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Service

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Meeting Strategic Milestones of the National Sclerotinia Research Initiative for 2013

**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 *Strategic Plan for the National Sclerotinia Initiative 2013-2017* 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 U.S. 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 2013* 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

	2013	2014	2015	2016	2017
	number of accomplishment citations				
Total Accomplishments	43				
Total Milestones	79				
Achievement Rating (%)	54.4				
Total Projects	21				
Accomplishments / project	2.0				
Total Publications	266				
Germplasm/Varieties released	41				

Achievement Rating: # cited accomplishments/ # published milestones *100

Distribution of Accomplishments across Strategic Plan

Milestone	2013	2014	2015	2016	2017
Crop Germplasm Resources & Genetics	number of accomplishment citations				
PM 1.1: Identify new sources of resistance.					
Germplasm of all NSI crops.	1				
Improved phenotypic methods for validating resistance.	2				
PM 1.2: Transfer new sources of resistance (pre-breeding).					
Common bean breeding lines from interspecific crosses.					
Canola, chickpea, lentil, pea, soybean and sunflower lines.	1				
Sunflower breeding lines from wild species	1				
PM 1.3: Genetic analysis and discovery of QTL					
Breeding pops in canola, bean, pea, soy, and sunflower.	1				
Backcross in sunflower/MAGIC populations in common bean.	1				
GWAS and linkage analysis in common bean & sunflower.	1				
QTL markers in NSI crops.	1				
Metabolic mechanisms with resistance QTL.	1				
PM 1.4: Pyramid white mold resistance and release cultivars.					
Canola, chickpea, lentil, and pea lines released.	1				
Pinto and other bean market classes released	1				
Interaction of QTL in common bean and soybean.	1				
Establish disease nurseries.					
Soybean breeding line with Sclerotinia resistance	1				
Commercial & experimental release of sunflower lines.					
Pathogen Biology & Mechanisms of Resistance					
PM 2.1: Characterize population structure & ecology					
Interaction of pathogen with environmental factors.					
Biotypes with resistance to new fungicide chemistry					
Characterization of the genetics of fungicide resistance					
Characterization of ecological types in the population.					
Associate activity in <i>Sclerotinia</i> with specific genetic markers.					
PM 2.2: Characterize virulence/aggressiveness					
Reactions of isolates on new sources of host resistance.	1				
Collection of isolates with broad aggressiveness	1				
New sources of host resistance isolates	2				
Criteria for virulence/aggressiveness on specific hosts.					
PM 2.3: Identify environmental & genetic factors in germination					
Factors that may enhance myceliogenic germination.	2				
Genetic control of myceliogenic/carpogenic germination					
Genetic events that lead to carpogenic germination.					
PM 2.4: Temporal gene expression profiles of Sclerotinia.					
Transcriptomic, genomic, and metabolomic data bases.	1				
Improved gene annotation using transcriptomic data.	1				
Genetic control of infection processes	1				
PM 2.5: Identification and verification of candidate genes.					
Development and maintenance of culture collections.					
Transcriptome profiling analyses.	1				
Promoters for RNAi constructs.					
Inventory of genes involved in pathogenesis.					
Functional verification of candidate genes.					
Gene Discovery & Phenotypic Association					
PM 3.1 Molecular marker resources for QTL Discovery.					
Core set for deployment in genotyping systems	1				
DNA markers for annotation of crop genomes	1				
Allele specific DNA markers for disease resistance					
Allele specific DNA markers for quality traits					

Milestone	2013	2014	2015	2016	2017
Allele specific DNA markers for yielding ability					
PM 3.2 Genetic and physical maps for Sclerotinia resistance.					
Compilation of genotypic data from mapping populations					
Improved genetic maps for Sclerotinia resistance genes	1				
Consensus genetic map for Sclerotinia resistance genes					
Core sets of markers for disease resistance					
Core sets of markers for quality traits					
Core sets of markers for agronomic traits					
Place candidate genes on consensus genetic map					
3.3 Characterize gene models for pathology & resistance.					
Standardized annotation of maps among crop species.					
Transcriptomic, proteomic & metabolomic annotation of QTL	1				
Biological mechanisms for resistance, such as: oxalic acid	2				
Gene atlas with a comprehensive list of all expressed genes.					
Identification of specific genes within QTL					
3.4 Genome mapping and allelic analysis through GWAS.					
High resolution exome maps of QTL					
Specific alleles that mediate Sclerotinia resistance.					
GWAS studies of phenotypic variation in disease resistance.	3				
Haplotype maps correlated with genetic variation for resistance.	1				
Allele specific markers for pyramiding genes					
PM 3.5 Develop improved resistance with biotechnology.					
Inventory validated resistance genes, promoters, & constructs					
Transcription factors and elements of gene regulation					
Functional tests in model plants to determine candidate genes	3				
Efficacy of transformed genes on defense control	2				
Genome editing to modify resistance to Sclerotinia					
Crop germplasm transformed with putative anti-fungal genes.					
Disease Management & Crop Production					
PM 4.1: Optimize fungicide application programs.					
Collection of <i>S. sclerotiorum</i> isolates for fungicide sensitivity	1				
Economic return of fungicide applications	1				
Management guides for fungicides	1				
Spray technologies for fungicide performance					
Timing of fungicide applications.	1				
PM 4.2: Develop bio-control alternatives.					
Efficacy of current bio control agents					
Novel antagonists of <i>S. sclerotiorum</i>					
Management guides for bio fungicides					
PM 4.3: Develop disease-warning systems.					
Models that calculate risk of disease development					
Effect of tillage practices on Sclerotinia survival;					
Economic loss models					
Define risk levels for crop-specific fungicide decisions					
PM 4.4: Optimize cultural practices for disease management.					
Variety selection based on disease reaction					
Publication of disease management information					
Epidemiological information on disease development.					
Total Accomplishments	43	..			
Total Milestones	79				
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Germplasm lines released	41				

2013 Accomplishment Highlights supported by publications

Application of advanced technologies:

