This QTL did not appear in 2008 and this is not surprising as the environment plays an important role in the development of disease in the field. However, a QTL for yield between markers Bmd17 and Pvat011 on linkage group 1 was present in both 2007 and 2008. This is consistent with yield data obtained from the three seasons and emphasizes the need to include yield under white mold pressure when selecting for resistance to white mold.

- *Marker-assisted selection protocol for more efficient genotyping*

ARS Scientists at Fargo ND used traditional breeding to develop F4 to F7 sunflower selfing generations for Sclerotinia head and stalk rots at two locations. Association mapping with 260 Plant Introductions from the North Central Regional Plant Introduction Station at Ames, IA are moving forward. Two locations were added with two replications. Based on the distribution of data, which is more broadly and normally distributed than we were expecting, we believe that the phenotypes will be adequate for association mapping. We are currently working on developing the marker set for our resistance candidate genes, which will contribute the genotypes for association mapping.

- *Field verification of resistance*

ARS scientists at Prosser WA conducted QTL interaction studies in common bean. 1) Inbred populations segregating for both Pv7 QTL and Pv8 QTL were characterized for disease reaction in the straw test. The QTL had an additive effect in one population, and in the other population the Pv8 QTL had major effect only. Field trials with these populations were conducted but stands were poor which reduced WM disease severity. 2) Field trials will be repeated in 2010. Field trial with a population segregating for Pv2 and Pv8 QTL showed a major effect for Pv2 QTL and minor effect for Pv8 QTL, a slight additive effect was shown. The field trial will be repeated and straw test initiated in 2010.

PM 1.6: GM improved resistance

- *GM oxalate oxidase expression, inheritance and field evaluation*

Scientists at the Ohio State University developed an improved transformation protocol for sunflower with RHA germplasm. Several new and effective promoters were identified. GFP expression was enhanced with isoxaflutole treatment.

- *Catalog of candidate resistance genes, promoters, and constructs for transformation*

- *Perlka-resistant soybean lines* ***Completed in 2008.***

- *Transgenic expression of antifungal peptides*

PM 1.7: Plant germplasm/cultivars with improved resistance

- *Enhanced adapted germplasm*

Scientists at NDSU, University of Minnesota, Agric. AgriFood Canada, and ARS at Fargo ND developed a system to generate reliable, reproducible data on the genetic resistance of sunflower germplasm to Sclerotinia head rot. The system was used to screen: 1) sunflower germplasm for improved resistance to head rot and stalk rot effort; 2) commercial and experimental sunflower hybrids for improved resistance to head rot at multiple sites. Head rot screening nurseries using the misting system procedures were utilized across all cooperating research sites. Commercial and experimental sunflower hybrids were screened for improved resistance to head rot. Sunflower companies with breeding programs submitted at least 75 hybrids for screening in an “Initial Test” at Carrington and Morden. A series of smaller screening nurseries referenced as the “Repeat Test” were established at Carrington, Langdon, Morden, and Oakes. The “Repeat Test” is designed to include a group of 20 hybrids that exhibited the best resistance to both Sclerotinia head and stalk rot based on the results from the previous year’s evaluations. In 2008, both the Carrington and Morden sites achieved very good head rot disease pressure for the evaluation of 75 hybrids in the “Initial Test”, a group of hybrids not previously screened. The sunflower hybrids in the Carrington site had head rot incidence levels of 26% to 97%, while the same hybrids at Morden ranged from 11% to 54% infection. The combined analysis indicated that 7 hybrids had head rot infection levels that were numerically lower than the best resistant check. A total of 43

hybrids had head rot incidence levels statistically equal to the resistant check hybrids. These results show that seed companies continue to develop hybrids that reflect improved tolerance to Sclerotinia head rot. The disease pressure among the four locations where the 'Repeat Test' was conducted in 2008 varied, however each site had adequate head rot incidence to effectively evaluate hybrid differences. The average head rot incidence at the four test locations was 68%, 44%, 27%, and 31% for the Carrington, Langdon, Morden, and Oakes sites respectively. The average incidence of head rot among hybrids in the "Repeat Test" across all four test sites
revealed that a number of hybrids continued to provide a high level of tolerance to head rot infections since these same hybrids were identified as having good resistance based on the 2007 "Initial Test" results. The project continues to move forward with the successful establishment of field trials and misting nurseries for the 2009 season. The USDA-ARS germplasm evaluations, "Initial Test", "Repeat Test", fungicide evaluations, and misting system methodology experiments were all established by the end of May.

