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Meeting Strategic Milestones of the National Sclerotinia Research Initiative for 2012

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 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 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 2012* 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	80
Milestones w/ multiple citations	17	10	14	18	21
Milestones w/ no citations	30	30	24	29	34
Milestones not funded	NA	14	2	3	2
Milestones completed	0	1	1	1	1
Total Milestones Rated	76	76	73	70	70
Achievement Rating (%)	56.7	62.2	67.2	73.4	80.9
Total Projects	34	28	19	15	21
Accomplishments / project	2.2	2.2	3.9	4.7	3.8
All Publications by SYs	47	53	214	235	303
Total SYs funded by NSI	20	33	35	29	27
Germplasm/Varieties released	10	10	15	9	13

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	0
• *Improved germplasm screening methods*	3	3	3	4	4
• *Increased availability of resistant germplasm*	1	3	1	5	3
• *Doubled haploid lines for resistance, tested in multiple environments*	1	1	1	1	1
PM 1.2: Transfer new resistance genes into plant germplasm					
• *Germplasm derived from interspecific crosses*	7	2	2	2	2
• *Resistant selections of un-adapted x agronomic traits*	0	1	1	2	4
PM 1.3: Genetic analysis & QTL discovery					
• *Highly inbred mapping populations with validated QTLs*	2	4	2	3	3
• *QTL analysis to generate a high density genetic maps*	3	2	2	2	4
• *Integrated linkage maps*	4	1	1	1	1
PM 1.4: Pyramid white mold resistance genes					
• *Improved conventional breeding methods for quantitative traits*	1	2	1	1	3
• *Breeding populations segregating for multiple resistance alleles and other traits*	3	1	1	1	2
PM 1.5: Marker-assisted selection					
• *Disease reaction of RILs in multiple field environments*	1	1	1	1	1
• *Marker-assisted selection protocol for more efficient genotyping*	4	1	1	3	1
• *Field verification of resistance*	3	1	1	2	3
PM 1.6: GM improved resistance					
• *GM oxalate oxidase expression, inheritance and field evaluation*	1	1	1	0	1
• *Catalog of candidate resistance genes, promoters, and constructs for transformation*	2	1	2	0	1
• *Perlka-resistant soybean lines (COMPLETED IN 2008)*	1				
• *Transgenic expression of antifungal peptides*	0	0	2		2
PM 1.7: Plant germplasm/cultivars with improved resistance					
• *Enhanced adapted germplasm*	2	1	1	4	3
• *Herbicide tolerance with resistance to Sclerotinia*	1	1	1	1	1
• *Agronomic resistant varieties for commercial production.*	1	2	3	2	5
• *Identify crop germplasm with partial resistance to virulent isolates*	1	1	12	3	2
Pathogen Biology & Mechanisms of Disease Resistance					
PM 2.1: Population structure & dynamics					
• *Standardized genotypic characterization on wild and cultivated crops*	1	1	1	1	0
• *Defined environmental requirements for pathogen biotype germination & disease*	1	0	0	0	0
• *Documented gene-flow or outcrossing contribution to population variability*	1	0	1	0	0
• *Geographical inventory of US populations*	1	1	1	1	1
PM 2.2: Durable host resistance					
• *Isolate virulence/aggressiveness across geographic areas and hosts*	2	0	1	1	1
• *Pathogen population dynamics on partially resistant crops*	0	1	1	0	0
• *Pathogen x environmental interaction*	0	1	0	0	1
• *Knowledge of plant x pathogen x environmental interactions*	1	1			0
• *Criteria for testing virulence/aggressiveness on specific hosts.*	1	1	1	0	0
PM 2.3: Factors that mediate sclerotia germination					
• *Host factors that mediate myceliogenic germination*	2	1	1	0	0
• *Defined environmental requirements for pathogen biotype germination & disease*	0	0	0	0	0
• *Effect of sclerotia-sphere microbes on germination and dormancy*	0	0	0	0	0
• *Effect of sclerotia-sphere microbes on mycelial growth*	0	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	0
• *Standard molecular protocols to genotype isolates*	2	1	1	1	1
• *Transformed isolates for host/pathogen & pathogen/microbe interactions*	1	2	1	1	0
PM 2.5: EST libraries from pathogen stains					
• *Useful cDNA libraries from pathogen expressed genes*	1	1	0	1	1
• *Useful genome sequence information*	1	0	0	1	0
• *Full length, normalized cDNA libraries*	1	0	0	0	0