Metabolic engineering of pathways associated with plant defense (common bean)
 Genome editing for specific genes (canola)
 Sequence independent amplification for RNA viruses that have infected Sclerotinia genomes
 SoySNP6K Illumina BeadChip for genotyping arrays (soybean)
 RNA-seq for transcriptome analysis of expressed genes (bean, soybean, canola, pea, pathogen)
 GWAS for SNP markers that define haplotypes and high density genetic maps (soybean, bean, canola)
 RAD-seq for SNP discovery in sunflower
 MAGIC for nested association studies with interspecific crosses (common bean)
 Reference genome sequences for Sclerotinia, soybean, Glycine latifolia, common bean
 Transgenic germplasm that overexpresses phytoalexins (soybean)
 Fast neutron induced mutagenesis (common bean, soybean)
 High-through put resequencing for Genotype x Sequencing in breeding populations
 Antisense transformation of the AOx2b gene for functional analysis of oxalate in disease prevention
 Regulatory control of selected viral genes that are present in the genome of Sclerotinia isolates
 Mechanisms of Sclerotinia sensitivity to commercial fungicides
 Biocontrol applications mediated by controlled expression of endogenous secondary metabolites
 Pathogen race specific DNA-markers (chickpea)
 High-density QTL maps for candidate gene discovery (pea, soybean, sunflower, bean)
 Molecular diagnostics for production decision guides (sunflower)
 Cytogenic maps based on BAC/BIBAC for genome sequence guided breeding selection (sunflower)

Released germplasm and varieties:

USPT-WM-12 (pinto bean)
 RioRojo (small red bean)
 ND 307 (pinto bean)
 Eldorado (pino bean)
 Lynx (winter pea)
 XRAV 40-4 (balck bean)
 Beniquez (white bean)
 Shiny Black Pearl (blackbean)
 8 high disease resistant germplasm (soybean)
 US14H BR6 (common bean)
 Cornell 607-612) (common bean)
 Lareat (pinot)
 Stampede (pinto)
 TARS-Tep22 (bean)
 TARS-Tep32 (bean)
 1 unnamed resistant germplasm (pea)
 HA R9 (sunflower)
 Essex (lentil)
 Seminis Seed cvs: (Hercules, Titan, Pony Express, Valentino, Secretariat, Green Valley, Zapata, Ulysses, Spartacus, Firstmate, Weapon, Gold Dust), BA1001, BA0999, SV1003, Sybaris)

Milestones for Sclerotinia Research - 2013

Crop Germplasm Resources & Genetics

Goal 1: Identify novel germplasm sources with higher levels of field resistance effective against a wide range of aggressive *Sclerotinia sclerotiorum* isolates.

PM 1.1: Identify new sources of resistance in plant germplasm.

- Germplasm accessions of canola, chickpea, lentil, pea, and sunflower, and wild crop relatives with resistance to *S. sclerotiorum* are identified and used in breeding programs.

1.1.21.01 Scientists at USDA-ARS Fargo, ND, North Dakota State University, Central Lakes College- Staples, MN, and University of Nebraska discovered a large group (260) of USDA Plant Introductions with superior resistance to head rot and stalk rot. These lines were used to identify candidate genes and in genome wide association mapping. This germplasm also exhibits resistance to Phomopsis stem canker. One gene family was associated with basal stalk rot resistance. Existing populations and breeding lines for stalk and head rot resistance were advanced. Current lines also have resistance to multiple races of rust and downy mildew. Several inbred lines that pyramid resistance with crop quality characteristics will be released.

- Improved phenotypic methods for identifying and validating resistance to *S. sclerotiorum*, in accessions from USDA and World germplasm collections.

1.1.19.01 Scientists at North Dakota State University amassed a diverged collection of 380 accessions of *Brassica napus* from 29 countries to screen for resistance to Sclerotinia stem rot in canola. These accessions were genotyped using an Illumina sequencing (GBS) platform. A library of single nucleotide polymorphisms (SNPs) was created. Disease scores and SNP markers will be used identify QTL associated with the Sclerotinia stem rot resistance genes.

1.1.21.02 Scientists at USDA-ARS Fargo, ND, North Dakota State University, Central Lakes College- Staples, MN, and University of Nebraska tested the ascospore production method developed by retired pathologist Dr. Michael Boosalis at three laboratories (Fargo, ND, Carrington, ND; Lincoln, NE; and Scottsbluff, NE) using six bean isolates of Sclerotinia. The original isolate (NEB-274) and one other produced ascospores with no preconditioning other than scarification, while four isolates failed to produce any apothecia. This method, while extremely effective, *may be applicable only to some fungal isolates*.

PM 1.2: Transfer and adapt new sources of resistance genes into useful plant germplasm (pre-breeding).

- Common bean breeding lines derived from interspecific crosses with effective resistance in multiple environments and against a range of aggressive isolates.
- Canola, chickpea, lentil, pea, soybean and sunflower lines selected from un-adapted accessions with confirmed resistance to Sclerotinia stem rot and evaluated for agronomic traits.

1.2.14.01 USDA-ARS scientists at Fargo ND developed sunflower germplasm with excellent stalk and head rot resistance in six amphiploids and wild perennials. Amphiploids resistant to stalk and head rot were crossed with HA 410, backcross progenies with $2n=34$, and BC2F4/BC3F3 families were evaluated in replicated trials in 2009-2013. Interspecific F1 progeny were produced between stalk rot resistant hexaploids *H. californicus* and *H. schweinitzii* and HA 410. Backcross progenies of *H. californicus* with HA 410 were evaluated in replicated trials in 2009-2013. Crosses between NMS HA 89 and head rot resistant *H. maximiliani* and *H. nuttallii* were advanced to BC1F4 and BC2F4 families for replicated field trials in 2009-2013. Stalk rot resistant diploid perennials *H. maximiliani*, *H. giganteus*, and *H. grosseserratus* were crossed with HA 410 and their BC1F4/ BC2F3 families were evaluated in replicated field trials in 2009-2013. Follow-up replicated field tests for head rot and greenhouse tests for stalk rot resistance in 2013 indicated moderate to good resistance, further confirming successful gene introgression.

- Sunflower breeding lines with enhanced resistance to Sclerotinia stalk rot derived from wild annual and perennial species via interspecific hybridizations. Alien chromosome addition stocks characterized and used for resistance breeding.

1.2.14.02 USDA-ARS scientists at Fargo ND conducted a molecular tracking study that indicated a higher frequency of gene introgression from diploid perennials than from hexaploid or interspecific amphiploids. A genomic in situ hybridization (GISH) technique distinguishing chromosomes of the perennials and cultivated sunflower was developed. New crosses using *H. strumosus*, *H. tuberosus*, and *H. decapetalus* were made in 2013 and are being backcrossed with HA 410. *Helianthus hirsutus*, *H. salicifolius*, *H. occidentalis*, *H. divaricatus*, and *H. resinosus* were crossed with HA 410, HA 451, or NMS HA 89 in 2012 to further diversify the pool of resistance genes and to increase the probability of identifying useful major resistance QTLs. Their early generation field seed was increased in 2013, which will provide more than 400 new families for replicated field test, in 2014.

PM 1.3: Genetic analysis and discovery of quantitative trait loci (QTL) that confer resistance to Sclerotinia

- Bi-parental breeding populations generated in canola, common bean, pea, soybean, and sunflower for identification of QTL associated with Sclerotinia resistance from diverse sources.