- *Herbicide tolerant germplasm with resistance to Sclerotinia*

Scientists a NDSU identified herbicide tolerant materials with enhanced resistance to *S. sclerotiorum via* evaluation of 95 advanced breeding lines. In 2008, three lines, 0330416, 30457, and 0427681 had higher levels of resistance to *S. sclerotiorum* than Invigor 5550 a commercial hybrid control. In 2009, three lines, 9023, 9028, and 9028 had the lowest incidence of Sclerotinia stem rot, although their incidence was not significantly different than that of two commercial controls (DKL 70-55 and DKL 52-41).

- *Identification of crop germplasm with partial resistance to virulent isolates (**from 2.2**)*

- *Agronomic resistant varieties for commercial production.*

Scientists at Oregon State University examined the phenotypic interaction among major QTL conferring partial resistance to WM in common bean**.** WM resistant common bean cultivars were bred using a backcross inbred approach to transferring resistance QTL from *P. coccineus* to *P. vulgaris*. A backcross-inbred (BCIB) approach combined with molecular marker analysis was devised to introgress the high degree of WM resistance found in *P. coccineus* into *P. vulgaris*. Certain parental combinations were more productive than others, leading to a focus on four populations 91G/PI 255956 (Gx25), 91G/PI 433251B Gx43, G122/PI 433251B (22/43), and MO162/PI433251B (Mx43). In FY06-07, we first advanced and characterized the Gx25 population (consisting of 115 lines) by straw testing three times, once with the oxalate test, and once in the field. Seventy-seven of 98 polymorphic SSRs were scorable in the progeny, and 2 revealed single introgressions. A single pair of AFLP primers amplified 59 scorable segregating fragments. The linkage map consisted of 11 linkage groups that corresponded to 9 of the 11 core map linkage groups. Twenty-nine potential loci associated with WM resistance were identified through single factor analysis. CIM revealed QTL on Pv2, Pv6 and Pv9 that collectively explained 34.7% of the phenotypic variation. The QTL on Pv2 and Pv6 were also associated with resistance in the straw test and explained 18.6% of phenotypic variation. Pv1, Pv5, and Pv8 were not represented in this population and a high level of segregation distortion was observed. All but three SSR loci had significantly significant residual heterozygosity. Recombination between species was greatly suppressed as shown by the fact that whole linkage groups were monomorphic for common bean, and that polymorphic loci were being maintained in a heterozygous state several generations after they would have been expected to have become fixed. Furthermore, a number of polymorphic SSRs were unlinked, and based on their position on the bean consensus map, we were able to infer that large regions of the mapped linkage groups had reverted to common bean. The Pv2 and Pv9 QTL are located near markers that are in chromosome regions associated with WM resistance QTL in common bean. 8 BCIB lines with WM resistance levels statistically similar to partially resistant common bean checks G122, NY6020, and Ex Rico were evaluated over three field seasons. One line was submitted for straw testing in the National WM Nursery in 2009. In FY08, the Gx43 and MO162/PI 433251B populations lines (263 and 120 respectively) were advanced with DNA being extracted in the BC2F4 , and straw and field tests performed in the BC2F5 and BC2F6. In general, the Mx43 population had a higher frequency of lines similar to or better than G122, while the Gx43 population was skewed towards a greater number of susceptible lines. In FY09, field and straw test evaluations were run on lines in the Gx43 and Mx43 populations. Populations have been now been subjected to four and five straw tests and have undergone two field trials. Ninety-two and 57 SSRs, respectively have been screened in the two populations. Preliminary mapping efforts revealed similar patterns for missing chromosomes as well as

markers associated with potential resistance QTL on some of the same linkage groups as the Gx25 map. Results to date provide insight into why prior efforts to introgress resistance from *P. coccineus* have had limited success. Secondly, the extraordinary retention of heterozygous loci suggests linked deleterious loci may hinder interspecific transfer and stabilization of introgressed resistance may take some time.

Scientists at Michigan State University have bred resistance to *S. sclerotiorum* in soybean. S19-90, A2506, Colfax, and Corsoy 79 with high levels of resistance were used as parents. Breeding lines derived from these resistance sources were evaluated. A variety, Skylla , with partial resistance to *S. sclerotiorum,* was released from the breeding program. A Sclerotinia stem rot resistant germplasm, AxN-1-55 (Diers et al., 2006), was co-released with the University of Illinois. New advanced lines were developed from crosses with Sclerotinia stem rot resistant parents and they are at different stages of evaluations for yield and for resistance to Sclerotinia stem rot. Complete resistance to *S. sclerotiorum* was not available in soybean, and commonly used sources of resistance including S19-90 are only partially resistant to the pathogen. Partial resistance to Sclerotinia stem rot is a quantitative trait.