	2008	2009	2010	2011	2012
Pathogen Biology & Mechanisms of Disease Resistance					
PM 2.6: Candidate genes for pathogenicity	number of accomplishment citations				
• *Large ATMT collections for phenotypic screens*	0	0	0	0	0
• *Transcriptome profiles and high through put functional analyses*	0	0	0	0	1
• *Promoters for RNAi constructs during infection*	0	0	0	0	0
• *Catalog genes from ATMT random mutagenesis*	0	0	0	0	0
• *Discovery of candidate gene function*	0	0	0	0	1
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	2
• *High density genetic map of DNA markers for resistance*	2	1	1	2	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	2
• *DNA markers from BAC-ends to anchor contigs to genetic maps*	1	1	2	2	3
• *Physical map of genomic regions containing resistance genes*	0	0	2	1	1
• *High through-put resequencing capacity and haplotype maps*	0	0	1	1	2
PM 3.3: Function of candidate resistance genes					
• *Microarrays for high throughput gene screening*	2	1	1	1	2
• *Sequenced cDNA libraries from infected host tissue*	0	0	1	1	3
• *Discovery of candidate genes for Sclerotinia resistance*	1	1	1	2	3
• *Function of candidate genes using gene silencing methods*	2	1			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	0
• *Conventional analysis of genetic mechanisms*	1	1	1	1	0
PM 3.5: Bioinformatic resources					
• *Web-based communication for the Sclerotinia Initiative*	1	1	1	1	0
• *Interactive website for genetic, genomic & biotech resources*	1	1	1	1	0
Disease Management & Pathogen Epidemiology					
PM 4.1: Optimized fungicide application programs					
• *S. sclerotiorum isolate collection to assess fungicide sensitivity*	0	1	2	0	1
• *Efficacy of new chemistries*	2	1	1	2	1
• *Updated management guides for disease management*	0	1	0	1	1
• *Improved spraying technologies*	0	0	0	0	0
PM 4.2: Bio-control alternatives for disease management					
• *Grower recommendations for commercial sclerotial antagonists*	0	1	0	0	0
• *Catalog of commercial microbial biocontrol agents*	0	0	0	0	0
• *Efficacy of Sporidesmium sclerotivorum as a biocontrol agent*	0	0	1	0	0
• *Updated management guides for biofungicides in disease management*	0	0	0	0	0
PM 4.3: Quantitative models for environmental and host-crop interactions					
• *Disease warning systems*	1	0	0	0	0
• *Validated predictive models in other crops.*	0	0	0	0	0
• *Yield loss models*	0	0	1	0	0
• *Threshold levels for decision aids*	0	0	0	0	1
PM 4.4: Optimized cultural practices for disease management					
• *Improved variety selection criteria*	0	0	1	2	1
• *Management decision aids*	1	1	1	1	0
• *Precision agriculture program*	0	0			

Total Accomlishments	**76**	**61**	**75**	**71**	**80**
Total Milestones with multiple accomplishments	**17**	**10**	**14**	**18**	**21**
Total Milestones with no accomplishment	**30**	**30**	**24**	**29**	**34**
Total Milestones not funded	**NA**	**14**	**2**	**3**	**3**
Total Milestones	**76**	**76**	**73**	**70**	**70**
Achievement Rating (%)	**56.7**	**61.5**	**67.2**	**73.4**	**80.9**
Total Projects	**34**	**28**	**19**	**15**	**21**
Accomplishments per project	**2.2**	**2.2**	**3.9**	**4.7**	**3.8**
Publications	**47**	**53**	**214**	**235**	**303**
Germplasm lines	**4**	**5**	**12**	**5**	**12**
Varieties	**6**	**5**	**3**	**4**	**1**

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,** and **soybean** in greenhouse and field settings.
- New sources of resistance in wild species of **pea, canola, common bean,** and **sunflower**.
- Transfer of resistance from wild species to cultivated types of **dry bean,** and **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**.
- Development of physical maps of **dry bean** and **canola** genomes.
- Development of transcriptome maps in **soybean, dry bean,** and **canola**.
- Discovery of candidate gene function in **soybean**.
- Marker Assisted Selection for resistance in **pinto bean, sunflower, common bean,** and **soybean**.
- A high density maps for Sclerotinia resistance in **soybean, common bean,** and **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,** and **soybean**.
- Germplasm releases with improved tolerance to white mold in **pinto bean (2), pea (3), sunflower (11), common bean (5), soybean (3), chickpea (1),** and **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),** and **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,** and **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-2012

Crop Germplasm Resources & Genetics

PM 1.1: New sources of resistant plant germplasm

- *Exploration trips to obtain seeds of wild species*

- *Improved germplasm screening methods*

1.1206 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND and the Discovery DuPont Crop Protection Stine Haskell Research Center, Newark DE sequenced the *S. sclerotiorum* isolate, NE 152 (also known as 1980) obtained from Dr. Jim Steadman in University of Nebraska. Experiments were conducted with double haploid canola lines differing in their susceptibility to white mold. The resistant (NEP 63) and susceptible (NEP326) 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* was 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. Inoculated petioles were harvested at 24 and 48 hours post inoculation and immediately flash frozen in liquid nitrogen to prevent degradation of genetic material and were stored at -80°C.

