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Meeting Strategic Milestones of the National Sclerotinia Research Initiative for 2011

Integrated Research for Disease Management in Sunflower, 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 2010* 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.ars.usda.gov/Research/docs.htm?docid=20317&page=3

Rating Summary:

Sclerotinia Initiative Research Progress Evaluation

	2008	2009	2010	2011	2012
	number of accomplishment citations				
Total Accomlishments Cited	76	61	75	71	
Milestones w/ multiple citations	17	10	14	18	
Milestones w/ no citations	30	30	24	29	
Milestones not funded	NA	14	2	3	
Milestones completed	0	1	1	1	
Total Milestones Rated	76	76	73	70	
Achievement Rating (%)	56.7	62.2	67.2	73.4	
Total Projects	34	28	19	15	
Accomplishments / project	2.2	2.2	3.9	5	
All Publications by SYs	47	53	214	235	
Total SYs funded by NSI	20	33	35	29	
Germplasm/Varieties released	10	10	15	9	

Achievement Rating:
((all accomps x (milestones (multi citations/(no citations-not funded)))/milestones(all-not funded) x 100)

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	1	2	
• *Improved germplasm screening methods*	3	3	3	4	
• *Increased availability of resistant germplasm*	1	3	1	5	
• *Doubled haploid lines for resistance, tested in multiple environments*	1	1	1	1	
PM 1.2: Transfer new resistance genes into plant germplasm					
• *Germplasm derived from interspecific crosses*	7	2	2	2	
• *Resistant selections of unadapted x agronomic traits*	0	1	1	2	
PM 1.3: Genetic analysis & QTL discovery					
• *Highly inbred mapping populations with validated QTLs*	2	4	2	3	
• *QTL analysis to generate a high density genetic maps*	3	2	2	2	
• *Integrated linkage maps*	4	1	1	1	
PM 1.4: Pyramid white mold resistance genes					
• *Improved conventional breeding methods for quantitative traits*	1	2	1	1	
• *Breeding populations segregating for multiple resistance alleles and other traits*	3	1	1	1	
PM 1.5: Marker-assisted selection					
• *Disease reaction of RILs in multiple field environments*	1	1	1	1	
• *Marker-assisted selection protocol for more efficient genotyping*	4	1	1	3	
• *Field verification of resistance*	3	1	1	2	
PM 1.6: GM improved resistance					
• *GM oxalate oxidase expression, inheritance and field evaluation*	1	1	1	0	
• *Catalog of candidate resistance genes, promoters, and constructs for transformation*	2	1	2	0	
• *Perlka-resistant soybean lines (COMPLTED IN 2008)*	1				
• *Transgenic expression of antifungal peptides*	0	0	2		
PM 1.7: Plant germplasm/cultivars with improved resistance					
• *Enhanced adapted germplasm*	2	1	1	4	
• *Herbicide tolerance with resistance to Sclerotinia*	1	1	1	1	
• *Agronomic resistant varieties for commercial production.*	1	2	3	2	
• *Identifycrop germplasm with partial resistance to virulent isolates*	1	1	12	3	
Pathogen Biology & Mechanisms of Disease Resistance					
PM 2.1: Population structure & dynamics					
• *Standardized genotypic characterization on wild and cultivated crops*	1	1	1	1	
• *Defined environmental requirements for pathogen biotype germination & disease*	1	0	0	0	
• *Documented gene-flow or outcrossing contribution to population variability*	1	0	1	0	
• *Geographical inventory of US populations*	1	1	1	1	
PM 2.2: Durable host resistance					
• *Isolate virulence/aggressiveness across geographic areas and hosts*	2	0	1	1	
• *Pathogen population dynamics on partially resistant crops*	0	1	1	0	
• *Pathogen x environmental interaction*	0	1	0	0	
• *Knowledge of plant x pathogen x environmental interactions*	1	1			
• *Criteria for testing virulence/aggressiveness on specific hosts.*	1	1	1	0	
PM 2.3: Factors that mediate sclerotia germination					
• *Host factors that mediate myceliogenic germination*	2	1	1	0	
• *Defined envronmental requirements for pathogen biotype germination & disease*	0	0	0	0	
• *Effect of sclerotiasphere microbes on germination and dormancy*	0	0	0	0	
• *Effect of sclerotiasphere microbes on mycelial growth*	0	0	0	0	
PM 2.4: Genetic markers and molecular tools for pathogen biology					
• *Reporter gene constructs with inducible promoters, insertional mutant libraries*	0	0	0	0	
• *Standard molecular protocols to genotype isolates*	2	1	1	1	
• *Transformed isolates for host/pathogen & pathogen/microbe interactions*	1	2	1	1	
PM 2.5: EST libraries from pathogen stains					
• *Useful cDNA libraries from pathogen expressed genes*	1	1	0	1	
• *Useful genome sequence information*	1	0	0	1	
• *Full length, normalized cDNA libraries*	1	0	0	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	0	0	
• *Transcriptome profiles and high through put functional analyses*	0	0	0	0	
• *Promoters for RNAi constructs during infection*	0	0	0	0	
• *Catalog genes from ATMT random mutagenesis*	0	0	0	0	
• *Discovery of candidate gene function*	0	0	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	2	1	
• *High density genetic map of DNA markers for resistance*	2	1	1	2	
PM 3.2: Structure of resistance gene enriched genomic regions					
• *Extensive cDNA libraries from host tissues at different stages of infection*	1	1	2	4	
• *DNA markers from BAC-ends to anchor contigs to genetic maps*	1	1	2	2	
• *Physical map of genomic regions containing resistance genes*	0	0	2	1	
• *High through-put resequencing capacity and haplotype maps*	0	0	1	1	
PM 3.3: Function of candidate resistance genes					
• *Microarrays for high throughput gene screening*	2	1	1	1	
• *Sequenced cDNA libraries from infected host tissue*	0	0	1	1	
• *Discovery of candidate genes for Sclerotinia resistance*	1	1	1	2	
• *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	2	0	
• *Conventional analysis of genetic mechanisms*	1	1	1	1	
PM 3.5: Bioinformatic resources					
• *Web-based communication for the Sclerotinia Initiative*	1	1	1	1	
• *Interactive website for genetic, genomic & biotech resources*	1	1	1	1	
Disease Management & Pathogen Epidemiology					
PM 4.1: Optimized fungicide application programs					
• *S. sclerotiorum isolate collection to assess fungicide sensitivity*	0	1	2	0	
• *Efficacy of new chemistries*	2	1	1	2	
• *Updated management guides for disease management*	0	1	0	1	
• *Improved spraying technologies*	0	0	0	0	
PM 4.2: Bio-control alternatives for disease management					
• *Grower recommendations for commercial sclerotial antagonists*	0	1	0	0	
• *Catalog of commercial microbial biocontrol agents*	0	0	0	0	
• *Efficacy of Sporidesmium sclerotivorum as a biocontrol agent*	0	0	1	0	
• *Updated management guides for biofungicides in disease management*	0	0	0	0	
PM 4.3: Quantitative models for environmental and host-crop interactions					
• *Disease warning systems*	1	0	0	0	
• *Validated predictive models in other crops.*	0	0	0	0	
• *Yield loss models*	0	0	1	0	
• *Threshold levels for decision aids*	0	0	0	0	
PM 4.4: Optimized cultural practices for disease management					
• *Improved variety selection criteria*	0	0	1	2	
• *Management decision aids*	1	1	1	1	
• *Precision agriculture program*	0	0			