Pathogen Biology & Mechanisms of Disease Resistance

PM 2.1: Population structure & dynamics

- *Standardized genotypic characterization on wild and cultivated crops*

Scientists at the University of Nebraska, Oregon State University, Michigan State University, Colorado State University, Cornell University, NDSU, Idaho State University, Seminis Seeds, Harris Moran Seed Co., Syngenta, and ARS at Prosser WA established a standardized common screening test was implemented with modified Petzoldt and Dickson scale for rating the greenhouse straw test, and the CIAT scale for rating field screening tests at all sites. Use of consistent rating scales provided results that were easily compared especially allowing fields with low infection to be identified. In four field tests reported so far in 2009, kidney bean lines Cornell 603, 607 and 611showed partial resistance and two pinto bean lines appear to have escape mechanisms similar to the control cultivar Bunsi. A195 was the best line for WM resistance

- *Documented gene-flow or outcrossing contribution to population variability*

- *Discovery of ecological and bio-types with fungicide resistance (same as 4.1)*

- *Geographical inventory of US populations*

Scientists at NDSU investigated genetic variation of *S. sclerotiorum* on six crops in the North Central Region. This project examined genetic variation and virulence for a set of isolates from the current population of the pathogen on canola, dry bean, field pea, lentil, soybean and sunflower in the North Central Region. Mycelial compatibility groups (MCGs) and microsatellite markers were used to characterize genetic diversity of the pathogen from the six susceptible crops in the northern tier of states. 149 isolates of the pathogen were collected from four crops (canola, dry bean, soybean, and sunflower) in twelve North Central states (North Dakota, South Dakota, Nebraska, Minnesota, Iowa, Missouri, Wisconsin, Michigan, Indiana, Ohio and Illinois) and Wyoming, Montana and Colorado. No isolates were obtained from pea and lentil. All isolates were evaluated for MCG and 46 MCG's were identified. The most common was MCG 9 found in nine states and on all four crops. Six of the most common MCG's represented 58% of the isolates and were found across crops. Most of the other MCG's had only one or several isolates. Twelve microsatellite markers with fluorescently labeled tags were used to characterize the isolates. The PCR product lengths were measured on an automated sequencer. There was a strong association between MCG's and microsatellites. For example, within MCG 9 all microsatellites were the same, while in MCG 8 there were three microsatellite haplotypes, but they only differed by one locus. The data thus far indicates little evidence of genetic differentiation across hosts or the region.

PM 2.2: Durable host resistance

- *Isolate virulence/aggressiveness across geographic areas and hosts*

- *Pathogen population dynamics on partially resistant crops*

NDSU scientists investigated virulence of *S. sclerotiorum* isolates collected from these crops was compared

on susceptible and partially resistant cultivars of all six crops when grown in the greenhouse. MCG and DNA fingerprinting was completed, but virulence testing was delayed. A pilot study compared lesion formation rates for two isolates on cultivars of the six crop species (susceptible lines of canola, dry bean, field pea, lentil, soybean, and sunflower, and partially resistant lines of canola and soybean).

- *Pathogen x environmental interaction*

Scientists at the University of Nebraska, Oregon State University, Michigan State University, Colorado State University, Cornell University, NDSU, Idaho State University, Seminis Seeds, Harris Moran Seed Co., Syngenta, and ARS at Prosser WA studied variation of *Sclerotinia sclerotiorum* isolates used to screen for resistance in the greenhouse. Isolates found in field plot nurseries in major bean production areas were tested for mycelial compatibility grouping (MCGs) or clonality and pathogen aggressiveness. Sixty-five MCGs were found in the 156 isolates: 37 of the MCGs were composed of a single isolate. Six MCGs were sampled frequently over multiple screening locations. Differences in isolate aggressiveness were detected using the straw test. Differences in aggressiveness were found between isolate MCGs, however no differences in isolate aggressiveness were found among isolates within the same mycelial compatibility grouping. This data supports the hypothesis that isolate aggressiveness is associated with MCGs. The pathogen variation found among isolates could affect interpretation of results from multi-site screening nurseries as indicated above. Isolates were also collected from three producer fields from each of three major bean production regions in the U.S. These producer field isolates were characterized using MCGs and a test of aggressiveness. When isolates from screening nurseries were compared with producer field isolates from ND, NE and CO, there were significant differences in aggressiveness. More sampling needs to be done to determine other relationships. A manuscript is being prepared summarizing the results from the MCG and virulence analyses of 245 *S. sclerotiorum* isolates from screening sites and grower fields. Presentations of the multi-site testing system and pathogen characterization were made at the 14th International Sclerotinia Workshop at Wilmington, NC in May, 2009.

- *Criteria for testing virulence/aggressiveness on specific hosts.*

Scientists at the University of Nebraska, Oregon State University, Michigan State University, Colorado State University, Cornell University, NDSU, Idaho State University, Seminis Seeds, Harris Moran Seed Co., Syngenta, and ARS at Prosser WA have collected 149 isolates from common bean grown in 8 States. MCGs were characterized. A high degree of variation was observed within and between locations.