1.3.06.02 Scientists at USDA-ARS Prosser WA, Oregon State University and North Dakota State University examined phenotypic interactions among major QTL conferring partial resistance to WM in common bean. A RI population consisting of 150 lines in F5:7 was developed. The resistant parent is USPT-WM- 312, a pinto germplasm line with a strong combination of field and greenhouse resistance. Great Northern Orion was the highly susceptible parent. This population is used to understand the nature of the greenhouse resistance based on multiple QTL (epistasis) and genetic mapping to understand how this unique resistance is being manifested in this new pinto germplasm line.

- Advanced backcross populations in sunflower and MAGIC populations in common bean used to identify, validate and fine map QTL identified from exotic sources including interspecific populations.

1.3.15.01 Scientists at North Dakota State University, Michigan State University and USDA-

ARS Prosser WA evaluated inoculation techniques for screening pea germplasm for *Sclerotinia* resistance. F1 populations among elite breeding lines or varieties and accessions showing increased levels of resistance were grown in the field in the summer of 2013 in space planted plots. Significant F2 populations were generated and will be used to study the genetics of the resistance mechanisms. Development of recombinant inbred line populations from previous crosses is progressing.

- Use of genome-wide association mapping and linkage analysis to identify and map QTL with major and minor effects in common bean and sunflower.

1.3.13.01 USDA-ARS scientists at Fargo ND developed populations to evaluate Genotype x Sequencing (GS) methods in sunflower for *Sclerotinia*, yield, and agronomic traits. DNA samples were taken from 308 experimental maintainer and restorer lines with testcross evaluation data in 2009 (308), 2010 (304), 2011 (137) and 2012 (281). Testcrosses were made to 'RHA 377' if the experimental was a maintainer, and 'CMS HA 412HO' if the experimental was a restorer. Testcross performance data for each phenotype in each year were obtained from one or two environments. No genotypes other than check hybrids were evaluated in more than one year; however, the self-pollinated progenies of selected lines were evaluated in sequential years. Released breeding lines, progenitors of current breeding germplasm were whole genome sequenced (10x coverage). GBS data will be aligned to existing whole genome scaffolds and parsed into flat data files using internal lab protocols at University of Colorado.

- Breeder friendly QTL-linked DNA markers generated in canola, chickpea, common bean, lentil, pea, soybean, and sunflower and validated for application in marker-assisted breeding.

1.3.16.01 Scientists at USDA-ARS Prosser WA, Oregon State University and North Dakota State University identified and validated white mold (WM) resistance QTL from *P. coccineus* and transferred them into common bean. During 2012-2013, 26 F4 RI populations of crosses between the *P. coccineus* derived experimental lines (WMG762, WMG 836, WMG853, WMG861, WMG897, WMG903, WMG904, WMM 619, WMM688, WMG 308, WMG327, WMG377, and WMG388) and two susceptible common bean parents (Great Northern „Spinel“ and OSU 5613) were increased. RI populations with WMG904 crossed to G122 and NY6020 were advanced to the F6 generation. These 28 individual populations are intended for validating and fine mapping *P. coccineus* derived WM resistance QTL.

- Define metabolic mechanisms associated with *Sclerotinia* resistance QTL in common bean, soybean, and sunflower.

1.3.12.01 Scientists at Colorado State University and Delaware State University identified resistant (A195) and susceptible (Sacramento) Andean bean lines that exhibit metabolic phenotypes associated with response to white mold. Metabolites from healthy tissue directly adjacent to the necrotic lesion were characterized using UPLC-MS and GC-MS non-targeted metabolomic workflows. 144 metabolites varied between A195 and Sacramento. This experiment showed: (i) reduction in many amino acids and saccharides, but increase in asparagine, (ii) increased abundance of bean phytoalexins and other secondary metabolites, (iii) variation in hormones and other molecules involved in cell signaling, (iv) increased abundance of many organic acids, (v) increased abundance of ureides, and decrease of a ureide precursor, (vi) variation in cell wall and glycerolipid composition. Some of the molecular phenotypes have been previously observed as a plant response to necrotrophic fungi (e.g.

asparagine) however, many changes are novel and appear specific to legumes, such as gibberellin A37 glycoside, soyasaponin, and phaseolin.

PM 1.4: Pyramid white mold resistance in plant germplasm using traditional and genome sequence guided approaches, and release germplasm lines and cultivars with enhanced resistance.

- Canola, chickpea, lentil, and pea lines with resistance to *Sclerotinia* and a broad portfolio of desirable agronomic traits developed and released.

1.4.05.01 Scientists from North Dakota State University developed 15 *B. napus* lines with resistance to *S. sclerotiorum* from parental line NEP 63, and 60 F6 lines derived from the cross between Ames 26628 and PI458940 that are considered resistant to *S. sclerotiorum*. However, these materials and the advanced breeding lines were not planted in Langdon as planned. All materials were planted in Prosper late in the summer with no misting system. No disease developed. The elite breeding lines are currently being evaluated in greenhouse conditions using the petiole inoculation technique. Double haploid production from *B. rapa* accessions PI426281 and Ames 21738 is under way. At the same time, crosses between these lines have been made in the greenhouse and F2 lines will be advanced.

- Breeding lines and cultivars of pinto and other bean market classes released with broadly effective resistance pyramided from diverse sources - Andean, Middle American, and secondary gene pools (*P. coccineus*), in combination with desirable agronomic traits.

1.4.22.01 Scientists at the University of Nebraska, USDA, ARS-Prosser WA, Michigan State University, Colorado State University, University of Idaho, Cornell University, Seminis Seeds, Oregon State University, and North Dakota State University identified sources of partial resistance to *S. sclerotiorum* in secondary gene pool derived as well as in *Phaseolus vulgaris* adapted dry and snap bean lines. A standardized screening test using the modified Petzoldt and Dickson scale was developed for rating the greenhouse straw test, and the CIAT scale was used for rating all field screening tests. 2012-13 greenhouse tests provided evidence for 11 lines with large cream, pinto, great northern, small red and cranberry seed types with intermediate levels of WM resistance. 9 entries had WM resistance ranging from similar to Bunsu (avoidance) to resistance similar to G122, the moderately resistant check. A snap bean, a pinto line, a bayo line and six kidney lines with WM resistance were released. New lines with high WM resistance from wide interspecific crosses are now in seed increases for greenhouse screening

- Interaction of combined QTL on level of disease reaction in common bean and soybean elucidated.