Total Accomlishments	**76**	**61**	**75**	**71**
Total Milestones with multiple accomplishments	**17**	**10**	**14**	**18**
Total Milestones with no accomplishment	**30**	**30**	**24**	**29**
Total Milestones not funded	**NA**	**14**	**2**	**3**
Total Milestones	**76**	**76**	**73**	**70**
Achievement Rating (%)	**56.7**	**61.5**	**67.2**	**73.4**
Total Projects	**34**	**28**	**19**	**15**
Accomplishments per project	**2.2**	**2.2**	**3.9**	**4.7**
Publications	**47**	**53**	**214**	**235**
Germplasm lines	**4**	**5**	**12**	**5**
Varieties	**6**	**5**	**3**	**4**

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, dry bean** and **soybean**
- Microarrays for expressed genes in soybean
- Marker Assisted Selection for resistance in **pinto bean, sunflower, common bean, soybean**
- A high density maps for Sclerotinia resistance in **soybean, common bean, sunflower**
- Protocol for transformation of **sunflower, soybean** and **lentil** germplasm
- Standardized protocol for genotypic characterization of warm and cool season legume crops
- First characterized geographic inventory of Sclerotinia isolates from **canola, dry bean, field 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**
- Germplasm releases with improved tolerance to white mold in **pinto bean (2), pea (3), sunflower (11), common bean (5), soybean (3), chickpea (1), lentil (1)**
- Variety releases with improved resistance to white mold in **pinto bean (5), soybean (3), common bean (5), lentil (2), great northern bean (2), sunflower (1).**
- Libraries of gene markers from RNA-seq gene expression profile analysis of transcriptomes in **pea, pinto bean, dry bean, and soybean**
- SNP association analyses for generation of haplotype maps in **soybean, common bean, sunflower, pea, lentil, canola**.
- A comprehensive **National Sclerotinia Initiative resource on the USDA-ARS website, and bioinformatic resources for soybean and cool season legume genomics** 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** and **sunflower**.
- 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-2011

Crop Germplasm Resources & Genetics

PM 1.1: New sources of resistant plant germplasm

- *Exploration trips to obtain seeds of wild species*

ARS scientists at Ames IA and Fargo ND increased the availability of wild sunflower accessions in the USDA sunflower germplasm collection. Excellent progress was made in germplasm availability through (a) new collections made by Drs. L. Marek and G. Seiler and (b) continued seed increase efforts. As of Dec-2011, 1309 of 1365 (95%) of the wild annual accessions were available for distribution and 590 of 824 (67%) wild perennials were available as seed.

ARS Scientists at Fargo ND established new perennials of H. silphioides, H. salicifolius, H. hirsutus, H. occidentalis, H. divaricatus, and H. resinosus to further diversify the resistance genes and to increase the probability of identifying useful major resistance QTLs

- *Improved germplasm screening methods*

ARS scientists at Ames IA and Fargo ND improved the efficiency and efficacy of a greenhouse screening method to evaluate a wide array of wild Helianthus accessions for resistance and to increase the number and variety of available wild Helianthus accessions in the USDA sunflower collection. The most critical factor for maintaining good disease pressure and differentiating resistance among accessions was to keep soil temperatures under 25C. Container size was the second important factor, with smaller pots contributing to greater disease pressure. The current method grows plants in pots ~2.5" sq., inoculated with Sclerotinia-infested millet, and incubated at soil temperatures between 21C and 23C.

ARS Scientists at Pullman WA developed an improved screen process for pea & lentil that consisted of inoculating the second inter-node of two-week old plants with a colonized agar plug from actively growing colonies of a virulent strain of S. sclerotiorum. Stem lesions appeared as bleaching or discoloration of the stems. Disease progress was monitored by measuring lesion lengths starting two days after inoculation and continued at appropriate time intervals until plants died. Although all germplasm lines were susceptible to Sclerotinia stem rot, significant differences were found among the germplasm lines in rate of lesion expansion.

Scientists at University of Nebraska, Colorado State University, Cornell University, Harris-Moran Seed Co., Michigan State University, University of Idaho, Seminis Seed Co., Oregon State University, North Dakota State University and USDA ARS-Prosser WA collaborated to identify resistance to S. sclerotiorum in the secondary gene pool derived as well as in Phaseolus vulgaris adapted dry and snap bean lines. Multiple location screening nurseries were used to improve understanding of the role of pathogen variation in the screening process. A standardized screening test using the modified Petzoldt and Dickson scale for rating the greenhouse straw test, and the CIAT scale for rating all field screening tests were developed..

Scientists at Michigan State University developed two new greenhouse evaluation methods, the spray-mycelium method and the drop-mycelium method, were developed for large scale evaluations of soybean breeding materials for resistance to Sclerotinia stem rot. The data obtained with these two methods have significant and good correlation with data obtained in field inoculation trials.

- *Doubled haploid lines for resistance, tested in multiple environments*

North Dakota State University evaluated double haploid lines with the S. sclerotiorum isolate NE 152 (also known as 1980). Double haploid canola lines belonging to the resistant (NEP 63) and susceptible (NEP32) lines were inoculated with a highly aggressive S. sclerotiorum isolate (NE152) in a growth chamber, an established petiole inoculation technique involving potato dextrose agar (PDA) plugs with actively growing S. sclerotiorum were utilized and samples were collected at 24 and 48 hours post inoculation. Non-inoculated, control canola plants were treated with PDA plugs containing no fungus. At both the time points examined,

there were no observable phenotypic differences between the susceptible and resistant canola lines and there was no difference in appearance of control petioles. However, four days post inoculation the plants from the susceptible line died whereas the resistant plants continued growing unharmed**.**

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- *Increased availability of resistant germplasm*

ARS scientists at Ames, IA and Fargo, ND evaluated diploid annual species H. agrestis, H. anomalus, H. bolanderi, H. deserticola, H. paradoxus, additional H. petiolaris, a 60-accession geographic cross-section of H. annuus, plus the perennials H. californicus and H. pauciflorus (183 acc. total). From Jun-10 to May-11, perennial collections of H. ciliaris, H. eggertii, H. resinosus (10 new accessions), H. tuberosus and H. salicifolius plus 88 cultivated H. annuus accessions were screened with some description of Sclerotinia tolerance (head or stalk rot) in their background or a backcross introgression from a non-H. annuus wild species (annual or perennial). The perennial species have been highly resistant.

ARS Scientists at Pullman WA identified new sources of resistance to Sclerotinia sclerotiorum stem and crown rot in wild relatives of grain legumes (chickpea, lentil and pea). Wild relatives of peas: Pisum fulvum (33 lines), Pisum sativum subsp. abyssinicum (27 lines), Pisum sativum subsp. elatius (45 lines), Pisum sativum subsp. transcaucasicum (4 lines) were collected. All the accessions of wild peas have been evaluated for resistance to Sclerotinia sclerotiorum. However, due to heterogeneity of wild lentil and chickpea accessions, it was necessary to select uniformed seeds and spend time to regenerate and increase seed quantity for pathogenicity assay to improve uniformity and reproducibility of the assay. Now with the newly generated seeds in hands, we are confident that we will complete the project in one more year.

ARS scientists at Urbana, IL evaluated 233 accessions from 18 Glycine species for sensitivity to Sclerotinia stem rot in replicated greenhouse trials. Highly resistant accessions of G. canescens, G. clandestina, G. latifolia, and G. tabacina were identified that showed little damage from the fungus after multiple inoculations. In contrast, susceptible accessions died after the first inoculation.