PM 2.3: Factors that mediate sclerotia germination

- *Defined environmental requirements for pathogen biotype germination and disease development.*

- *Host factors that mediate myceliogenic germination*

Scientists at NDSU identified some of the environmental and biological factors that affect development of Sclerotinia stem rot on canola by studying factors influencing apothecia formation (carpogenic germination of sclerotia) and the role that the source, concentration and timing of inoculation, as well as moisture on disease development. The role in carpogenic germination was conducted under controlled conditions (texture and soil moisture). Clay, sandy, and sandy loam soil types were created at constant or fluctuating moisture between 25 and 100% of matrix potential. Soils kept at constant 25% of saturation produced the highest percentage of carpogenic germination and produced apothecia faster (within 22 days) than any other treatment. With fluctuating moisture, the optimum range was between 50 and 75% of saturation. The minimum moisture requirement for carpogenic germination was calculated. In 2009 field experiments at Langdon compared the impact of supplemental water as irrigation vs. misting and natural precipitation. No statistical differences in disease intensity were detected between plots that had sclerotia (20 sclerotia per square meter) and controls. Misted plots developed significantly higher amounts of disease compared to other treatments (mean incidence of 35%). Plots that depended on natural precipitation averaged 27%. Supplemental ground irrigation did not increase disease incidence compared to natural precipitation and had an average incidence of 25%. The highest disease intensity was obtained when ascospores were inoculated at 100% bloom stage. The lowest amounts of disease were produced when ascospores were inoculated at 50% bloom. Weather conditions may have heavy influence on results. Controlled environment studies are being conducted to characterize the association between soil moisture and apothecia formation.

- *Effect of sclerotiasphere microbes on germination and dormancy*

- *Effect of sclerotiasphere microbes on mycelial growth*

PM 2.4: Genetic markers and molecular tools for pathogen biology

- *Reporter gene constructs with inducible promoters, organelle specific targets; insertional mutant libraries*

- *Standard molecular protocols to genotype isolates*

Scientists at the University of Nebraska, Oregon State University, Michigan State University, Colorado State University, Cornell University, NDSU, Idaho State University, Seminis Seeds, Harris Moran Seed Co., Syngenta, and ARS at Prosser WA developed informative microsatellite primers to characterize 239 isolates from greenhouse and field screening tests and producer fields. There are various numbers of haplotypes at different locations. There are six haplotypes that are more common than the other 72. This pattern is similar to MCG results where there were MCGs that were more frequent than the larger number that were found across locations. Pathogen variability exists in both greenhouse and field screening isolates. Use of multi-sites can provide more convincing evidence for putative resistance in bean lines. Results impact crop germplasm resources and genetics (resistance identification) and pathogen biology (phenotypic variability impacting resistance screening).

- *Transformed isolates for host/pathogen & pathogen/microbe interactions*

Scientists at the University of Florida developed OxOx minus mutants via transformation. Tests were conducted on tomato, sunflower, pinto bean and canola. Isolates retained pathogenicity in the absences of oxylate, but oxalate appears to be important in stimulating virulence.

PM 2.5: EST libraries from pathogen stains

- *Useful cDNA libraries from pathogen expressed genes*

Scientists at NDSU discovered potential pathogenicity genes expressed by *S.sclerotiorum* during the infection process. The gene sequences generated can be used to develop markers to help in identification of sequences appropriate for marker development as well as *S. sclerotiorum* genes or pathways that can be targeted for development of disease control strategies. Some of the pathogen genes identified through this effort could serve as targets for the development of control measures ranging from chemical control to the development of white mold resistant plants through conventional breeding or transgenic approaches. This may be the first use of next-generation sequencing to study interaction between *S. sclerotiorum* and its host.