1.4.23.02 Scientists at Michigan State University evaluated two greenhouse methods, the spray-mycelium method and the drop-mycelium method, for large scale evaluations of soybean germplasm for resistance to *Sclerotinia* stem rot. Two new QTLs were identified from PI 391589A and PI 391589B. Five resistance sources PI 089001, PI 153259, PI 437764, PI 548404, and PI 548312 were tested for QTLs for resistance to *Sclerotinia* stem rot. Nine reported QTLs were found in these resistance sources. 432 lines were evaluated with over 52,000 SNP DNA markers in an attempt to identify DNA markers closely linked to the disease resistance genes. The three new resistance sources, PI 416805, PI 361059B, and FC 030233,

have been used as sources of resistance. They were crossed with high yielding lines with other desirable traits such as resistance to soybean cyst nematode, phytophthora root rot, and sudden death syndrome. The progenies from these crosses are currently at various generations from F1 to F4.

- Establish disease nurseries for characterizing field and greenhouse resistance to all pathogenic forms of *Sclerotinia* in common bean, soybean and sunflower.
- At least one released soybean breeding line with *Sclerotinia* resistance from multiple sources of resistance as verified by QTL-linked markers, including high yield, and resistance to other diseases or insects.

1.4.23.01 Scientists at Michigan State University released the soybean cultivar Skylla and a germplasm AxN-1-55 with partial resistance to *Sclerotinia* stem rot. 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, E06161, E06164, E06240, and E08310, with yield similar to the yield check IA2094 and with resistance similar or better than the resistant check S19-90 were developed.

- Commercial & experimental release of sunflower lines exhibiting both *Sclerotinia* head rot and stalk rot resistance.

Pathogen Biology & Mechanisms of Resistance

Goal 2: Understand *Sclerotinia sclerotiorum* biology and development

PM 2.1: Characterize migration/population structure and ecological variability of genotypes.

- Understanding the interaction of pathogen with environmental factors such as temperature and light.
- Identification of biotypes with resistance to new fungicide chemistry
- Characterization of the genetics of fungicide resistance
- Characterization of ecological types in the population.
- Associate activity in *Sclerotinia* with specific genetic markers.

PM 2.2: Characterize virulence/aggressiveness within the population, identify isolates for

use in screening, and monitor durability of host resistance.

- Documented reactions of a broad spectrum of isolates on new sources of host resistance.

2.2.22.02 Scientists at the University of Nebraska, USDA, ARS-Prosser WA, Michigan State University, Colorado State University, University of Idaho, Cornell University, Seminis Seeds, Oregon State University, and North Dakota State University collected 366 isolates of *S. sclerotiorum* from nine bean production regions in the USA as well as regions in Mexico and France: all were genotyped with 16 polymorphic microsatellites and by UPGMA cluster analysis. These isolates exhibited no significant differences in aggressiveness using the straw test under greenhouse conditions. However, there were significant differences in aggressiveness between screening nurseries in MN, MI, ND, NE and CA. There was also significant variation in aggressiveness between isolates collected in Red River Valley compared to Trail County. When isolates collected from nurseries and grower fields within a state were compared, MI isolates did not differ in aggressiveness. However, ND isolates compared in the same way were significantly different, as were those from WA where the year of collection also was a significant factor in aggressiveness variation.

- Diverse collection of isolates with a broad spectrum of aggressiveness and other characteristics

2.2.22.03 Scientists at the University of Nebraska, USDA, ARS-Prosser WA, Michigan State University, Colorado State University, University of Idaho, Cornell University, Seminis Seeds, Oregon State University, and North Dakota State University constructed a dendrogram using 16 polymorphic microsatellites and UPGMA (unweighted pair-group method with arithmetic mean) cluster analysis that defined 20 gene clusters. The 20 clusters were similar for the three control hosts in the screening nurseries with isolates for Beryl, Bunsu and G122 respectively. State or country origin isolates exhibited variability in total clusters and distribution between the 20 clusters. Grower field isolates were also variable when compared by state origin, where isolates were distributed in only 10 clusters.

- Identification of new sources of host resistance using a new set of aggressive isolates

2.2.08.01 Scientists at USDA-ARS Urbana, IL, University of Illinois and North Dakota State University are testing the hypothesis that viruses infecting *Sclerotinia sclerotiorum* have the ability to reduce severity of white mold disease in crops. Viruses infecting *S. sclerotiorum* were identified in total RNA extracted from pure cultures of 138 *S. sclerotiorum* field isolates and analyzed by high-throughput sequencing. Twenty novel *S. sclerotiorum* viruses were identified, more than doubling the number of viruses known to infect *S. sclerotiorum*. One of the viruses had a double-stranded RNA (dsRNA) genome, three had negative-sense single-stranded RNA [ssRNA(-)] genomes and 16 had positive-sense ssRNA [ssRNA(+)] genomes. No viruses with DNA genomes were detected. Among the viruses with ssRNA(+) genomes, mitochondria-infecting viruses were the most numerous. Isolates of *S. sclerotiorum* were confirmed to be infected with the viruses by real-time reverse-transcriptase polymerase chain reaction using primers specific for each of the new viruses.

2.2.08.02 Scientists at USDA-ARS Urbana, IL, University of Illinois and North Dakota State University genome sequenced selected viruses to facilitate production of infectious cloned copies of the viruses. Four of the viruses selected for further analysis included a ssRNA(-) virus that is

related to the plant pathogen Maize mosaic virus and other arthropod-transmitted viruses that infect plants and animals. The *S. sclerotiorum* isolate infected with the ssRNA(-) virus grew more slowly and produced fewer sclerotia in culture than isolates not infected with the virus. A large (>14 kb) dsRNA virus infecting *S. sclerotiorum* related to *Cryphonectria hypovirus* 1 reduced the virulence of the chestnut blight fungus. The *S. sclerotiorum* isolate infected with the new hypovirus produced dense mycelial mats with extensive hyphae branching in culture. Finally, two viruses (<5 kb) ssRNA(+) genomes were identified as *Diaporthe ambigua* RNA virus 1 and a group of soil-borne plant viruses in the family Tombusviridae. The *S. sclerotiorum* isolates infected with the viruses did not show obvious phenotypes. However, the two viruses may be more suitable than non-encapsidated dsRNA viruses for development of persistent biological fungicides.

- Criteria for testing virulence/aggressiveness on specific hosts and tissue types.

PM 2.3: Identify environmental and genetic factors involved in myceliogenic and carpogenic germination of sclerotia.

- Identification of host factors that may enhance myceliogenic germination.

2.1.11.01 Scientists at USDA ARS Urbana IL and the University of Illinois demonstrated growth suppression of *S. sclerotiorum* by varied levels of the native soybean phytoalexin glyceollin and the non-native phytoalexins resveratrol and pterostilbene in vitro and in vivo. Transgenic soybean plants with genes enabling biosynthesis of non-native phytoalexins resveratrol or pterostilbene have been developed for evaluation of resistance to *S. sclerotiorum* in planta.