1.1210 Scientists at the USDA-ARS Sunflower & Plant Biology Research Unit, Fargo ND; the Ag Research Center, Central Lakes College, Staples MN, the Panhandle Res. & Exten. Center, University of Nebraska, Scottsbluff NE; the NDSU Res. & Exten. Center, Carrington ND; and Plant Pathology Department, University of Nebraska-Lincoln collaborated on improving the ascospore production method developed by retired pathologist Dr. Michael Boosalis. The method was tested at four laboratories (Fargo and 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. Thus, this method may be applicable only to some fungal isolates. Preliminary work is in progress to see whether sunflower meal, substituted for corn meal, will enhance ascospore production, and if vermiculite can be replaced in the media to minimize the health hazard associated with it. An initial publication documenting the Boosalis protocol is being drafted.

1.1220 Scientists in the Department of Plant, Soil and Microbial Sciences, Michigan State University. East Lansing MI evaluated two new greenhouse methods, the spray-mycelium method and the drop-mycelium method. These methods were developed for large scale evaluations of breeding materials for resistance to Sclerotinia stem rot. The data obtained with these two methods have a significant correlation with data obtained in field inoculation trials.

1.1221 Scientists at the NDSU - Carrington Research Extension Center, Carrington ND; NDSU - Langdon Research Extension Center, Langdon ND; NDSU - Carrington REC Oakes Irrigation Research Site, Oakes ND; and University of Nebraska Panhandle Research and Extension Center, Scottsbluff NE used disease screening nurseries established in 2012 to successfully differentiate the relative susceptibility of commercial sunflower hybrids and breeding lines to Sclerotinia head rot. In the large screening nursery conducted in Carrington ND, 13 of 28 entries submitted by breeders showed significantly more resistance to Sclerotinia head rot than the susceptible checks. In the multi-location screening nurseries conducted in Carrington, Oakes, and Langdon ND, 5 entries (of 22 entries submitting for testing) consistently exhibited significantly lower levels of Sclerotinia head rot than the most susceptible lines .

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

. 1.1405 Scientists in the Department of Plant Pathology, Department of Plant Sciences, Langdon Research Extension Center, at North Dakota State University successfully produced five sets of F2 seeds from the cross

between NEP63 and NEP32, another doubled haploid line considered susceptible to Sclerotinia stem rot. Collaborators have already planted the seeds of one set containing 180 seeds and have extracted DNA samples from the plants produced. These plants are approaching the flowering stage in greenhouse and will be inoculated in the first week of December to record phenotypic data. DNA of these plants will be characterized using molecular markers to identify QTL associated with resistance.

- *Increased availability of resistant germplasm*

1.1308 USDA-ARS scientists in the Department of Crop Sciences, University of Illinois evaluated 233 accessions from 18 wild perennial Glycine species for sensitivity to Sclerotinia stem rot in replicated trials. Highly resistant accessions of *G. canescens*, *G. clandestina*, *G. latifolia*, and *G. tabacina* (all 2n=40) were identified that showed little damage from the fungus after multiple inoculations.

1.1310 Scientists at the USDA-ARS Sunflower & Plant Biology Research Unit, Fargo ND; the Ag Research Center, Central Lakes College, Staples MN, the Panhandle Res. & Exten. Center, University of Nebraska, Scottsbluff NE; the NDSU Res. & Exten. Center, Carrington ND; and Plant Pathology Department, University of Nebraska-Lincoln discovered new sources of resistance and broadened the genetic base of sunflower breeding material. Two hundred fifty USDA Plant Introductions were phenotyped and compared with elite USDA released germplasm. The Plant Introductions (PIs) are genetically very diverse, and originate from 30 countries. Over a two- year period, six datasets of stalk rot were generated on this germplasm. The top 25 entries included 5 of the 11 elite USDA lines, and 20 Plant Introductions from ten different countries, ranging from Paraguay to Mexico to Russia to Zambia. This same group of PIs and USDA inbreds were used for selection of new germplasm and for association mapping.

1.1312 USDA-ARS scientists at the Northern Crop Science Laboratory, Fargo ND crossed *Helianthus hirsutus*, *H. salicifolius*, *H. occidentalis*, *H. divaricatus*, and *H. resinosus* 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.

PM 1.2: Transfer new resistance genes into plant germplasm

- *Germplasm derived from interspecific crosses*

1.2112 USDA-ARS scientists at the Northern Crop Science Laboratory, Fargo ND crossed amphiploids resistant to stalk and head rot with HA 410. Backcross progenies with 2n=34 were established in the field, and BC2F4/BC3F3 families were evaluated in replicated trials in 2009-2012. In addition, interspecific F1 progeny were produced between stalk rot resistant hexaploids *H. californicus* and *H. schweinitzii* and HA 410. Backcross progenies of *H. californicus* crosses with HA 410 were evaluated in replicated trials in 2009-2012. A molecular tracking study indicated a higher frequency of gene introgression from diploid perennials than from hexaploid or interspecific amphiploids.