- *Useful genome sequence information*

- *Full length, normalized cDNA libraries*

PM 2.6: Candidate genes for pathogenicity

- *Large ATMT collections for phenotypic screens*

- *Transcriptome profiles and high through put functional analyses*

- *Promoters for RNAi constructs during infection*

- *Catalog genes from ATMT random mutagenesis*

- *Discovery of candidate gene function*

Crop Genome Analysis and Genomic Tools

PM 3.1: DNA markers for QTL identification and marker assisted selection

- *Affordable high-throughput genotyping and phenotyping technology*

ARS Scientists at Urbana IL discovered that resistance to Sclerotinia stem rot (*S. sclerotiorum*) in soybean is quantitatively inherited. Based on our previous work through funds from the Sclerotinia Initiative (2004 and 2005) some QTL were found associated with Sclerotinia stem rot resistance; however, large gaps without genetic markers occur in these chromosome lineage regions. Two (F4:5) 155 and 100 recombinant inbred lines (RILs), developed by crossing the Sclerotinia stem rot-susceptible cultivar Merit to two partially resistant parents (PI 194639 and PI 194634) were evaluated for Sclerotinia stem rot resistance in the greenhouse in a previous project. DNA from these populations will be bulked in two groups (most resistant and susceptible lines) based on the phenotypic response to Sclerotinia stem rot. Significant lineages in the genome will be identified with SNP Golden Gate Assay, and polymorphic SSR markers will be selected to genotype the regions and fine map any new QTL linked with resistance genes. Linkage analysis and composite interval mapping will be performed to identify QTL that explain a major portion of the phenotypic variation for disease resistance in the populations.

ARS scientists at Fargo ND used SSR markers to track introgressed genes from wild sunflower parents in interspecific populations with HA410. Diploid, hexaploid and amphiploid populations were screened with 20-22 codominant markers. Results showed that 10X more genes were transferred from diploid that hexaploid parents, and 3X more genes were transferred from amphidiploids than hexaploid parents.

- *High density genetic map of DNA markers for resistance*

Scientists at the University of Nebraska and the University of Missouri developed a SNP map of 37 LysM domain genes that mapped close to 6 WM QTL in soybean (D1a, L, E, F, G, K). Other LysM domain coding genes were located on 10 linkage groups. QTL on LG F, J, N, O will be investigated..

- *Mechanisms for host-pathogen interactions*

PM 3.2: Structure of resistance gene enriched genomic regions

- *Extensive cDNA libraries from host tissues at different stages of infection*

Scientists at Michigan State University, NDSU, Washington State University and Dow Agrosciences collaborated to develop a large expressed sequence tag (EST) data set with massively parallel sequencing on a 454 Roche platform. Post-trimming the data set consisted of 145,049 reads with an average read length of >200 nucleotides. The sequence reads were assigned by the use of BLAST analysis and parsing script to one of four categories; *S. sclerotiorum*, *P. sativum*, *S. sclerotiorum* and *P. sativum*, and unassigned. BLAST alignment was performed against the *S. sclerotiorum* and soybean genome. As no *P. sativum* genome sequence is available the soybean genome was used as a proxy. A threshold for similarity of *P. sativum* to soybean was determined by blasting the c.a. 5,000 pea ESTs that are available on GenBank. Assignment of the reads demonstrated that most (71%) of the reads could be assigned to *P. sativum* and/or *S. sclerotiorum*. Twenty-nine percent of the reads were assigned to *S. sclerotiorum* and 46% of the reads assigned to *P. sativum*. Only 1% of the reads were assigned to both *P. sativum* and *S.sclerotiorum,* while 24% of the reads were unassigned. Unassigned reads may have resulted from ESTs that are not represented by genes in the soybean or *S. sclerotiorum* genomes, or did not provide significant match due to sequence variation. It is also possible that the unassigned reads were the result of PCR or sequencing artifact. Further analysis will aim to resolve the identity of these unassigned reads. Reads that were assigned to both *P. sativum* and *S. sclerotiorum* may be due to similarity between some regions of genes. Future analysis of the data set will include assembly of contiguous sequences prior to sorting reads to species. This will enable assembly of consensus sequences relevant to host-pathogen interaction. The project is now focused on using an RNAseq approach to perform expression profiling of the Sclerotinia-pea host pathogen interaction. A susceptible and resistant pea line will be examined at multiple time points to identify genes involved in resistance response. The EST data set described above will assist in sorting the reads as currently there is no reference pea genome sequence and only ~5000 pea ESTs are publicly available in GenBank.

Scientists at the University of Illinois and Ag AgriFoods Canada demonstrated the association of 4526 expressed genes in OxOx minus soybeans. Oxalic acid treated plants provided a differential gene expression profile of 5093 out of 38,000 genes on microarray chips.

- *DNA markers from BAC-ends to anchor contigs to genetic maps*

- *Physical map of genomic regions containing resistance genes*

- *High through-put resequencing capacity and haplotype maps*

PM 3.3: Function of candidate resistance genes

- *Microarrays for high throughput gene screening*

Scientists at ARS Urbana and the Eastern Cereal & Oilseed Research Centre in Ottawa CN used gene expression profiling and gene clustering to identify candidate genes with an active role in defense to Sclerotinia. Microarray gene expression studies compared the partially resistant PI194639 to susceptible Williams 82, a resistant oxalate oxidase (OxO) transgenic to susceptible control, and infiltrated OA to water at neutral and acid pH. 60 candidate defense-related genes were selected based on response to OA and Sclerotinia in the transgenic OXO line 80(30)-1. In addition to this set of 60 candidate genes, 105 genes (19 of which are located within 500 kb of a known white-mold resistance QTL) were significantly differentially expressed between resistant and susceptible lines. Candidate defense genes were confirmed by select qRT-PCR. Stable transformation systems routinely used in the Simmonds will be used instead of VIGS. Selected candidate gene homologs in Arabidopsis, a plant for which a mutant collection exists of defined T-DNA insertions will be studied. Efforts to identify the best candidate defense genes continue with more microarray gene expression screens and by running autoBLAST to the soybean genome, allowing us to determine if candidate genes identified in the expression studies also are located close to known Sclerotinia defense QTL.