Scientists at ARS-Fargo ND; Ag Research Center, Central Lakes College, Staples, MN; Panhandle Res. & Exten. Center UNEB) Scottsbluff, NE; University of Nebraska; and NDSU Res. & Exten. Center, Carrington, ND broadened the genetic base of sunflower by phenotyping a large group (250) of USDA Plant Introductions and elite USDA released germplasm. These Plant Introductions are genetically very diverse, and originate from 30 countries. Over a two year period, four datasets of stalk rot were generated on this germplasm. 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

Scientists at Idaho University and Colorado State University developed 24 white mold resistant breeding lines from interspecific crosses between common bean and Phaseolus species of the secondary gene pool, namely P. coccineus (23 interspecific breeding lines derived from three different germplasm accessions, namely G 35006, G 35172, and PI 433246) and P. costaricensis (one interspecific breeding line derived from germplasm accession G40604). Four partially white mold resistant large-seeded Andean genotypes, namely A 195, G 122, MO 162, and VA 19, were crossed together to develop a second group of six white mold resistant breeding lines. The third group of 12 breeding lines with pinto and other seed colors was developed from broad-based multiple-parent crosses between white mold resistant small- and medium-seeded Middle American and large-seeded Andean common bean, and interspecific breeding lines derived from the secondary gene pool species.

PM 1.2: Transfer new resistance genes into plant germplasm

- *Germplasm derived from interspecific crosses*

ARS scientists at Urbana IL confirmed putative F1 plants derived from resistant and susceptible accessions of G. latifolia to be hybrids using the SNPs that were identified in DCL3 genes. The putative G. canescens F1 plants were determined to be selfed and not hybrids. Crosses were performed with susceptible and resistant accessions of G. tabacina and G. clandestina, but none produced hybrid F1 plants. Over 300 F3 G. latifolia lines were developed and are currently in the greenhouse and are being advanced by single seed descent to produce recombinant inbred lines (RILs).

Scientists at ARS-Prosser WA, Oregon State University and North Dakota State University exploited WM resistance QTL from interspecific crosses in common bean by examining phenotypic interactions among new and existing QTL, followed by fine-mapping QTL using whole genome sequence and gene expression transcript profiling, represents a continuous QTL investigation pipeline from discovery to implementation for marker-assisted breeding. Several lines stable for resistance have been characterized for agronomic traits, and will be released as germplasm during the spring of 2012 for distribution to other bean breeding programs.

- *Resistant selections of unadapted x agronomic traits*

ARS Scientists at Fargo ND used sunflower amphiploids resistant to stalk and head rot in crosses with HA 410, and backcross progenies with 2n=34 were established in the field, and BC2F4/BC3F3 families were evaluated in replicated trials in 2009-2011. Interspecific F1 progeny were produced between stalk rot resistant hexaploid H. californicus and H. schweinitzii and HA 410. Backcross progenies of H. californicus crosses with HA 410 were evaluated in replicated trials in 2009-2011. Crosses between NMS HA89 and head rot resistant H. maximiliani and H. nuttallii were advanced to BC1F4 and BC2F4 families for replicated field trials in 2009-2011. Stalk rot resistant diploid perennial H. maximiliani, H. giganteus, and H. grosseserratus were crossed with HA 410 in 2007, and their BC1F4/ BC2F3 families evaluated in replicated field trials in 2009-2011. Replicated field tests in 2009 and 2011 for head and stalk rot resistance indicated moderate to good resistance indicating successful gene introgression for head rot tests.

ARS scientists at Fargo ND transferred Sclerotinia stalk rot resistance from wild sunflower species to 21 cultivated F1 hybrids and their BC1 and BC2 progenies. Reactions to Sclerotinia stalk rot were evaluated in greenhouse trials in 2010. A total of 504 BC2F1 plants from these crosses were screened in 2011, and the 68 most resistant plants were advanced to the BC2F2 generation. Given the quantitative nature of stalk rot resistance, 3000-4000 plants from BC2F2 populations will be screened in the greenhouse and growth chambers from now to early 2012.

PM 1.3: Genetic analysis & QTL discovery

- *Highly inbred mapping populations with validated QTLs*

ARS Scientists at Fargo ND conducted a molecular tracking study to enhance higher frequency of gene introgression from diploid perennials than from hexaploid or interspecific sunflower amphiploids. Replicated field tests screened for head and stalk rot resistance, respectively, failed to produce usable results due to midge damage and adverse environmental conditions. A genomic in situ hybridization technique (GISH) distinguishing chromosomes of the perennials and cultivated sunflower has been developed

Scientists at the Michigan State University identified four novel QTL (WM3.3TW, WM7.5TL, WM9.2TW and WM11.1TL) associated with white mold resistance in wide crosses with Tacana black bean. Those results were recently published (Mkwaila et al., 2011). These QTL were mapped to linkage groups 3, 7, 9 and 11, respectively and two previously mapped QTL were also validated on LGs 2 and 4. In addition to identifying QTL associated with physiological resistance to white mold resistance, a QTL that accounted for 19 to 37% of the variation for yield under white mold pressure over three years, was detected on LG2 in the Tacana/Landrace population. These QTL will be pyramided with QTL from other sources to further enhance resistance to white mold in navy and black beans.

ARS Scientists at Fargo ND continued to develop an advanced backcross (AB) population to facilitate genetic characterization of novel QTL for stalk rot resistance derived from H. argophyllus. A total of 300 plants from 14 BC2F1 plants were advanced to BC2F2 generation by single-seed descent. The goal is to produce an AB population of BC2F2:6 lines by an additional four cycles of single-seed descent.

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

Scientists at Michigan State University found two new QTLs for resistance to Sclerotinia stem rot in PI 391589A and PI 391589B. Five resistance soybean sources PI 089001, PI 153259, PI 437764, PI 548404, and PI 548312 were tested for procession of any reported QTLs for resistance to Sclerotinia stem rot. Nine reported QTLs were found in these resistance sources

Scientists at USDA-ARS, Prosser, WA; Oregon State University, and North Dakota State University investigated WM resistance QTL in common bean from Phaseolus coccineus. Validation and

characterization of QTL identified in these populations is under way. During the winter of 2011 a greenhouse straw test showed that 18 lines were more resistant than 91G, and one was more resistant than the resistant check G122. Results also indicated that the majority of lines were fixed for resistance. Crosses were made during the spring of 2011 with 13 interspecific lines possessing markers for identified interspecific QTL to produce 55 cross combinations. The populations will be used to fine map and validate the QTL. Inbred populations segregating for different QTL combinations were successfully characterized for disease reaction by the straw test and in the field. The WM7.1 and WM8.3 QTL exhibited a partial additive effect in the field and greenhouse. The WM2.2 and WM8.3 QTL exhibited a partial additive effect in the field but not the greenhouse, which was expected given WM2.2 from Bunsi is not expressed in the straw test. Clearly the WM2.2 and WM8.3 QTL have different modes of action, with WM2.2 having the greatest effect in the field and WM8.3 in the straw test.

- *Integrated linkage maps*

Scientists at the University of Nebraska and the University of Missouri identified 28 putative QTL on 15 different chromosomes in soybean. Five RIL populations were evaluated using Williams 82 as the common susceptible parent. Seven QTL were associated with disease resistance. A mutation in the LysM-RLK1 gene resulted in susceptibility to fungal infection. Enhanced expression of this gene enhanced innate immunity. Research revealed: 1) 37 LysM genes on various linkage groups, 2) At least 6 LysM genes on white mold QTL, 3) SNPs in 8 LysM-encoding genes, 4) the LysMe11 gene LG I was associated with smaller lesion size from the more resistant parent. SNP genotypes from the 1536 SNP chip for soybean will provide a more complete QTL analysis.