1.2119 Scientists in the Crop & Soil Sciences Dept, Michigan State University, USDA ARS, Prosser WA; BioAg Science & Pest Management Dept, Colorado State University, Ft. Collins CO; University of Idaho, Kimberly ID; Cornell University, Geneva NY; NDSU Carrington Res Ext Center, Carrington ND; Seminis Seeds, DeForest WI: Dept Plant Pathology University of Nebraska, Lincoln NE; and Dept Horticulture, Oregon State University, Corvallis OR found that the 2012 field nursery provided evidence that all 9 entries had white mold resistance ranging from similar to Bunsi (avoidance) to resistance superior to G122, the moderately resistant check. The field entries are generally adapted and cover navy, pinto, great northern, pink and small red seed classes. These results illustrate the progress that the NSI has made in identifying functional white mold resistance.

- *Resistant selections of un-adapted x agronomic traits*

1.2208 USDA-ARS scientists in the Department of Crop Sciences, University of Illinois performed reciprocal crosses between resistant and susceptible accessions to produce putative F1 plants. Single nucleotide polymorphisms (SNPs) identified in the DCL3 genes of the parental lines were used to confirm that putative *G. latifolia* F1 plants were true hybrids. Attempts to produce F1 plants from the other three Glycine species were not successful. A population of F3 *G. latifolia* lines was developed by single-seed descent for production of recombinant inbred lines (RILs).

1.2210 Scientists at the USDA-ARS Sunflower & Plant Biology Research Unit, Fargo ND; the Ag Research Center, Central Lakes College, Staples MN, the Panhandle Res. & Exten. Center, University of Nebraska, Scottsbluff NE; the NDSU Res. & Exten. Center, Carrington ND; and Plant Pathology Department, University of Nebraska-Lincoln used PIs to produce four datasets of Phomopsis resistance from field trials. These data will be used to do association mapping for all three diseases.

1.2215 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University developed backcross populations, generated in an attempt to introgress resistance from WM7.1 and WM8.3 into higher yielding commercially acceptable pinto bean. Progeny will be tested during 2013.

1.2217 USDA, ARS scientists in the Sunflower and Plant Biology Research Unit, Fargo ND made a series of paired crosses between 13 released, elite inbred lines from the USDA sunflower breeding program and 20 plant introductions in the top tier of Sclerotinia resistance as evidenced by replicated field testing. The F1s were random mated in the greenhouse, and the random mated progeny were grown in the field in the summer of 2012. The plants in the field were self-pollinated to form S1 lines for visual observation in the 2013 field season, as well as marker assisted selection during the winter in the greenhouse.

PM 1.3: Genetic analysis & QTL discovery

- *Highly inbred mapping populations with validated QTLs*

1.3105 Scientists in the Department of Plant Pathology, Department of Plant Sciences, Langdon Research Extension Center, at North Dakota State University evaluated thirty three Fs lines from the cross between *B. napus* accessions PI458939xPI649136 for their reaction to *S. sclerotiorum* and *Leptosphaeria maculans*, the causal agent of blackleg. Lines 71 and 153 were moderately resistant to PG-3 and PG-4 strains of *L. maculans* and at the same time have some resistance against *S. sclerotiorum*. These lines will also be used to develop breeding lines.

1.3113 Scientists in the Plant, Soil and Microbial Sciences Department, Michigan State University, East Lansing MI located significant Quantitative Trait Loci (QTL) for field disease incidence on Pv01, Pv03 and Pv08 based on disease scores collected in 2007, 2008 and 2010. These QTL explained between 19 to 22% of total variation observed and the favorable alleles associated with these QTL originated from the AN37 parent. Significant QTL for flowering on Pv03 accounted for 23% total variation observed and this originated from the P02630 parent. QTL associated with the number of days to maturity explaining up to 31% variation were mapped on Pv05, and Pv08. Longer days to maturity were associated with alleles from the AN37 parent. There were also significant QTL for physiological resistance detected in the greenhouse straw test on chromosomes Pv02, and Pv07 that explained up to 42% of total variation and most of the positive alleles came from the AN37 parent.

1.3115 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University validated QTL and examined interactions among identified QTL. Different QTL combinations have been characterized for resistance response in the straw test and in the field. Some QTL combinations exhibited a partial additive effect in the field (WM2.2 and WM8.3) or in both field and greenhouse environments (WM7.1 and WM8.3 QTL). Andean origin of the deployed WM7.1 and WM8.3 QTL contributes to the negative linkage drag effect on yield observed in pinto (Middle American), but not in snap beans, which are predominately of similar Andean origin. Scientists also tested the Middle American Diversity Panel (MDP) composed of 300 cultivars/lines for partial resistance to white mold in the straw test for three of six planned replications. The other three straw test replications were completed in February 2013. The field trial will be conducted in the summer of 2013. Given the new importance for avoidance traits in field control traits affecting canopy architecture will be measured in addition to disease severity.

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

1.3205 Scientists in the Department of Plant Pathology, Department of Plant Sciences, Langdon Research Extension Center, at North Dakota State University evaluated a total of 278 *B. napus* accessions for their reaction to *S. sclerotiorum* using the PIT. DNA was collected and screened using 3072 molecular markers. Thirty two markers were significantly associated with resistance and their effects ranged between 1.5 and 4.22%. Eight

markers that provided the highest R2 values were mapped at the chromosome level, but some could be identified only to genome level. As soon as the C genome becomes available, collaborators will locate these markers. The most resistant accessions have been identified and will be used for future development of breeding lines.