- *Sequenced cDNA libraries from infected host tissue*

- *Discovery of candidate genes for Sclerotinia resistance*

Scientists at NDSU identified resistance and pathogenicity genes expressed during *Sclerotinia sclerotiorum* infection on canola. Gene expression analysis was conducted in double haploid lines showing significant resistance to white mold. Resistant and susceptible plants from these nearly homozygous lines were inoculated with an aggressive isolate of *S. sclerotiorum* to identify potential resistance genes expressed in the plants.

- *Functional RNAi and VIGS systems*

- *Function of candidate genes using gene silencing methods*

PM 3.4: Mechanisms of Sclerotinia resistance

- *Yeast screens for ecotypes and defense-related mutants for oxalate sensitivity*

- *Transgenic incorporation of genes into crops and determine their effectiveness against Sclerotinia*

Scientists at the University of Illinois and Ag AgriFoods Canada overexpressed OxOx in transgenic soybean. Disease development was arrested early in OxOx minus plants, but spread rapidly through leaves of parent lines.

- Scientists at NDSU and Washington State University characterized two partial resistance mechanisms in pea., node and stem.

PM 3.5: Bioinformatic resources

- *Web-based communication for the Sclerotinia Initiative*
- *Interactive website for genetic, genomic & biotech resources*

Disease Management & Pathogen Epidemiology

PM 4.1: Optimized fungicide application programs

- *S. sclerotiorum isolate collection to assess fungicide sensitivity*

ARS scientists at Pullman WA collected 235 isolates Sclerotinia sclerotiorum from pea and lentil fields and fields that are planted with other crops in the dry land and irrigated production areas in the Pacific Northwest. These are from seven different geographic locations representing the diversity of cropping systems in the Pacific Northwest. In addition to the experiments of pathogenicity and aggressiveness of 235 isolates of *S. sclerotiorum* that were reported last year, 57 isolates (31 from a commercial dry pea field and 26 from a commercial potato field) were used in the comparison of MCG and fungicide sensitivity. Twenty-three MCGs were found among 31 pea isolates (G:N ratio 0.74), and 17 MCGs in 26 isolates of the potato population (G:N ratio 0.65), suggesting that relatively higher genetic diversity exists in the pea population. Variation in sensitivity to two fungicides between the two populations was also compared. Colony diameters were determined 36 hrs after inoculation on PDA plates amended with Benomyl (0.2 !g a.i./ml) and Quadris (0.8 !g a.i./ml). Pea field population showed greater variance than did the potato population for both the fungicides, suggesting that the pea population has higher diversity for loci controlling fungicide sensitivity and has more potential to adapt into new environments. No variation was found in sclerotial dry weight between the two populations suggesting that life history traits are less prone to selection pressure. To assess genetic variation in neutral genetic marker loci, thirteen microsatellite markers are being tested on the populations.

- *Efficacy of new chemistries*

Scientists at NDSU discovered that *Sclerotinia sclerotiorum* are capable of developing resistance in canola fields against carbendazim (thiophanate methyl) fungicide even after two applications in the same growing season. A baseline for fungicide sensitivity was developed for North Dakota *S. sclerotiorum* isolates to thiophanate methyl, prothioconazole, and azoxystrobin. A steady increase in tolerance to thiophanate methyl occurred within the last 10 years. Six isolates collected before 1999 had a mean EC50 of 1.17 parts per million (ppm), whereas isolates collected prior to 2004 (55 isolates evaluated) had an average EC50 of 1.53 ppm, and isolates collected between 2005 and 2007 had an EC50 of 1.56 ppm. The latter change represented an increase of almost 30% in the level of EC50 since 1999. Regional baseline sensitivity of *S. sclerotiorum* to these was tested with 148 isolates of *S. sclerotiorum* representing 40 mycelial compatibility groups from at least four different crops from over 10 states. In addition, these isolates have been characterized using microsatellites analysis. Field trials suggest that mixing thiophanate methyl (Topsin) with boscalid (Endura) or prothioconazole (Proline) could provide similar levels of control than some of the newer compounds but with up to almost 25% less cost.