2.3.10.01 Scientists at USDA, ARS Fargo ND and North Dakota State University conducted exploratory experiments using *Sclerotinia sclerotiorum* strain 1980 to: 1) examine the effect of a synthetic strigolactone called GR24 on germination, and 2) examine the effect of growth and conditioning temperatures on germination. GR24 incorporated into water agar or PDA media did not increase the onset or rate of myceliogenic germination relative the control. A new strain sun-87 was obtained as an additional source of experimental material.

- Genetic control and required environmental conditions governing the processes of myceliogenic and carpogenic germination
- Determination of common and unique genetic events that lead to carpogenic germination in different *Sclerotinia* spp.

PM 2.4: Identify genes that are functional at specific growth and infection stages of Sclerotinia.

- Transcriptomic, genomic, and metabolomic data bases for growth stage-specific genes and infection-related genes from both host and pathogen.

2.4.03.01 Scientists from Michigan State University, North Dakota State University and Dow AgroSciences, LLC investigated the host-pathogen interaction of *Pisum sativum* and *Sclerotinia*

sclerotiorum and reported the first gene expression profiling data via RNA-seq on a susceptible (Lifter) and a partially resistant (PI240515) line inoculated with *S. sclerotiorum*. Comparative analysis of differentially expressed genes revealed five genes encoding: two putative precursors of peroxidases (Psat_118093 and Psat_116532), a chalcone synthase (Psat_107301), a ferulate 5-hydroxylase (Psat_117663) and a β -1,3-hydrolase (Psat_111657) that are linked to host defenses. These genes were up-regulated in the partial resistance PI240515 line and influence production of phytoalexins and cell wall lignin. 540 SSR markers were validated for selection of these traits.

- Improved gene annotation using transcriptomic data.

2.4.03.02 Scientists from Michigan State University, North Dakota State University and Dow AgroSciences, LLC characterized unique expressed genes during pea-*S. sclerotiorum* interaction. The results showed that PI240515 pea line had a higher number of specific unique genes than Lifter that were associated with cell wall, death, immune system and regulation of transcription functions. PI240515 seemed to favor programmed cell death (PCD) related events, as a means to impede the spread of the disease.

- Genetic control of differential infection processes of the *Sclerotinia* spp. in response to different host plants

2.4.17.01 Scientists at North Dakota State University resequenced genomic (g) DNA from the mycelium of 120 isolates from a collection of *S. sclerotiorum* from 22 hosts and 25 states. Two genotype-by-sequencing (GBS) libraries were constructed containing 50,000 -100,000 barcoded and sequestered sequence reads for each *S. sclerotiorum* isolate. Double digested gDNA was size selected (200 bp fragments) and ligated to barcoded Ion Torrent sequencing adaptors specific to each isolate. The barcoded and pooled libraries (40 multiplexed isolates per library) were sequenced on three separate Ion Torrent 318 microprocessor chips. The sequencing reactions yielded 15.6 million sequences at an average of 160 bases per read for a total of ~2.5 billion bases. The sequence alignment identified 16,320 unique sequence tags/loci with ~30,000 SNPs present on ~60% of the sequence tags. The 16,320 GBS tags were randomly spread throughout the 38 Mb genome. This preliminary analysis predicts 1 SNP marker every 3.9 Kb throughout the *S. sclerotiorum* genome. Preliminary BLAST analysis suggested these markers hit over 50% of the predicted genes within the genome.

PM 2.5: Identification and verification of candidate genes involved in *Sclerotinia* pathogenicity.

- Development and maintenance of relevant natural and derived culture collections for use in phenotypic association.
- Transcriptome profiling approaches for a variety of gene targets and high through put functional analyses.

2.5.01.01 USDA scientists at Pullman WA conducted inoculation experiments under environmentally controlled growth conditions. Three-week old lentil and chickpea plants were inoculated with actively growing mycelium of *S. sclerotiorum*. The tissue at the interface of disease lesions and healthy tissue were harvested at three different times after inoculation (24, 48

and 72 hours post inoculation). Total RNAs were isolated using the TRIzol method from freshly harvested plant tissues. The mRNAs were isolated using a GenElute™ mRNA Miniprep Kit (Sigma) from the total RNAs, and sequenced with the 454 GS FLX Titanium pyrosequencing.

- Promoters useful for expressing RNAi constructs during infection (e.g., plant-inducible promoters).
- Inventory of genes potentially involved in pathogenesis recovered from ATMT random mutagenesis and transcriptome profiling.
- Functional verification of candidate genes using a systems biology approach to gene silencing and quantitative expression assays.

Gene Discovery & Phenotypic Association

Goal 3: Develop molecular technologies that facilitate breeding progress

PM 3.1 Develop useful molecular marker resources for QTL Discovery.

- Identification of a core set of informative markers for deployment in genotyping systems suitable for use in breeding programs

3.2.18.01 USDA ARS scientists at Fargo ND transferred Sclerotinia stalk rot resistance from four wild annual Helianthus species (*H. argophyllus*, *H. debilis*, *H. praecox*, and *H. petiolaris*) into cultivated sunflower. Resistant plants were selected from 21 accessions of four wild species, and were first crossed in 2009 to a nuclear male-sterile line (NMS) HA 89, susceptible to Sclerotinia stalk rot. Selected F1 resistant plants were backcrossed to HA 458 (a susceptible elite cultivar), and the resistant BC1s were again backcrossed to HA 89 to produce BC2s followed by self-pollination to advance to BC2F2. Sclerotinia stalk rot tests were applied in each generation in the greenhouse trials to narrow down population size. 4,288 BC2F2 plants derived from eight original resistant accessions of the four wild species and advanced 302 BC2F3 families from the selected resistant individuals were screened in 2011. The 302 BC2F3 families were screened for stalk rot resistance in field and/or greenhouse trials in 2012 and 2013. The resistant individuals from those families with resistance similar or superior to the resistant checks Croplan 305 and HA 441 were selected to advance BC2F4 lines. One BC2F4 line with good seed set and other traits from each of the best BC2F3 families were further evaluated for stalk rot resistance. Overall, 28 resistant BC2F4 lines were obtained by combining field and greenhouse data, out of which, 12 derived from *H. petiolaris* PI 435843, *H. argophyllus* PI 494573, and *H. praecox* PI 468853 were tested in field trials at two locations both in 2012 and 2013, and were identified with higher levels of resistance. Whole genome scans of the resistant line 11-275-037 from *H. argophyllus* PI 494573 with 256 polymorphic SSR markers revealed the presence of introgressed chromosome segments located on linkage groups 8, 9, 10, and 11, indicating some of these chromosomes probably associate with stalk rot resistance. Novel QTL for stalk rot resistance derived from *H. argophyllus* were characterized with an advanced backcross (AB) population. 140 plants from 11 BC2F1 rows were advanced to the BC2F5 generation by single-seed descent.