1.3213 Scientists in the Plant, Soil and Microbial Sciences Department, Michigan State University, East Lansing MI verified QTL with the same microsatellite markers that were screened on the second half-sib population (AP647). Screening revealed that 43 polymorphic markers associated with resistance from the first population (AP630) were also segregating in the AP647 population. Analysis of segregation patterns with significant markers in the AP647 population showed that the AN37 allele associated with BMd- 34 marker, which was previously unlinked in AP630 population, now mapped near markers on Pv02 and increased field resistance significantly. This QTL on Pv02 is likely the same WM 2.2 QTL that was previously mapped by other authors (Soule et al., 2011). The favorable alleles from the AN37 parent associated with the marker BMd-1 in the QTL interval on Pv03 contributed an average of 10% increase in resistance in the greenhouse straw test in three separate tests. The QTL on Pv03 could be the same as WM3.1 that was mapped in the Aztec/ND population from which AN37 parent was originally selected. Confirmation of existing QTL is valuable as it provides bean breeders with more robust QTL markers to use in enhancing white mold resistance in future bean varieties.

1.3215 Scientists at USDA-ARS, Prosser WA., Oregon State University, and North Dakota State University identified the most resistant *P. coccineus* accessions in the USDA PI collection, and then crossed them to *P. vulgaris* to create three backcross populations (91G/PI255956 BC2F8, 91G/PI433251 BC2F6, and M0162/PI433251 BC2F6) to characterize white mold resistance QTL. Five QTL, some apparently unique from *P. vulgaris* QTL were identified. One significant finding was that like *P. vulgaris*, white mold resistance in *P. coccineus* is quantitatively inherited and controlled by multiple factors.

1.3220 Scientists in the Department of Plant, Soil and Microbial Sciences, Michigan State University. East Lansing MI identified two new QTLs for resistance to Sclerotinia stem rot from the resistance sources PI 391589A and PI 391589B. Five resistance 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.

- *Integrated linkage maps*

1.3315 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University generated a comparative QTL map to integrate QTL identified from new studies, validate QTL, and to identify independent QTL with major effects for gene pyramiding. The comparative map generated by Soule et al. (2011) was expanded to include QTL related to disease avoidance traits. This new map contains 79 QTL, 27 for partial resistance, 36 for disease avoidance traits, and 16 for root traits (Miklas et al., 2012). In summary, 13 QTL conferring resistance to white mold co-located with QTL conferring disease avoidance traits, six with strong and seven with weak associations.

PM 1.4: Pyramid white mold resistance genes

- *Improved conventional breeding methods for quantitative traits*

1.4112 USDA-ARS scientists at the Northern Crop Science Laboratory, Fargo ND developed a genomic in situ hybridization (GISH) technique to distinguish chromosomes of the perennials and cultivated sunflower.

1.4115 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University developed a set of 66 families by crossing resistant x resistant parents. The parents include five lines well known for resistance (G122, Cornell 501, Ex Rico, NY6020-5, and A195), four parents that have shown promise in Oregon trials (M0070, M0169, PI 207130, and PI 290990), and one interspecific line (WMG904). These populations advanced by single seed descent are in F5 are now ready for phenotypic and genotypic characterization.

1.4117 USDA, ARS scientists in the Sunflower and Plant Biology Research Unit, 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 250 plants from 14 BC2F1 plants were advanced to the

BC2F3 generation by single-seed descent. The goal is to produce an AB population of BC2F2:6 lines by an additional three cycles of single- seed descent.

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

1.4215 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University found that interspecific lines do not approach homozygosity at the same rate as intraspecific lines when inbred over generations. In addition, interspecific derived lines show a higher rate of outcrossing than do *P. vulgaris* lines. This last observation in combination with interspecific incompatibilities may help explain why approach to homozgyosity with inbreeding is slower than expected.

1.4217 USDA, ARS scientists in the Sunflower and Plant Biology Research Unit, Fargo ND transferred Sclerotinia stalk rot resistance from wild sunflower species to a cultivated background. In 2012, a total of 4,288 F2 plants were screened from the crosses of cultivated sunflower with eight wild species accessions, and 262 resistant plants were selected for advance to the BC2F3 generation. Seventy-one BC2F3 families from four cross combinations were tested for their reaction to stalk rot in our field nurseries. Overall, seven lines had no infection and 10 lines had a disease incidence lower than 10% at both locations.

PM 1.5: Marker-assisted selection

- *Disease reaction of RILs in multiple field environments*

1.5112 USDA-ARS scientists at the Northern Crop Science Laboratory, Fargo ND crossed stalk rot resistant diploid perennials *H. maximiliani*, *H. giganteus*, and *H. grosseserratus* with HA 410 in 2007, and their BC1F4/ BC2F3 families were evaluated in replicated field trials in 2009-2012. Replicated field tests in 2012 for stalk rot and head rot resistance indicated moderate to good resistance indicating successful gene introgression.