PM 1.4: Pyramid white mold resistance genes

- *Improved conventional breeding methods for quantitative traits*

Scientists at the University of Idaho and Colorado State University made progress in pyramiding white mold resistance from across Phaseolus species of the primary and secondary gene pool and introgressing high level of pyramided resistance into Pinto bean. Four single or bi-parental crosses between diverse breeding lines and germplasm accessions with partial WM resistance were made between June and September 2010. These were used to make three-way and double-crosses between October and December 2010, which subsequently will be used to make multiple-parent crosses (January to May, 2011). All crosses will be made only among parents possessing partial resistance to WM and of diverse evolutionary origins (A 195, G 122, 'Chase', CORN 501, CORN 601, I9365-25, MO 162, USPT-WM-1, VA 19, VCW 54, VCW 55, VRW 32, 92BG-7, 0785-220-1, 0785-221-1, and 0785-127-1). These crosses should allow simultaneous pyramiding of high levels of WM resistance from across Phaseolus species of the primary and secondary gene pools and transfer into pinto bean. Also, 78 F5 families developed from two double-crosses, namely USPT-WM-1/CORNELL 601//USPT-CBB-1/92BG-7 and Chase/I9365-25//ABL 15/A 195 made for a doctoral dissertation, and over two thousand early generation progenies from additional crosses were screened in the greenhouse. From the initial screenings it is encouraging to note that some of the recombinants exhibited higher levels of WM resistance than the individual parents. Also, some of these recombinants had pinto-like seed. However, because these are only in early segregating generations it will take several selection and progeny testing cycles to develop breeding lines uniform for WM resistance reaction and assess their true potential.

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

ARS scientists at Fargo ND transferred Sclerotinia stalk rot resistance from wild sunflower species to 21 cultivated F1 hybrids and their BC1 and BC2 progenies. Reactions to Sclerotinia stalk rot were evaluated in greenhouse trials in 2010. A total of 504 BC2F1 plants from these crosses were screened in 2011, and the 68 most resistant plants were advanced to the BC2F2 generation. Given the quantitative nature of stalk rot resistance, 3000-4000 plants from BC2F2 populations will be screened in the greenhouse and growth chambers from now to early 2012.

PM 1.5: Marker-assisted selection

- *Disease reaction of RILs in multiple field environments*

Scientists at the Michigan State University grew the 96-entry pinto bean RIL population AP647 at the Montcalm Research Farm (MRF). Yields ranged from 16 to 45 cwt/acre with a test mean of 32.2 cwt/acre. The parental lines AN37 and P02647 yielded 27.4 and 38.1cwt/a respectively. The highest yielding entry P07721 exceeded the test mean by 40% in the 2011 trial. White mold scores at MRF were lower in 2011 and ranged

from 16 to 83% with a mean of 48%. Greenhouse straw test scores in this population ranged from 1.5 to 7.0 with a mean of 3.7 (rated on a 1-9 scale) and both greenhouse and field data is being used in QTL analysis.

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

Scientists at the Michigan State University used microsatellite and InDel marker data to reveal 43 polymorphic markers were associated with white mold resistance from the full-sib RIL population (AP630) were also segregating in this AP647 population. The 34 SSR's represent 31% polymorphism while InDels represent 22% polymorphism out of a total of 40 screened. The markers were run in Join map software. Single marker analysis will be performed to validate if any of markers are also co-segregating with resistance in the AP647 population. These results indicate that these markers can be used to validate the QTL from the first population and possibly be used in marker-assisted selection to enhance resistance to white mold in common bean.

Scientists from North Dakota State University, USDA-ARS Prosser, WA; and North Dakota State University continue to use marker-assisted backcrossing with new indel markers identified by fine mapping to move WM7.1 and WM8.3 QTL into a higher yielding commercially acceptable pinto bean market type.

ARS scientists at Fargo ND screened the polymorphism between HA 89 with six wild species accessions with more than 500 mapped SSR markers. A high polymorphism rate was detected in these populations, ranging from 44% to 60% with the most of them being co-dominant markers. These markers will be used to monitor the resistance introgressions

- *Field verification of resistance*

Scientists at the Michigan State University grew the 96-entry pinto bean RIL population AP647 at the Montcalm Research Farm (MRF). Yields ranged from 16 to 45 cwt/acre with a test mean of 32.2 cwt/acre. The parental lines AN37 and P02647 yielded 27.4 and 38.1cwt/a respectively. The highest yielding entry P07721 exceeded the test mean by 40% in the 2011 trial. White mold scores at MRF were lower in 2011 and ranged from 16 to 83% with a mean of 48%. Greenhouse straw test scores in this population ranged from 1.5 to 7.0 with a mean of 3.7 (rated on a 1-9 scale) and both greenhouse and field data is being used in QTL analysis.

Scientists at Michigan State University used Skylla, AxN-1-55, three lines from Dr. Craig Grau from University of Wisconsin, and five soybean plant introductions (PIs) with partial resistance to Sclerotinia stem rot were used as resistant parents to improve soybean for resistance to the disease. Four progeny lines with yield similar to the yield check IA2094 and with resistance similar or better than the resistant check S19-90 were developed..

PM 1.6: GM improved resistance

- GM oxalate oxidase expression, inheritance and field evaluation (**Not funded)**

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

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

- *Transgenic expression of antifungal peptides (****Not funded****)*

PM 1.7: Plant germplasm/cultivars with improved resistance

- *Enhanced adapted germplasm*

Scientists at North Dakota State University crossed Brassica napus plant introductions with higher resistance to S. sclerotiorum than the commercial cultivars to develop breeding populations, one conventional and the other a doubled haploid. The conventional population was developed from the cross between Ames 26628 and PI458939, the DH population was created from the cross between Ames 26628 and PI458940. In the last two years seedlings from the Ames 26628xPI458939 population were challenged using the petiole inoculation technique. Surviving plants were advanced to the F6 generation. With each new generation plants became more tolerant of infection and those plants that died took longer and longer to do so. The percentage of surviving plants increased from 30% in the F1 to 85% in the F4 generation.

ARS Scientists at Fargo ND used amphi-ploids resistant to stalk and head rot in crosses with HA 410, and backcross progenies with 2n=34 were established in the field, and BC2F4/BC3F3 families were evaluated in replicated trials in 2009-2011. Interspecific F1 progeny were produced between stalk rot resistant hexaploid H. californicus and H. schweinitzii and HA 410. Backcross progenies of H. californicus crosses with HA 410 were evaluated in replicated trials in 2009-2011. Crosses between NMS HA89 and head rot resistant H. maximiliani and H. nuttallii were advanced to BC1F4 and BC2F4 families for replicated field trials in 2009-2011. Stalk rot resistant diploid perennial H. maximiliani, H. giganteus, and H. grosseserratus were crossed with HA 410 in 2007, and their BC1F4/ BC2F3 families evaluated in replicated field trials in 2009-2011. Replicated field tests in 2009 and 2011 for head and stalk rot resistance indicated moderate to good resistance indicating successful gene introgression for head rot tests

Scientists from North Dakota State University, USDA-ARS Prosser, WA; and North Dakota State University moved the WM2.2 QTL, derived from Bunsi, and originally transferred to the pinto bean USPT-WM-1 (released in 2005) into a higher yielding pinto bean with better seed size, earlier maturity, and less problems with stay-green trait. This high yielding pinto bean with partial resistance to white mold will be released as an improved germplasm line USPT-WM-12 in 2012 in collaboration with Michigan State University AgBioResearch. The national Bean White Mold Nursery (BWMN) administered by James Steadman (University of Nebraska) also contributed to this new release. USPT-WM-12 had the highest yield out of 64 entries in the Michigan white mold nursery (data courtesy of Jim Kelly) for two consecutive years (2010 and 2011). It also possesses partial resistance in the straw test (Table 4) which was unexpected. Genetic populations are being generated to characterize the partial resistance of USPT-WM-12 in the straw test. USPT-WM-12 will be released as a germplasm line because the seed coat hue is slightly too dark for commercial success as a cultivar. Crosses between USPT-WM-12 and new slow dark pinto beans were conducted in 2011 to overcome this dark seed coat problem

Scientists at Idaho University and Colorado State University released a total of 42 breeding lines with high levels of white mold resistance have been developed in the past 10 years. Highly white mold resistant A 195 (large-seeded Andean) and VCW 54 and VCW 55 (interspecific breeding lines derived from P. coccineus accession G 35172) have been released, registered, and are being distributed to public and private researchers nationally and internationally. This material also has exhibited high levels of resistance in the National Bean White Mold Nursery for the past few years. The release and registration document for the P. costaricensis derived interspecific white mold resistant breeding line VRW 32 is under preparation.