- DNA markers that contribute to the annotation of the crop genomes

3.1.06a.01 Scientists from North Dakota State University and DuPont Inc. evaluated the reaction of two doubled haploid lines, NEP32 and NEP63, to inoculations with *S. sclerotiorum*. The lines which are considered susceptible and resistant to *S. sclerotiorum*, respectively, were derived from Ames 26628, a *B. napus* plant introduction. A 180 line F2 population from the DH lines was inoculated with *S. sclerotiorum* and DNA was extracted in greenhouse conditions. Within two weeks from inoculation 50% the lines were dead; the remaining F2 lines developed lesions of different sizes. Marker analyses using GAPIT and TASSEL revealed two SNP markers significantly associated with resistance to *S. sclerotiorum*. Of these, marker S4_14654875 was located in chromosome 4 (*B. rapa* genome) while marker S20_1260815 was ascribed to a scaffold that could not be anchored to any chromosome at this time. 44 mutants were generated in these genes (deleted or silenced). Pathogenicity trials are being conducted.

- Allele specific DNA markers that can be used in pre-breeding for disease resistance

- Allele specific DNA markers that can be used in pre-breeding for quality traits
- Allele specific DNA markers for yielding ability and other agronomic traits

PM 3.2 Genetic and physical maps for Sclerotinia resistance.

- Compilation of marker genotyping data for different mapping populations
- Improved genetic maps for Sclerotinia resistance genes

3.2.16.01 Scientists at USDA-ARS Prosser WA, Oregon State University and North Dakota State University use SNPs from RNA-seq in Introgression mapping of WM7.1 QTL. The number of SNPs within each 500kb window defined the introgressions from the donor parent. In this case, it is the WM7.1. Significant introgression also was observed from Pv08 and Pv11. The majority of the introgressions are located on Pv07 in the range of 1.0 to 8.3 Mb. All totaled, 314 genes resided in the three introgressed regions, and among these, 119 are located in the Pv07 WM7.1 region. A refined WM7.1 QTL map, with eight polymorphic indel markers, narrowed the gene search within the WM7.1 region to 4.5 cM.

- A consensus genetic/QTL map for Sclerotinia resistance genes
- Core sets of markers for discovery of candidate genes for disease resistance
- Core sets of markers for discovery of candidate genes for quality traits
- Core sets of markers for discovery of candidate genes for agronomic traits
- Placement of candidate genes on the consensus genetic map

3.3 Characterize gene models associated with pathology and resistance.

- A standardized methodology for annotation of maps among specified crop species.
- Transcriptomic, proteomic and metabolomic annotation of genome sequences in QTL associate with resistance to Sclerotinia diseases

3.3.16.01 Scientists at USDA-ARS Prosser WA, Oregon State University and North Dakota State University used RNA-seq to identify candidate genes at the WM7.1 QTL locus in resistant and susceptible lines. Differentially expressed RNA revealed 179 up-regulated genes in the susceptible

line and 9 up-regulated genes in the resistant line.

- Characterization of candidate genes involved in biological mechanisms for resistance, such as: oxalic acid

3.3.04.01 Scientists from USDA-ARS Urbana IL and Agriculture and Agri-Food Canada generated gene expression data from soybean infected with Sclerotinia or OA. This expression data identified Sclerotinia responsive genes closely associated with defense. Differential expression suggests that PR5 is a good candidate defense gene that is located close to a QTL for Sclerotinia resistance.

3.3.16.02 Scientists at USDA-ARS Prosser WA, Oregon State University and North Dakota State University Identified 168 genes within WM7.1 region of Pv07. Among these genes is Phvul.007G067300 which is overexpressed in the resistance response. This gene is a U-Box ubiquitin ligase protein, and its homology in Arabidopsis is associated with the disease response to Pseudomonas infection. These proteins target invading proteins for destruction, a mechanism that halts the effects of invading organisms. Another U-box ubiquitin ligase (Phvul.008g099500) is located within the WM8.3 QTL, and also was up-regulated in white mold resistance response. Ubiquitin ligases could play an important role in the white mold resistance response.

- A commodity-based gene atlas with a comprehensive list of all expressed genes, alternative splice products, identification of co-regulated genes and gene networks.
- Identification of specific genes within QTL of importance to Sclerotinia-host interactions

3.4 Genome mapping and allelic analysis through Genome-Wide Association Studies.

- High resolution exome maps of genomic regions that harbor QTL for Sclerotinia resistance
- Identification of specific alleles in gene families that mediate Sclerotinia resistance.
- GWAS studies of the trait associated with phenotypic variation in disease resistance.

3.4.07.01 USDA ARS scientists at Urbana IL evaluated highly resistant accessions of *G. canescens*, *G. clandestina*, *G. latifolia*, and *G. tabacina*. Reciprocal crosses were performed between resistant and susceptible accessions of *G. latifolia* to produce F2 plants and recombinant inbred lines (RILs). Single nucleotide polymorphisms (SNPs) were identified by sequencing reduced representations of genomic DNAs of resistant and susceptible accessions of *G. latifolia*. Many of the SNP markers that aligned to soybean chromosomes mapped in similar orders in the two species. 186 F2 individuals were evaluated for segregation of sensitivity to oxalic acid and over 2,500 genotyping-by-sequencing (GBS) markers identified a locus for oxalic acid sensitivity on *G. latifolia* linkage group 17. RIL population development was continued, and a draft genome sequence was assembled for *G. latifolia*. Additional loci for oxalic acid sensitivity were found on *G. latifolia* linkage groups 2, 10, and 17, which corresponded to soybean chromosomes 19, 10, and 17, respectively. Comparison of SNP data and the draft genome sequence *G. latifolia* showed that the 3.4-cM genetic interval containing the locus on chromosome 2 represented a region of 2.7 Mbp that contained at

least 68 predicted genes. An F5 RIL population was analyzed for segregation of over 5,000 GBS markers and evaluations of the population for sensitivity to oxalic acid and inoculation with *S. sclerotiorum* were initiated. These experiments identified an accession of *Glycine latifolia* with high levels of resistance to Sclerotinia stem rot and produced the genetic and molecular tools for identification and characterization of genomic regions responsible for resistance to infection by *S. sclerotiorum* in a perennial wild relative of soybean.