- *Updated management guides for disease management*

ARS scientists at Prosser WA and NDSU demonstrated that lines with partial resistance to white mold exhibited significantly enhanced yields without application of Topsin, Endura or Ormega fungicides. However, the gain in yielding ability was not at a commercially acceptable level.

- *Improved spraying technologies*

PM 4.2: Bio-control alternatives for disease management

- *Grower recommendations for commercial sclerotial antagonists*

Contans WG, a commercial formulation of the *Coniothyrium minitans* strain CON/M/91-08, was developed by Prophyta (Malchow, Poel, Germany) as a biocontrol agent for the control of diseases caused by *Sclerotinia* spp. Contans WG is currently marketed in the United States by SipcamAdvan (Durham, NC). Contans WG was applied in fall for three years for control of *Sclerotinia minor* on peanut. No effect of *C. minitans* application was observed in the first crop planted following treatment. However, by the third growing year, there was no difference in the level of control between plots treated with *C. minitans* for one, two or three consecutive years. Gerlagh et al. observed significantly less disease on bean after 5 growing seasons. Application of *C. minitans* at seeding reduced the number of apothecia under bean, canola canopies. Soil type influences survival of *C. minitans*. *C. minitans* cannot germinate and grow through soil to reach sclerotia. Transmission to previously uninfected sclerotia was much more efficient (58% transmission) in sand than in loamy soil (10% transmission). This influences spread of *C. minitans* in soil, and efficacy of Contans WG

- *Catalog of commercial microbial biocontrol agents*

- *Efficacy of Sporidesmium sclerotivorum as a biocontrol agent*
- *Updated management guides for biofungicides in disease management*

PM 4.3: Quantitative models for environmental and host-crop interactions

- *Disease warning systems*
- *Validated predictive models in other crops.*
- *Yield loss models*
- *Threshold levels for decision aids*

PM 4.4: Optimized cultural practices for disease management

- *Improved variety selection criteria*
- *Management decision aids*

Scientists at Colorado State University and Idaho State University provided critical data on cultural practices and responses of bean cultivars and breeding lines in dry bean. Agronomic responses revealed that there was a variable effect on plot yield when the plant population of commercially-grown pinto cultivars was increased 50% from 1 line to 2 lines per bed. The percentage increase in yield during 2007 – 2008 when comparing 1 or 2 lines with fungicide protection was + 21% or + 12% for pinto Montrose which is a susceptible, prostrate vine-type III cultivar. Similarly, Vision is a susceptible, upright-vine type II cultivar which yielded + 27% or + 23% with fungicide protection when data were combined across years and locations. Disease intensity in Vision was lower (43% vs 62% and 78% vs 87%) for 1 vs 2 lines with and without fungicide protection, respectively, illustrating that its more upright plant architecture did contribute to reduced disease pressure in dry bean production systems in semiarid regions of the western U.S. and the fungicide treatment provided additional protection for this genetically susceptible cultivar. As an example of the pinto breeding lines from CSU with improved levels of single stem development and a degree of physiological resistance, CO23704 yielded better than CO54150 in different plant populations with or without additional fungicide protection. As an example of the interspecific breeding lines from Idaho with improved levels of physiological resistance, WM 55 yielded better than WM 54 in different plant populations with or without additional fungicide protection. With an average yield of 20 cwt/A (2240 kg/ha) and average grower price of $0.25/pound ($0.55/kg), the increased plant population (2 rows) could provide an additional gross return of 10% (224 kg valued at $123/ha) up to 20% (448 kg valued at $247/ha) for currently grown commercial cultivars like Montrose and Vision in the absence of white mold or if protected from damage by white mold by a fungicide (at a cost of $30/A or $74/ha). During 2009, we encountered low white mold pressure in fields with a history of the disease apparently due to delayed plantings (early-spring rains) which delayed flowering until late July when weather conditions were warm and dry. In addition, one of the commercial fields was severely damaged by common rust which opened up the canopy of the commercial variety surrounding our trial and prevented the production of ascospore inoculum. However, the preliminary analysis of data from the 3 locations revealed that there were no interactions between fungicide treatment or nitrogen by entries or locations. Yields of the 4 entries averaged 2015 lb/acre (37.9 g/100 seed) at the research station and 3299 lb/acre (36.7 g/seed) at the better commercial field in the absence of white mold and with trace presence of bacterial diseases. When combined over locations, yield ($P < 0.01$) and seed size ($P < 0.001$) differences between entries were significant. Common rust was not a problem since the 4 entries possess genetic resistance against the common races of rust present in this region. The experiment will be repeated in 2010.