- *Herbicide tolerant germplasm with resistance to Sclerotinia*

Scientists at North Dakota State University identified glyphosate-tolerant canola breeding lines with less disease than commercial controls. In 2011, Sclerotinia stem rot incidence ranged between 3% and 40% although most lines evaluated had <25% incidence. Seven breeding lines had SSR incidences below 15%. Some of these lines have performed well in previous years and were advanced to the next level by the breeder.

- *Identification of crop germplasm with partial resistance to virulent isolates*

ARS scientists at Fargo ND released 4 inbred lines with improved Sclerotinia resistance this year (RHA 472, RHA 473, RHA 474, and RHA 475). These are currently available to researchers and breeders.

Scientists at University of Nebraska, Colorado State University, Cornell University, Harris-Moran Seed Co., Michigan State University, University of Idaho, Seminis Seed Co., Oregon State University, North Dakota State University and USDA ARS-Prosser WA conducted 2011 greenhouse tests of 12 lines from five seed classes with WM resistance and increased seed of adaptation crosses. In field nursery trials all nine test lines had significantly better WM resistance than the susceptible check. These results confirmed the progress that the SI has made in identifying functional WM resistance. Aided by the multi-site nursery data over the past 6 years, a snap bean, two pinto lines, a bayo line and six kidney lines with WM resistance were released. At least five more lines are scheduled for release in 2012. New lines with WM resistance from wide interspecific crosses are now in seed increases for greenhouse screening. Superior WM resistance has been found in wild beans and other Phaseolus spp.

Scientists at Michigan State University evaluated over 2,000 lines derived from crosses in which either or both parents were partially resistant to Sclerotinia stem rot were evaluated for yield and other agronomic traits. Lines with acceptable yield and other agronomic traits were further evaluated for resistance to Sclerotinia stem rot. A cultivar Skylla and a germplasm AxN-1-55 with partial resistance to Sclerotinia stem rot were released.

- *Agronomic resistant varieties for commercial production.*

Scientists at ARS-Fargo ND; Ag Research Center, Central Lakes College, Staples, MN; Panhandle Res. & Exten. Center UNEB) Scottsbluff, NE; University of Nebraska; and NDSU Res. & Exten. Center, Carrington, ND evaluated 52 lines over 4 years for stalk rot, head rot, and yield tests. This resulted in the 2011 release of four oilseed lines, RHA 472, 473, 474, and 475. Current lines under multiple years of selection include lines with resistance to multiple races of rust and downy mildew so that subsequent releases will embody resistance to Sclerotinia head and stalk rot plus other diseases.

Scientists at North Dakota State University Carrington Research Extension Center; NDSU Langdon Research Extension Center; NDSU Carrington Research Extension Center, Oakes, ND; University of Nebraska Panhandle Research Extension Ctr, NDSU Williston Research Extension Ctr.; and North Dakota State University evaluated sunflower hybrids for resistance to Sclerotinia head rot. This resulted in the identification of sources of resistance to Sclerotinia head rot in commercial breeding programs, facilitated the development of commercial hybrids with partial resistance to Sclerotinia head rot, and improved the methodologies used to screen sunflowers for resistance to head rot. In 2011, susceptibility to Sclerotinia head rot was evaluated in Carrington, ND and Morden, MB for 72 commercial breeding lines and hybrids whose resistance to Sclerotinia head rot had not been properly characterized previously. In Morden, disease ranged from 0 to 40% across entries; in Carrington, disease ranged from 67 to 100% across entries. Disease severity was higher than ideal in Carrington, and misting will be conducted less aggressively in 2012. Sunflower midge, which was a problem in 2010, was successfully managed in Carrington with a late planting date and insecticide applications, but the trial was severely damaged by hail.

Pathogen Biology & Mechanisms of Disease Resistance

PM 2.1: Population structure & dynamics

- *Standardized genotypic characterization on wild and cultivated crops*

Scientists at ARS-Fargo ND; Ag Research Center, Central Lakes College, Staples, MN; Panhandle Res. & Exten. Center UNEB) Scottsbluff, NE; University of Nebraska; and NDSU Res. & Exten. Center, Carrington, ND optimized the ascospore production method developed by retired pathologist Dr. Michael Boosalis. Sclerotinia isolates from different hosts and geographic origins were compared to determine the effect of physical scarification on apothecial production. With technical staff at the three locations occupied with field research during the summer months, this project task is done from October to April, and is expected to be completed by December, 2012.

- *Documented gene-flow or outcrossing contribution to population variability (**not funded**)*

- *Discovery of ecological and bio-types with fungicide resistance*

- *Geographical inventory of US populations*

Scientists at University of Nebraska, Colorado State University, Cornell University, Harris-Moran Seed Co., Michigan State University, University of Idaho, Seminis Seed Co., Oregon State University, North Dakota State University and USDA ARS-Prosser WA characterized many of the isolates of S. sclerotiorum collected over the past 6 years from nine bean production regions in the USA as well as regions in Australia, Mexico and France using mycelial compatibility groups. A total of 65 MCGs have been found in the USA isolates. These same isolates have also under greenhouse conditions were found to have varying degrees of aggressiveness (2.8 to 7.9) based on a scale of 1 = no disease and to 9 = death of the plant.

PM 2.2: Durable host resistance

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

Scientists at University of Nebraska, Colorado State University, Cornell University, Harris-Moran Seed Co., Michigan State University, University of Idaho, Seminis Seed Co., Oregon State University, North Dakota State University and USDA ARS-Prosser WA determined aggressiveness variation of the USA characterized bean isolates. Differences in isolate aggressiveness were found among the isolates ($p<0.0001$) and isolates that are clones had similar aggressiveness. Clonality varied from 12 clones from 62 MI isolates to 27 from 59 isolates from WA. Shared clones also varied from seven to one across screening sites. There is evidence that pathogen variation can influence resistance screening results. A summary of MCG and aggressiveness characterization of 157 screening isolates collected across the USA was published (Otto-Hanson et al., 2011).

- *Pathogen population dynamics on partially resistant crops*

- *Pathogen x environmental interaction*

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

PM 2.3: Factors that mediate sclerotia germination

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

- *Host factors that mediate myceliogenic germination*

- *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 from University of Nebraska, NDSU-Carrington, Colorado State University, Michigan State University, University of Idaho, Oregon State University, Cornell University, USDA ARS at Prosser WA, Seminis Seeds and Harris Moran Seeds used four polymorphic microsatellite markers to genotype the isolate populations collected from 2003-2007. A total of 240 isolates have been sequenced and formed 65 microsatellite haplotypes which associated closely with the MCGs. Of the 81 MCGs formed by the 2003-2007 isolates, 68 have only one microsatellite haplotype. Using analysis of molecular variance analysis (AMOVA) with the microsatellite haplotype data, 75% of the haplotype variation comes from within the nine USA locations, 21% from among the locations within regions (East, Midwest or West) and only 4% of the variation is found among the regions. This variability data does not address aggressiveness.

- *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. Filtrate activity was not quantitatively associated with the virulence level exhibited by plate cultures of the oxalate minus mutant. Variations in plate culture virulence were associated with penetration efficiency that appear to be epigenetically controlled. This variation could be eliminated by wounding plants prior to inoculation. As such all oxalate minus mutants displayed attenuated virulence relative to the wild-type, yet culture filtrate activities were comparable between wild type and the oxalate minus mutant. This indicates that a toxic factor exists that is independent of oxalic acid production and not correlated with quantitative measures of virulence.