3.4.16.01 Scientists at USDA-ARS Prosser WA, Oregon State University and North Dakota State University conducted genome-wide association mapping (GWAS) for identification of white mold tolerance QTL in the Dry bean WM8.3 region. 13 indel markers that co-segregated in our mapping population were physically mapped onto the version 1.0 of the common bean genome, over a distance of 41 Mb. The reason for this large physical distance is that the most of these markers are located in the low recombination heterochromatic region of the chromosome. This QTL may have ~1000 genes.

3.4.16.02 Scientists at USDA-ARS Prosser WA, Oregon State University and North Dakota State University conducted a GWAS analysis of the Mesoamerican Diversity Panel (MDP; n=252, consisting of pinto, navy, black, great northern, pink, and small red market cultivars) under field conditions in 2013 to discover the genetic factors underlying disease resistance. Major association peaks were discovered on chromosomes Pv01, Pv02, Pv03, Pv04, Pv06, and Pv08. The GWAS peaks correspond with a number of previously defined QTL: WM1.1, WM2.1, WM5.1 or WM5.2, and WM8.1 or WM8.3. A multi-locus mixed model (MLMM) analysis was applied to gain a good estimate of the number and location of factors affecting the field reaction to white mold.

- Haplotype maps correlated with genetic variation for resistance to Sclerotinia diseases.

3.4.16.03 Scientists at USDA-ARS Prosser WA, Oregon State University and North Dakota State University conducted GWAS on 131 snap bean lines. A wide range in variation for resistance was observed with some lines showing high levels of field resistance. The 131 genotypes were genotyped with the 6998 SNP array, and a GWAS analysis was performed for white mold severity, white mold incidence, and white mold geometric mean. Association peaks were detected on Pv02, Pv09, and Pv10 for all three traits. Coordinates of the Pv02 peak at 3.2 Mb places it near the WM2.1 QTL. The other Pv02 peak located at ~34.8 Mb is physically near the WM2.3 QTL.

- Allele specific markers and high-throughput screening methods for pyramiding genes that mediate resistance to Sclerotinia diseases.

PM 3.5 Develop plant germplasm with improved resistance using biotechnology and other novel genetic methods.

- An inventory of validated disease resistance genes, promoters, and constructs for transformation into crop germplasm.
- Discovery of transcription factors and elements of gene regulation that mediate expression of disease resistance genes.
- Functional tests in model plants to determine potential importance of candidate defense genes

3.5.20.01 Scientists at the University of Florida identified an *Arabidopsis thaliana* gene that is hyper-susceptible to *S. sclerotiorum* (HSS1) and the pathogen encoded oxalate decarboxylase1 (ODC1) protein. The HSS1 gene was cloned. 130 F2 plants with a homozygous *hss1* were used in linkage analysis. The HSS1 gene was positioned between the molecular markers *Ciw5* and *Ciw6* on Chromosome 4. Further recombination analysis of 1479 F2 mutant plants placed the HSS1 gene between markers *m602* and *m268*. Candidate genes were sequenced and several mutations were found. The *hss1* mutation is being confirmed by cloning the candidate wild-type genes in this region in plant expression vectors. Genetic complementation constructs were developed to characterize candidates for the HSS1 gene.

3.5.20.02 Scientists at the University of Florida performed a microarray experiment to monitor *S. sclerotiorum*-induced transcriptome changes in *hss1* and wild-type plants. Microarray data indicated that the *hss1* mutation significantly shifted *S. sclerotiorum*-induced transcriptome changes in the host. Compared with the wild type, 102, 1107, 642, and 1322 genes were up regulated in *hss1*, respectively, and 391, 994, 279, and 1239 genes were down-regulated. Analysis of the gene annotations revealed that induction of a group of ethylene (ET) pathway genes (*ORA59*, *PDF1.2*, *CHIB*, *PR4/HEL*) was significantly inhibited in the *hss1* mutant, suggesting that the HSS1 gene may play an important role in ET signaling. Moreover, the glucosinolate biosynthetic genes *CYP79B2* and *CYP79B3* were also down-regulated in *hss1*, indicating that HSS1 may regulate glucosinolate biosynthesis as well.

3.5.04.01 Scientists from USDA-ARS Urbana IL and Agriculture and Agri-Food Canada used soybean transformation to validate candidate defense-associated genes by means of silencing. Three RNAi genes were delivered to soybean in multiple experiments. Data show that G-protein coupled receptor (GPCR), is enhanced susceptibility when GPCR is silenced, indicating that this gene is playing a role in defense when active. Similarly, 14-3-3 RNAi transgenic plants show enhanced susceptibility. T2 seed carrying RNAi of the matrix metalloproteinase (MMP) now enables infection assays to evaluate this gene for its role in defense. These genes or paralogs are initial pieces of the metabolic network for resistance.

- Determination of the efficacy of transformed genes on defense control in crop germplasm.

3.5.20.03 Scientists at the University of Florida cloned the *S. sclerotiorum* ODC1 gene into the T-DNA vector pCAMBIA1300S and transformed *Arabidopsis* plants with *Agrobacterium* carrying the T-DNA vector.

3.5.09.01 Scientists at The Ohio State University increased regeneration efficiency of transformed RHA280 plantlets up to 70% of cotyledon explants producing more than 40 shoots each. An alternative shoot induction protocol using primary leaves from RHA280 seedlings was developed following seed germination on cytokinin-containing medium. Primary leaves became responsive to the shoot induction medium used for shoot induction in cotyledons. Adventitious shoots from leaves were successfully elongated and plantlets were recovered following micro grafting. Transgenic shoots obtained from leaves show less long-term potential for improvement. Use of cotyledon tissue, cultured in a liquid cytokinin-containing medium was more responsive to Sonication Assisted *Agrobacterium*-mediated Transformation (SAAT) than cotyledons that were not pre-cultured with cytokinin. Multiple transgenic shoots and shoot clusters with GFP expression were obtained using SAAT. The percentage of explants with transgenic shoots ranged from 3% to 27%. Results suggest that early selection after transformation yields more consistent transgenic shoots than our

previously-used selection scheme.

- Effective use of genome editing technologies to genetically modify genomic regions in ways that enhance resistance to Sclerotinia diseases
- Development and testing of agronomic crop germplasm transformed with putative anti-fungal genes or RNA interfering constructs for reaction to white mold.

Disease Management & Crop Production

Goal 4: Broaden knowledge of *Sclerotinia sclerotiorum* epidemiology and improve disease management strategies

PM 4.1: Optimize fungicide application programs.