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

1.5217 USDA, ARS scientists in the Sunflower and Plant Biology Research Unit, Fargo ND tested 23 families derived from *H. petiolaris* PI 435843 and *H. argrophyllus* PI 494573 for their reaction to stalk rot in the growth chamber in the summer of 2012. Molecular marker tracking of the introgressed segments indicated that one promising line, 11-275-037, had *H. argrophyllus* alleles at 8.98% of the SSR markers, mostly on linkage groups 9, 10, and 11, indicating these chromosomes may associate with stalk rot resistance.

- *Field verification of resistance*

1.5312 USDA-ARS scientists at the Northern Crop Science Laboratory, Fargo ND made crosses between NMS HA 89 and head rot resistant *H. maximiliani* and *H. nuttallii* which were advanced to BC1F4 and BC2F4 families for replicated field trials in 2009-2012. Scientists also conducted replicated field tests in 2012 that identified 56 immune stalk rot entries with 0% infection compared to 16% for the most resistant check, and 23 head rot entries with disease ratings of 1 compared to 2.9 for the resistant checks.

1.5315 Scientists at USDA-ARS, Prosser WA., Oregon State University, and North Dakota State University found that canopy porosity and resistance to lodging were extremely important for reducing disease severity in both dry and snap bean ($r = 0.61$) across 11 trials conducted in MI, OR, and WA, from 2000 to 2011 (Miklas et al., 2012). Some avoidance traits were less effective in reducing disease severity in trials with heavy disease pressure. Dry bean lines (eg. USPT-WM-1, USPT- WM-12) with physiological resistance in combination with disease avoidance traits did not require fungicide application to protect yield potential under moderate and heavy disease pressure. Given the complexity of disease resistance as evidenced by the comparative QTL map, marker-assisted breeding for disease avoidance is not recommended at this time. Instead, selecting for resistance to white mold in the field, in combination with high yield potential and acceptable maturity, is the recommended strategy for improving both disease avoidance and physiological resistance to white mold in cultivars with commercially acceptable agronomic traits. This approach resulted in the new white mold resistant pinto bean release USPT- WM-12 . Scientists also screened 134 of the 150 snap bean lines included in the Bean CAP set for white mold resistance in Oregon in 2012. A wide range in variation for resistance was observed with some lines showing high levels of field resistance and on par with partially resistant checks included in the trial. Some of the top performing lines may have avoidance traits, but others such as ‘Corbette Refugee’ and ‘US Refugee #5’ have a type III growth habit and would be expected to have higher levels of disease than observed. The 134 set can be used for association mapping in the snap bean background.

1.5319 Scientists in the Crop & Soil Sciences Dept, Michigan State University, USDA ARS, Prosser WA; BioAg Science & Pest Management Dept, Colorado State University, Ft. Collins CO; University of Idaho, Kemperly ID; Cornell University, Geneva NY; NDSU Carrington Res Ext Center, Carrington ND; Seminis Seeds, DeForest WI: Dept Plant Pathology University of Nebraska, Lincoln NE; and Dept Horticulture, Oregon State University, Corvallis OR coordinated the development of 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. The results from 2012 greenhouse tests completed to date support four lines with large cream, pinto, great northern and cranberry seed types having high levels of white mold resistance.

PM 1.6: GM improved resistance

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

1.6118 Scientists in the Department of Plant Pathology, University of Florida, Gainesville, FL made progress toward effective and durable disease resistance for Sclerotinia stem rot of canola/rapeseed (*Brassica napus*) through transgenic and/or cisgenic engineering of the host. A strategy that is built on two genes, one from a host plant and the other from the pathogen, that hold the potential to block disease when over-expressed in canola. The first of these is the hypersusceptible to *S. sclerotiorum* 1 (HSS1) gene that scientists have identified by mapping an *Arabidopsis thaliana* mutation conferring extreme susceptibility to Sclerotinia infection. The second is the oxalate decarboxylase1 (ODC1) gene from the pathogen that functions in the enzymatic breakdown of oxalate, a major virulence factor. Results should show whether coexpression of HSS1 and ODC1 will provide effective and durable resistance to Sclerotinia stem rot. Scientists will complete fine genetic mapping and isolation of the HSS1 gene from *A. thaliana*. This gene will be cloned into a plant expression vector, over-expressed in *A. thaliana*, and evaluated for conferring Sclerotinia disease resistance. Simultaneously, they will clone and characterize the ODC1 gene from *S. sclerotiorum*, over-express it in *A. thaliana*, and evaluate transgenic lines for disease resistance. Additionally, using available genome sequence resources, collaborating scientists will isolate corresponding HSS1 homolog(s) from *B. napus* to be used in year two and three for engineering resistance in canola.