PM 2.5: EST libraries from pathogen stains

- *Useful cDNA libraries from pathogen expressed genes*

Scientists at North Dakota State University created cDNA libraries from Sclerotinia isoltates from inoculated canola petioles and stems. cDNAs were sequenced at the University of Minnesota. Differential expression

patterns were characterized using DESeq. Analysis. Comparison of fungal genes that are expressed during infection process and in culture will allow us to gain understanding of mechanisms employed by S. sclerotiorum during pathogenesis. Information obtained from this analysis can be useful in designing alternative disease management strategies. Analysis of the above two mentioned comparisons are initiated and in progress. Moreover, while the sequencing and sequence data analysis was being conducted, the expression of selected S. sclerotiorum genes, oxaloacetate acetylhydrolase, metallothionein, and cellobiohydrolase I, which were expected to be involved with virulence or pathogenicity was analyzed by RT-PCR. The actin gene was used as a control. Synthesis of cDNA from total RNA and PCR was completed using commercially available kits. Results indicate that all analyzed S. sclerotiorum genes were expressed at all the time points. There were no observable differences in gene expression of S. sclerotiorum retrieved from NEP32 (susceptible) and NEP63 (resistant) canola lines, likely indicating that these genes are not affected by the differential reaction occurring between the canola lines during infection.

- *Useful genome sequence information*

Scientists at University of Nebraska, Colorado State University, Cornell University, Harris-Moran Seed Co., Michigan State University, University of Idaho, Seminis Seed Co., Oregon State University, North Dakota State University and USDA ARS-Prosser WA identified 18 polymorphic microsatellite markers for differentiating Sclerotinia isolates. 239 isolates were sequenced, and formed 65 microsatellite haplotypes which associated closely with the MCGs. Of the 81 MCGs formed by screening nursery isolates, 66 have only one microsatellite haplotype. Using analysis of molecular variance analysis (AMOVA) with the microsatellite haplotype data, 64% had a single haplotype associated with an MCG. Total molecular variation was composed of 70% in populations within geographic regions, 20% was among populations within regions and 10% associated with differences among regions (Table 5). We have developed a database that soon will be able to recommend and supply characterized isolates that breeders/pathologists can use to screen for WM resistance in local areas or across regions and selecting for high, moderate or low levels of resistance.

- *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*

Scientists at the University of Nebraska and the University of Missouri identified 28 putative QTL on 15 different chromosomes in soybean. using a detached leaf assay. Five RIL populations of 100 lines each were evaluated, using Williams 82 as the common susceptible parent. Seven of the QTL identified in the study were significant in at least 2 of the 5 populations. Subsequent reports from other research groups have supported many of these QTL .

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

Scientists at ARS-Fargo ND; Ag Research Center, Central Lakes College, Staples, MN; Panhandle Res. & Exten. Center UNEB) Scottsbluff, NE; University of Nebraska; and NDSU Res. & Exten. Center, Carrington, ND conducted association mapping on sunflower Plant Introductions and USDA inbreds that exhibit

Sclerotinia resistance. The same material was evaluated for Phomopsis resistance in four field trials. SNPs identified were integrated into high density genetic maps.

Scientists from North Dakota State University, USDA-ARS Prosser, WA; and North Dakota State University fine mapped WM8.3 QTL in the region of chromosome Pv08 for partial resistance to white mold. The region was saturated with indel-based markers that were generated from common bean and soybean sequence synteny information. This approach generated an additional 61 markers (55 at > LOD 2.0) to a 60.2 cM region of Pv08 on the community-wide BAT93 x Jalo EEP558 linkage map. This resulted in a high density map of the region consisting of ~one marker per cM. Those markers were then used to remap the QTL in the PS02-029 population developed from backcrosses between the great northern cultivar Matterhorn (susceptible recurrent parent) and the snap bean NY6020-4 (partially resistant donor parent). Utilizing the indel markers polymorphic between those two parents, the QTL was mapped, using composite interval mapping, to an interval of 3.1 cM which is narrower than the previously defined ~25 cM interval.

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, North Dakota State University and Dow AgroSciences used parents of a pea mapping population that Dr. McPhee established in an RNA-seq approach to examine the expression profile of the Sclerotinia-pea host pathogen interaction. The parents, Lifter a susceptible cultivar and PI240515 a partially resistant line were inoculated with S. sclerotiorum under conditions similar to those under which the population was phenotyped. cDNA was massively parallel sequenced. The expression profile of both pea and S. sclerotiorum are being examined at multiple time points to identify genes involved in the resistance response as well as S. sclerotiorum genes involved in pathogenicity. By utilizing similar environmental and inoculation conditions as used to phenotype the mapping population we hope to identify potential resistance genes that will map to identify QTL's . Software called Trans-ABySS and Trinity allowed de novo assembly and analysis of RNA-seq data without a reference genome. The RNA-seq approach has generated a large amount of transcript sequence data for each pea parent, which will be valuable for the development of gene-linked markers such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs).

Scientists from North Dakota State University, USDA-ARS Prosser, WA; and North Dakota State University conducted RNAseq analysis of WM8.3 QTL in dry bean: Expression patterns associated with the WM8.3 QTL were evaluated in near isogenic lines that contain either the resistance or susceptible QTL. Using the straw test, RNA was collected from pools of WM8.3 resistant and susceptible lines 24 and 48 hrs after inoculation. Mock inoculation and non wounded controls were included. cDNA libraries were developed and sequenced using the Illumina GAIIx technology. 131 million reads of 32 nt (4.2 bp billion) were collected and associated with the common bean sequencing project maps to analyze gene expression along chromosome Pv08. Utilizing the gene models for that chromosome, and after correcting for genotype and treatment effects (such as wounding), 20 genes were found to be differentially expressed at 24 hrs and 17 genes were differentially expressed at 48 hrs. Of these 37 genes, the Arabidopsis homolog for 12 was associated with a disease defense response to some pathogen. Two of these were especially intriguing. One LRR is a classic disease defense gene model often associated with qualitative resistance. The second gene, PUB13 is of particular interest because it is known to be a member of a signal cascade pathway that begins with pathogen recognition and ends with activation of the general disease defense response.

Scientists from North Dakota State University, USDA-ARS Prosser, WA; and North Dakota State University correlated RNA-seq results with QTL mapping in dry bean. Mapped scaffolds from the sequencing project relative to the genetic map was integrated using the indel markers. One mapped to scaffold 00093, while PUB13 mapped to scaffold 00361. These loci mapped just outside the QTL peak. It should be noted that our mapping of WM8.3 QTL in PS02-029 mapping population only used the available indel markers. That marker set did not extend the entire length of the chromosome, rather it only was localized to one end of the chromosome. Therefore, the actual mapping position of the QTL is bound by the marker locations and indeed may extend further along the chromosome and include one or both of the LRR and PUB13 genes. An additional 16 indel markers (recently available from the USDA/NIFA BeanCAP project were used to extend the map the full length of the chromosome.

Scientists from North Dakota State University, USDA-ARS Prosser, WA; and North Dakota State University conducted RNAseq analysis of WM7.1 QTL in dry bean: The tissue for the first biological replication has been collected from near isogenic lines and the second replication has been planted, and tissue from that run should be available within the next 20 days. The National Center for Genome Resources will perform next generation (next-gen) sequencing of our RNA samples. This year, 100 base-pair reads (vs. 36 from last year) will be collected. Advances in sequencing technology will result in a greater sequencing depth for this experiment.

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

Scientists at Michigan State University, North Dakota State University and Dow AgroSciences develop[ed s 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. This data set has been analyzed and a manuscript has been prepared for submission to Genome Biology. SSR (microsattelite) markers were identified in the 454 sequence data from pea and were utilized to develop markers that were screened for polymorphism across pea parents from 4 recombinant inbred lines developed by Dr. McPhee.