- *Precision agriculture program*

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FY2009 Funded Projects

2009 Sclerotinia Funding Matrix

H = 5; M = 3; L = 1

	Project	PI	Cooperator	Commodity	Requested	PM	Aveage Rating
1	Evaluation of Wild Helianthus Species for Resistance to Sclerotinia Stalk Rot	Block	ARS	Sunflower	$17,427	1.1	5.0
2	Genetic Resistance to White Mold Derived from Multiple Sources of Common Bean and Scarlet Runner Bean	Brick	CO	Dry Bean	$29,422	1.3	4.7
3	Variation in pathogenicity and fungicide sensitivity in relation to variation of neutral markers of Sclerotinia sclerotiorum	Chen	ARS	All	$76,268	2.4	4.3
4	Searching for resistance to sources to Sclerotinia in wild relatives of cool season grain legumes	Chen	ARS	All	$72,630	1.1	3.0
5	Expression profiling of the pea-Sclerotinia sclerotiorum interaction for genomics assisted breeding	Chilvers	MI	Pea & Lentil	$55,426	3.1	4.7
6	Identification and functional analysis of candidate defense-related genes to Sclerotinia sclerotiorum in soybean and Arabidopsis	Clough	ARS	Soybean	$71,599	3.1	4.3
7	Defining critical environmental and biological parameters needed to develop Sclerotinia stem rot on canola	del Rio	ND	Canola	$26,580	2.3	4.3
8	Evaluation of fungicide alternatives for control of Sclerotinia stem rot of canola	del Rio	ND	Canola	$26,411	4.1	4.7
10	Identification of novel loci for resistance to Sclerotinia stem rot in perennial soybean accessions	Domier	ARS	Soybean	$188,380	3.2	3.5
12	Use of a transformation system in sunflower for Sclerotinia resistance studies	Finer	OH	Sunflower	$50,000	1.6	4.7
14	Candidate genes for fungal resistance: Mapping and SNP development for LysM-domain encoding genes in soybean	Graef	NE	Soybean	$76,840	3.1	5.0
16	Discovery of novel sources of resistance to head rot and stalk rot in Cultivated sunflower and wil Helianthus	Gulya	ARS	Sunflower	$67,847	1.1	4.3
17	2009 Uniform sunflower germplasm evaluation for resistance to Sclerotinia gead rot in Minnesota	Hollings-worth	MN	Sunflower	$18,000	1.7	4.3
18	Pyramiding Sclerotinia head rot and stalk rot resistances into elite sunflower breeding lines with the aid of DNA markers	Hulke	ARS	Sunflower	$95,000	1.4	4.3
19	Transferring Sclerotinia resistance genes from wild Helianthus species into cultivated sunflower	Jan	ARS	Sunflower	$102,000	1.2	4.3
20	Identification of QTL for white mold resistance in pinto bean	Kelly	MI	Dry Bean	$34,396	1.3	4.7
21	Characterization of the genetic basis for partial resistance to Sclerotinia sclerotiorum in pea	McPhee	ND	Pea & Lentil	$69,250	1.3	4.0
22	Identification of defense-response genes conferring partial resistance to white mold in dry bean	Miklas	ARS	Dry Bean	$75,000	1.3	5.0
23	Contribution of partial genetic resistance to white mold disease management in pinto and great northern beans	Miklas	ARS	Dry Bean	$15,000	4.4	4.3
24	Transfer and characterization of white mold resistance from P. coccineus into P. vulgaris	Myers	OR	Dry Bean	$55,210	1.4	4.7
25	Genetic variation and virulence of S. sclerotiorum on six crops in the North Central Region	Nelson	ND	All	$59,490	2.2	3.3
27	The requirement for oxalate during pathogenesis on multiple crops	Rollins	FL	All	$18,400	2.2	4.7
28	Evaluation of Sunflower Hybrids and Germplasm for Resistance to Sclerotinia	Schatz	ND	Sunflower	$72,500	1.7	4.3
29	On-Farm Validation of Cultural Practice Adjustments to Improve White Mold Management in Dry Bean Irrigation Systems	Schwartz	CO	Dry Bean	$76,190	4.4	3.7
30	Introgressing white mold resistance from the secondary gene pool of common bean	Singh	ID	Dry Bean	$59,404	1.4	5.0
32	Improved resistance in common bean through multi-site screening and pathogen characterization throughout major production areas	Steadman	NE	Dry Bean	$59,050	2.2	5.0
33	Identifying molecular markers linked in lentil (Lens culinaris Medik.) to white mold resistance derived from the lentil cultivar Pennell	Vandemar k	ARS	Pea & Lentil	$57,920	3.1	4.7
34	Enhancing soybean for resistance to Sclerotinia stem rot	Wang	MI	Soybean	$40,001	1.5	5.0