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

1.6209 Scientists at the Department of Horticulture and Crop Science, The Ohio State University identified line RHA280 as responsive to shoot induction. They increased the percentage of explants forming shoots and consistent recovery of large numbers of shoots formed on each piece of cotyledonary tissue. For the optimized response, dry seed material, from freshly harvested seeds, was sectioned into 2-3 mm explants, and placed cut-surface down on our shoot induction medium (SIM), which contains MS salts, B5 vitamins, 1.5 mg/l BA, 0.2 mg/l NAA, 3% sucrose and 0.2% Gelrite. This medium has also been successfully used for the production of shoots from primary leaf tissue. Shoot induction from leaf tissue was lower than from cotyledonary tissue but it was felt that evaluation of an additional target tissue for transformation was warranted, due to the poor response of the cotyledonary tissue to transformation. Shoots were formed on the non-cut surface of the cotyledonary explant and on the base of the leaf blade, just above the point of contact of the cut surface of the explant with the medium. Using Agrobacterium-mediated transformation of leaves and cotyledonary tissue, we have been able to recently recover GFP-expressing shoots tips (left). Only a few GFP expressing shoots were recovered from leaf tissue but these results led us to re-explore cotyledonary tissue, using a different approach, for transgenic shoot production. Although transgenic plants have not yet been recovered, the improvement in transgenic shoot production has been striking (left, GFP in shoots).

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

- *Transgenic expression of antifungal peptides*

1.6404 Scientists at USDA-ARS, Urbana IL and Agriculture and Agri-Food Canada, Ottawa Ontario used infected OxO transgenic plants and parent in RNA sequencing (RNA-seq), allowing for the verification of the candidate gene expressions and expansion of the expression analysis to those genes not present on the microarrays.

1.6406 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND and the Discovery DuPont Crop Protection Stine Haskell Research Center, Newark DE developed a viable *S.*

sclerotiorum transformation protocol. The rationale w as based on the idea that once *S. sclerotiorum* genes differentially expressed during infection of susceptible (NEP32) and resistant (NEP63) canola lines are identified by Illumina sequencing, those genes could be targeted for gene disruption. The resulting gene "knock-out" mutant *S. sclerotiorum* clones could then be assessed for phenotypic changes, including effects on virulence or pathogenicity. This is one way to determine whether a particular gene of interest is, in fact, a virulence or pathogenicity factor and could be of particular value when studying a gene that has not been functionally characterized. The targeted gene replacement protocol developed during these studies was successfully used to replace the metallothionein gene that was analyzed in RT-PCR experiments and will be used to determine the role of selected genes of interest identified during the Illumina-based sequencing of petiole and leaf cDNA libraries.

PM 1.7: Plant germplasm/cultivars with improved resistance

- *Enhanced adapted germplasm*

1.7105 Scientists in the Department of Plant Pathology, Department of Plant Sciences, Langdon Research Extension Center, at North Dakota State University increased seed of *B. napus* lines with resistance to *S. sclerotforum*. Seeds of NEP 63, a doubled haploid line and the F6 line of Ames 26628xPI458539 were intended for field trials to be planted during the summer of2012. We encountered difficulties in producing enough seed for evaluations in our disease nursery trials and only managed to establish observation plots in Prosper. We continued seed production of these lines in greenhouse and will have enough for field trials in the 2013 growing season.

1.7113 Scientists in the Plant, Soil and Microbial Sciences Department, Michigan State University, East Lansing MI observed significant variation in the data ranges among all the traits in two pinto bean RIL populations (AP630 and AP647). All traits exhibited fairly normal distribution except for the straw test in AP647 population where about 30% of the genotypes were skewed towards the lower disease scores. Broad sense heritabilities (h2) for most traits in AP630 population were low to moderate except for the straw test, yield, seed weight and days to flowering. Field disease incidence (h2=0.23) was noticeably low in comparison to greenhouse tests (h2=0.46) indicating that only 23% of the observed variation in the field was due to genotypic differences. Plant height and lodging also exhibited low heritability estimates (h2=0.25 and 0.23 respectively). Seed weight and yield exhibited relatively higher heritability estimates (h2=0.74 and 0.53 respectively). AP647 in general had higher heritability estimates for seed weight (h2=0.88), days to flowering (h2=0.74) and maturity (h2=0.72) showing relatively more genetic control of these traits than disease incidence or lodging (h2=0.49 and 0.46 respectively). In the AP630 population all the traits were negatively correlated with disease incidence in the field except for lodging (r=0.44). In the AP647 population yield was positively and significantly correlated with all traits except lodging (r=-0.17) and white mold incidence (r=-0.36). Similarly white mold affected all traits negatively except days to maturity (r=-0.41) where higher disease incidence was associated with late maturity. Scientists also observed in field trials that the new white mold germplasm line USPT-WM-12 did very well as did entry ND080547 from North Dakota. Progress is being made in combining yield potential with white mold resistance in different seed types. Included in the trial were the TW and TL lines derived from crosses with Tacana with wild and landrace accessions (Mkwaila et al., 2011) that the author proposed to use as resistance sources to pyramid QTL for white mold resistance. Most of these lines are later maturing but they combine yield potential and disease resistance in seed types that would be easy to incorporate into elite navy and bean breeding materials. The 12 elite navy and black lines listed as parents in the proposal were also evaluated in the test but were not included in the study due to space limitations. Those data can be found on line at:
http://www.css.msu.edu/VarietyTrials/DryBean_HomePage.html