ARS scientists at Urbana IL developed reduced representations were prepared from genomic DNA of G. latifolia accessions PI559298 and PI559300 and sequenced on an Illumina sequencer, which produced over 35 million 100-nt reads from each line. Over 350,000 SNPs were identified between the two parental lines, about 10% of which aligned to the soybean genome. To test the usefulness of the SNPs and the synteny between the G. latifolia and G. max genomes, nine SNPs were selected that aligned to soybean chromosome 4 or 13. All of the G. latifolia SNP markers segregated in the expected 3:1 manner in 94 lines of a G. latifolia F2 population, formed two distinct linkage groups and mapped in similar orders in G. latifolia and G. max. These results confirmed that the SNPs identified from the genome sequences of the two G. latifolia lines will be useful for gene mapping and comparing gene locations between G. latifolia and G. max.

- *Physical map of genomic regions containing resistance genes*

Scientists at the University of Nebraska and University of Missouri mapped LysM genes in soybean near genomic regions that did not have significant QTL in the Corsoy 79 or Dassel populations, but are found on LGs where significant QTL were identified in one or more of the 3 other populations. For example, the LYK4NFR5b gene maps near Satt129 and Satt147 on LG-D1a, which are significant in the DSR 173 population and NK S19-90 populations. Linkage groups F, G, J, and N contain QTL for white mold resistance as well as resistance genes for other fungal pathogens such as fusarium, phytophthora, and powdery mildew. A search of SoyBase (www.soybease.org) indicates that QTLs for partial resistance to phytophthora have been identified on LG F associated with Satt252 and Satt423, which are within 5 cM of Lyk11.

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

Scientists at ARS-Fargo ND; Ag Research Center, Central Lakes College, Staples, MN; Panhandle Res. & Exten. Center UNEB) Scottsbluff, NE; University of Nebraska; and NDSU Res. & Exten. Center, Carrington, ND genotyped the entire set of sunflower PIs with 10,000 SNP markers and re-sequenced resistance gene candidates from the PIs, which has been used to map stalk rot resistance and can now be applied to head rot. This facilitated selection of populations and breeding lines for stalk and head rot resistance, and introduction of new populations to breeding programs.

PM 3.3: Function of candidate resistance genes

- Microarrays for high throughput gene screening

Scientists from North Dakota State University created cDNA libraries from inoculated canola petioles for NGS at the DNA Sequencing and Analysis Facility, BioMedical Genomics Center at the University of Minnesota using the Illumina GA IIx. Comparisons were made to determine ESTs differentially expressed in resistant line NEP63 compared to the susceptible line NEP32 during infection process. Preliminary analysis of the petiole inoculation libraries revealed a total of 4350 differentially expressed ESTs, of which 1946 are up-regulated. Interestingly, more than 400 ESTs seem to be turned on exclusively in resistant line, 436 ESTs are up-regulated by 10 fold change. From the leaf inoculated libraries, a total of 477 ESTs were found to be differentially expressed in the resistant line NEP63, 320 of them were up-regulated and 157 were down-regulated. However, from the leaf inoculated conditions no genes that are exclusively turned on in

resistant line have been found in the initial analysis. Functional categorization of the up-regulated gene set was performed using Blast2Go. Of the 320 ESTs only 163 have a known role in biological processes. 24 of the 163 ESTs were found to be involved in defense response.

- *Sequenced cDNA libraries from infected host tissue*

Scientists from Michigan State University, North Dakota State University and Dow AgroSciences, LLC used RNA-seq to examine expression profiles of Sclerotinia-pea host pathogen interactions in susceptible X partially resistant lines The expression profile of both pea and *S. sclerotiorum were* examined to identify genes involved in host resistance as well as pathogen pathogenicity. Trans-ABySS software was used for *de novo* assembly and analysis of RNA-seq data. Phenotypic association was used to map resistance gene QTL. RNA-seq revealed a large number of gene-linked markers (SSRs and SNPs). EST sequence reads were assigned by BLAST analysis. Fifty eight percent of reads were assigned to pea, 25% were assigned to *S. sclerotiorum*, 1% were assigned to both pea and *S. sclerotiorum* and only 16% were unassigned. The pea transcripts were assembled into 11,810 contigs and 17,619 singletons. Seventy three putative SSRs with a length of repeat greater than 20 nucleotides were identified in the pea contigs and will be evaluated as potential makers for pea genetic mapping.

- *Discovery and function of candidate genes for Sclerotinia resistance*

ARS scientists at Urbana IL, Agriculture and Agri-Food Canada and University of Florida used gene expression profiling and gene clustering to identify candidate defense-associated genes. Expression analyses were conducted on partially resistant PI194639/susceptible Williams 82; a resistant oxalate oxidase (OxO) transgenic/susceptible control; and infiltrated OA to water at neutral and acid pH. 60 candidate defense-related genes were selected based on their response to OA and Sclerotinia in the transgenic OXO line 80(30)-1. 105 genes (19 of which are located within 500 kb of a known white-mold resistance QTL) also were significantly differentially expressed between resistant and susceptible lines. qRT-PCR confirmed the microarray results. The transformation system routinely used in the Simmonds' lab will be used instead of the VIGS system. Selected candidate gene homologs in Arabidopsis, a plant for which a mutant collection exists of defined T-DNA insertions, and for which transformation is straightforward was studied. Identification of the best candidate defense genes close to Sclerotinia defense QTL was conducted by running autoBLAST to the soybean genome

ARS scientists at Fargo ND developed a population derived from 36 sunflower Plant Introductions with high Sclerotinia resistance and elite inbred lines with improved yield and other traits of importance. Genome-wide and candidate gene analyses revealed that sunflower homologues of significant genes from Arabidopsis-Sclerotinia research are significantly associated with Sclerotinia stalk rot resistance in sunflower, particularly the two Coronatine Insensitive (Coi1) homologues. This information will guide marker-assisted improvement of the aforementioned sunflower population.

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PM 3.4: Mechanisms of Sclerotinia resistance

- *Yeast screens for ecotypes and defense-related mutants for oxalate sensitivity* ***(not funded)***

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

- *Conventional analysis of genetic mechanisms*

Scientists at the University of Nebraska and University of Missouri explored plant defense mechanisms in soybean that involve chitin degradation by a family of Lys-M domain containing proteins. LysM is a protein domain originally described in bacterial enzymes known to be involved in hydrolysis of the peptidoglycan component of the bacterial cell wall. Peptidoglycan is structurally similar to chitin, and, therefore, the presence of the LysM domain suggests that the LysM-RLKs (receptor-like kinases) are involved in chitin recognition. A mutation in the Arabidopsis LysM-RLK1 (also called AtCERK1) gene completely abolished the plant response to chitin resulting in increased susceptibility to fungal infection Chitin is an established pathogen-associated molecular pattern (PAMP) and is an important part of the fungal response pathway. Plant cells do not contain chitin although they do have chitinase enzymes that degrade chitin in fungal cell walls in response to fungal infection. AtLysM RLK1 (AtCERK1) is the receptor for chitin found on the plasma membrane and has been shown to directly bind to chitin, activating a protein phosphorylation cascade The net result is activation of the transcriptional response leading to enhanced innate immunity.