- A region-wide collection of *S. sclerotiorum* isolates to establish a baseline of fungicide sensitivity

4.1.01.01 USDA Scientists at Pullman WA investigated fungal genetic resistance mechanisms to various fungicides in *S. sclerotiorum* isolates that had not been exposed to fungicides. Mutations in genes that encode: 1) β -tubulin renders pathogen resistance to benzimidazole fungicides; 2) mitochondrial cytochrome b confers resistance to strobilurin (QoI) fungicides; 3) 14 α -demethylase (CYP51) confers resistance to DMI fungicides; 4) histidine kinase or cAMP-dependent protein kinase (*ubc1*) confers resistance to dicarboximide fungicides. High levels of resistance in *S. sclerotiorum* to several classes of fungicides were reported in China. Significant difference in sensitivity among US isolates was found to three fungicides (Iprodione, Benzoyl and Fluanzinam).

- Identification of the economic return of fungicide applications relative to timing of disease onset

4.1.24.03 Scientists at North Dakota State University and the University of Nebraska achieved intermediate levels of disease that normally would be ideal for differentiating fungicide efficacy, fungicides did not show efficacy against *Sclerotinia* head rot. Most of the fungicides that were evaluated have efficacy against *Sclerotinia* on other crops, and the poor results observed in the sunflower head rot trials are likely due to the difficulty of achieving satisfactory fungicide coverage on the front of the heads with the available application technology.

- Updated management guides for growers on use of fungicides for disease management

4.1.24.01 Scientists at North Dakota State University and the University of Nebraska facilitated management of *Sclerotinia* head rot of sunflowers through screening hybrids for resistance and evaluating fungicides for efficacy. Screening nurseries were highly successful at differentiating the relative susceptibility of commercial sunflower hybrids and breeding lines to *Sclerotinia* head rot. At Carrington, ND, eight of 30 entries, including one confectionary hybrid, were significantly more resistant to head rot than the susceptible checks. Results were significantly correlated across the Carrington, Landon, and Oakes screening locations.

- New spraying technologies that improve fungicide performance by enhancing canopy penetration, plant coverage, and fungicide deposition
- Determine most effective timing of fungicide applications relative to canopy closure after blooming.

4.1.24.02 Scientists at North Dakota State University and the University of Nebraska evaluated the susceptibility of sunflowers to *Sclerotinia* head rot during and after bloom. There was a sharp drop in

susceptibility to head rot between the R5 (bloom) and R6 (flowering complete, ray flowers wilted) growth stages in both susceptible and partially resistant hybrids. Only inoculations at R5 resulted in a significant increase in *Sclerotinia* head rot relative to the non-inoculated control. Other inoculation timing studies showed susceptibility to head rot increased as bloom progressed, with sunflowers significantly more susceptible to head rot in the last third of bloom (R5.7 to R5.9; 70 to 90% of the disk flowers in bloom or having completed bloom) than in the first third of bloom (R5.1 to R5.3). These results suggest that inoculations be conducted at the same stage of bloom and that no inoculations be conducted after bloom.

PM 4.2: Develop bio-control alternatives for disease management.

- Identification of application strategies that will maximize the efficacy of currently available bio control agents for control of *S. sclerotiorum*
- Identification of novel antagonists of *S. sclerotiorum* and assessment of their efficacy in field trials
- Updated management guides for growers on use of bio fungicides for disease management

PM 4.3: Develop disease-warning systems to optimize management of *S. sclerotiorum*.

- Models that calculate risk of disease development as functions of leaf wetness duration and temperature, and risk of apothecia formation as function of soil moisture conditions
- Effect of tillage practices on *Sclerotinia* survival;
- Economic loss models based on plant density at time of disease onset
- Define risk levels to guide crop-specific fungicide selection decisions

PM 4.4: Optimize cultural practices for disease management.

- Variety selection using disease reaction measured as the amount of sclerotia produced
- Collate disease management information and distribute to growers through print media, internet postings and extension publications
- Epidemiological information on disease development (spatial distribution, remote sensing, etc.) that could be used to support precision agriculture programs for disease control.

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2013 Sclerotinia Research Projects			
Project	PI	Cooperator	Commodity
Discovery and use of novel sources of resistance to head rot and stalk rot in cultivated sunflower & wild <i>Helianthus</i>	Gulya	ARS	Sunflower
White mold resistance-QTL: Identification, interactions & fine mapping in common bean	Miklas	ARS	Dry Bean
Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas	Steadman	NE	Dry Bean
Transferring <i>Sclerotinia</i> resistance genes from wild <i>Helianthus</i> species into cultivated sunflower	Jan	ARS	Sunflower
Deployment of novel sources of <i>Sclerotinia</i> resistance and tools for breeding resistance in sunflower	Qi	ARS	Sunflower
Pyramiding QTL for white mold resistance into Mesoamerican beans	Kelly	MI	Dry Bean
Enhancing soybean for resistance to <i>Sclerotinia</i> stem rot	Wang	MI	Soybean
Synergistic enhancement of resistance to <i>Sclerotinia sclerotiorum</i>	Rollins	FL	All
High density genotyping of a diverse population of <i>Sclerotinia sclerotiorum</i>	Nelson	ND	All
Identifying and verifying genes for defense to <i>Sclerotinia</i>	Clough	ARS	Soybean
Identification of resistance and pathogenicity genes associated with <i>Sclerotinia sclerotiorum</i> infection using next-generation sequencing	del Rio	ND	Canola
Fine mapping of loci for resistance to <i>Sclerotinia</i> stem rot in the wild perennial <i>Glycine latifolia</i>	Domier	ARS	Soybean
Characterization and validation of two distinct mechanisms for partial resistance to <i>Sclerotinia sclerotiorum</i> in pea	McPhee	ND	Pea & Lentil
Use of a transformation system in sunflower for <i>Sclerotinia</i> resistance studies	Finer	OH	Sunflower
Expression profiling of the pea- <i>Sclerotinia sclerotiorum</i> interaction for genomics assisted breeding	Chilvers	MI	Pea & Lentil
Development and evaluation of canola breeding populations for resistance to <i>Sclerotinia sclerotiorum</i>	del Rio	ND	Canola
Characterization of growth, pathogenicity, and apothecial development of <i>Sclerotinia sclerotiorum</i> isolates from different geographic regions in contrasting temperature regimes	Hartman	ARS	Pathogen
Facilitating management of <i>Sclerotinia</i> head rot of sunflowers through screening hybrids for resistance and evaluating fungicides for efficacy	Wunsch	ND	Sunflower
Comparative transcriptomics of <i>Sclerotinia sclerotiorum</i> infecting grain legumes for genomics assisted breeding	Chen	ARS	Pea & Lentil
Preventing development of fungicide resistance in <i>Sclerotinia sclerotiorum</i> by investigating resistance mechanisms	Chen	ARS	Pea & Lentil
Managing <i>Sclerotinia</i> stem rot of canola with fungicides	del Rio	ND	Canola