PM 3.5: Bioinformatic resources

- *Web-based communication for the Sclerotinia Initiative*

See: *http://www.ars.usda.gov/Research/docs.htm?docid=20317&page=3*

- *Interactive website for genetic, genomic & biotech resources*

See: http://lis.comparative-legumes.org/lisg/lis_links.html

Disease Management & Pathogen Epidemiology

PM 4.1: Optimized fungicide application programs

- *S. sclerotiorum isolate collection to assess fungicide sensitivity (not funded)*

- *Efficacy of new chemistries*

Scientists at North Dakota State University and Langdon ND evaluated the efficacy of chemical fungicide tank mixtures and biological control agents as alternatives to mange S stem rot in canola. Five chemical fungicides that were applied alone or in combinations of two fungicides at a time and three biological fungicides for control of Sclerotinia stem rot of canola. Sensitivity data was taken for thiophanate methyl and metconazole for 96 S. sclerotiorum isolates collected from 14 states in North central US. Sensitivity to boscalid and pyraclostrobin is in progress. Efficacy of fungicides for disease control was evaluated in field trials whereas fungicide sensitivity was assessed in laboratory. In field trials, compounds were applied alone or in combinations of two compounds at a time each at 50% of the field doses. Efficacy of control varies from year to year. Most treatments, either applied alone or in tank mixes with other compounds, on average reduced disease incidence by almost 40% when compared to the non-protected plots although in some years a few mixes reduced incidence by up to 80%.

Scientists at North Dakota State University Carrington Research Extension Center; NDSU Langdon Research Extension Center; NDSU Carrington Research Extension Center, Oakes, ND; University of Nebraska Panhandle Research Extension Ctr, NDSU Williston Research Extension Ctr.; and North Dakota State University evaluated fungicide efficacy of 11 to 13 fungicides in sunflower field trials in Carrington, ND, Langdon, ND, and Scottsbluff, NE. In the non-treated check, disease incidence was 35% in Langdon, 67% in Carrington, and near 0% in Scottsbluff in mid- to late-September. Fungicide treatments resulted in differences in disease incidence and severity in Langdon but not Carrington, and they had no impact on seed yield or quality in any of the trials

- *Updated management guides for disease management*

Scientists at Colorado State University encountered low white mold pressure in fields with a history of the disease due to delayed plantings (early-spring rains) which delayed flowering until late July when weather conditions were warm and dry. In addition, one or more of the commercial fields was severely damaged by common rust and/or bacterial brown spot which opened up the canopy of the commercial cultivar surrounding the trial and significantly reduced the production of ascospore inoculum. Analysis of data from the multiple locations revealed that there were no interactions between fungicide treatment or nitrogen by entries or locations during 2009 and 2010; nitrogen was not compared in 2011. Yields of the 4 entries averaged 1135-3641 lb/acre at the research station and 2966-3433 lb/acre at the better commercial fields in the absence of white mold and with varying presence of bacterial diseases and rust in the surrounding fields of commercial pinto beans. 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 during the 2009 study

- *Improved spraying technologies*

PM 4.2: Bio-control alternatives for disease management

- *Grower recommendations for commercial sclerotial antagonists*
- *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*

Scientists at Colorado State University Plant found that canopy monitoring during late vegetative to seed fill periods of crop growth showed that average daily relative humidity was higher in a prostrate vine cultivar (Montrose) than an upright semi-vine type (Stampede); and canopy temperature showed the reverse trend during 2010-2011. These observations improved understanding of the effects of plant spacing and architecture (prostrate versus upright) upon pathogen development and forecasting with the goal of enhancing disease forecast models and IPM approaches to deal with white mold.

Scientists at North Dakota State University Carrington Research Extension Center; NDSU Langdon Research Extension Center; NDSU Carrington Research Extension Center, Oakes, ND; University of Nebraska Panhandle Research Extension Ctr, NDSU Williston Research Extension Ctr.; and North Dakota State University evaluated sunflower susceptibility to Sclerotinia head rot across diverse environments for 22 commercial breeding lines and hybrids that exhibited reduced susceptibility to head rot or stalk rot in 2010, and multiple lines were identified with reduced resistance. The trials conducted in Langdon, Oakes, and Crookston were particularly informative, showing highly consistent results and clear differentiation of treatments. The first assessment of the susceptibility of sunflowers to head rot after flowering was conducted. Inoculations at R5 (flowering) and R6 (immediately after flowering) resulted in significant increases in disease relative to the control in Carrington and Langdon, respectively. A resistant and a susceptible hybrid were evaluated. In Carrington but not Langdon or Oakes, the hybrids differed in their susceptibility to Sclerotinia head rot across inoculation timings, with the resistant but not the susceptible hybrid showing near-immunity to head rot when inoculated after flowering.

- *Management decision aids*

Scientists at North Dakota State University used new compounds in tank mix trials and to fine tune the combinations we identified as most effective and consistent, i.e. thiophanate methyl and boscalid. Tank mixes with concentrations >50% of the doses of some of the compounds were also evaluated. A regional data base on sensitivity to thiophanate methyl and metconazole was developed using 96 S. sclerotiorum collected from 14 states in North central US. Information on sensitivity to boscalid is in progress. Most isolates were sensitive to thiophanate methyl, but a few from Minnesota and Nebraska had EC50 values > 2 ppm, which is considered the resistance threshold.

- *Precision agriculture program* ***(not funded)***

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2011 Sclerotinia Funding Matrix

Project	PI	2010	Cooperator	Commodity	2011	Aveage Rating
Evaluation of Wild *Helianthus* Species for Resistance to Sclerotinia Stalk Rot	**Block**	**$14,767**	ARS	Sunflower	$14,029	**4.83**
Searching for resistance sources to Sclerotinia in wild relatives of cool season grain legumes	**Chen**	**$73,912**	ARS	Pea & Lentil	$55,000	**4.12**
Expression profiling of the pea-Sclerotinia sclerotiorum interaction for genomics assisted breeding	**Chilvers**	**$63,870**	MI	Pea & Lentil	$58,044	**4.00**
Functional verification of candidate defense-related genes in *Sclerotinia sclerotiorum* in soybean and arabidopsis	**Clough**	**$66,099**	ARS	Soybean	$84,512	**4.83**
Development of Canola Breeding Populations and Identification of Herbicide-tolerant Breeding Lines with Resistance to Sclerotinia Sclerotiorum	**del Rio**	**$66,955**	ND	Canola	$72,973	**3.87**
Optimizing Management of Sclerotinia Stem Rot of Canola Through Fungicide Use	**del Rio**	**$45,112**	ND	Canola	$37,370	**3.22**
Identification of novel loci for partial resistance to sclerotinia stem rot in perennial soybean accessions	**Domier**		ARS	Soybean	$45,000	**3.54**
Identification of Resistance and Pathogenicity Genes Associated with Sclerotinia Sclerotiorum Infection using Next-generation Sequencing	**Goswami**	**$65,100**	ND	Canola	$60,306	**4.33**
eQTL analysis in soybean populations to elucidate genetic architecture of host response to infection by *Sclerotinia sclerotiorum*	**Graef**		NE	Soybean	$69,096	**4.63**
Discovery and use of novel sources of resistance to head rot and stalk rot in cultivated sunflower & wild *Helianthus*	**Gulya**	**$81,012**	ARS	Sunflower	$78,699	**4.21**
Transferring Sclerotinia resistance genes from wild *Helianthus* species into cultivated sunflower	**Jan**	**$106,000**	ARS	Sunflower	$119,700	**4.42**
White mold resistance-QTL: Identification, interactions & fine mapping in common bean	**Miklas**	**$160,000**	ARS	Dry Bean	$154,096	**4.63**
Deployment of novel sources of Sclerotinia resistance and tools for breeding resistance in sunflower	**Qi**		ARS	Sunflower	$109,250	**4.46**
Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas	**Steadman**	**$58,907**	NE	Dry Bean	$60,202	**4.59**
Evaluation of Sunflower Hybrids for Resistance to sclerotinia Head Rot	**Wunsch**		ND	Sunflower	$70,856	**4.25**
Outreach (summit meeting, website, etc.)		**$28,994**	ARS	All	$28,994	