1.7115 Scientists at USDA-ARS, Prosser WA., Oregon State University, and North Dakota State University released USPT- WM-12 pinto bean germplasm with partial resistance to white mold in 2012 (Miklas et al., 2012). It 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) and third highest in 2012. Although high yielding, it was released as a germplasm line because the seed coat is too dark for commercialization. To overcome this dark seed coat problem, crosses between USPT-WM- 12 and ‘bright’ pinto beans were initiated in 2011. Scientists know that USPT-WM-12 possesses WM2.2 QTL for partial field resistance, but it also possesses partial

resistance in the straw test which was unexpected. Genetic populations are being generated to characterize this extremely rare occurrence of straw test resistance in a high yielding pinto bean background.

- *Herbicide tolerant germplasm with resistance to Sclerotinia*

1.7205 Scientists in the Department of Plant Pathology, Department of Plant Sciences, Langdon Research Extension Center, at North Dakota State University produced double haploids from *B. rapa* PI426281 and Ames 21738. They did not make crosses between NDSU lines and elite glyphosate tolerant lines as originally proposed due to proprietary limitations; instead, they made crosses between NDSU lines and public lines, for example, 'Jet Neuf', 'Quantum', and 'Samourai' which could be useful sources of resistance against Sclerotinia stem rot and blackleg. Glyphosate-tolerant canola breeding lines were identified as having statistically less disease than three of the four commercial controls included in the study. In 2012, Sclerotinia stem rot incidence ranged between 34% and 74% with 2 breeding lines below the 40% mark. The two best breeding lines are hybrids that have not been evaluated previously.

- *Agronomic resistant varieties for commercial production*

1.7310 Scientists at the USDA-ARS Sunflower & Plant Biology Research Unit, Fargo ND; the Ag Research Center, Central Lakes College, Staples MN, the Panhandle Res. & Exten. Center, University of Nebraska, Scottsbluff NE; the NDSU Res. & Exten. Center, Carrington ND; and Plant Pathology Department, University of Nebraska-Lincoln. Scientists continue to advance existing populations and breeding lines for stalk and head rot resistance, and introduce new populations. 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. In the next few months, scientists plan to complete data analysis from 2012 on yield, quality, and disease resistance of several lines, and they will release lines in early 2013 that are high yielding with Sclerotinia and Phomopsis resistance, high oleic acid, and IMI herbicide resistance.

1.7313 Scientists in the Plant, Soil and Microbial Sciences Department, Michigan State University, East Lansing MI conducted 2012 field trials at Montcalm Research Farm. Included in the 64-entry trial were the 12-entries from the National Sclerotinia Trial coordinated by Dr. Steadman. In that test the new pinto variety Eldorado (Kelly et al., 2012) continued to perform well and showed overall low white mold scores. The traditional resistance sources rated low for white mold, but were among the lowest yielding materials.

1.7319 Scientists in the Crop & Soil Sciences Dept, Michigan State University, USDA ARS, Prosser WA; BioAg Science & Pest Management Dept, Colorado State University, Ft. Collins CO; University of Idaho, Kemperly ID; Cornell University, Geneva NY; NDSU Carrington Res Ext Center, Carrington ND; Seminis Seeds, DeForest WI; Dept Plant Pathology University of Nebraska, Lincoln NE; and Dept Horticulture, Oregon State University, Corvallis OR facilitated a multi-site NSI nursery test over the past 6 years. A snap bean, two pinto lines, a bayo line and six kidney lines with white mold resistance have been released. At least five more lines are scheduled for release in the next year. New lines with white mold resistance from wide interspecific crosses are now in seed increases for greenhouse screening. High levels of white mold resistance have been found in some wild beans and in other *Phaseolus* spp such as *P. coccineus*. Of the 364 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, all have been characterized using mycelial compatibility groups and 82 MCG's have been found in the USA isolates with 85 total MCGs.

1.7320 Scientists in the Department of Plant, Soil and Microbial Sciences, Michigan State University. East Lansing MI evaluated over 2,000 lines derived from crosses in which either or both parents were partially resistant to Sclerotinia stem rot 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.

1.7321 Scientists at the NDSU - Carrington Research Extension Center, Carrington ND; NDSU - Langdon Research Extension Center, Langdon ND; NDSU - Carrington REC Oakes Irrigation Research Site, Oakes ND; and University of Nebraska Panhandle Research and Extension Center, Scottsbluff, NE evaluated the susceptibility of sunflowers to Sclerotinia head rot during and after bloom. This work provided insights on how

to improve the methods used to screen sunflowers for resistance to this disease. In the trial conducted in Langdon in 2012, inoculations conducted at bloom (R5 growth stage) resulted in a significant increase in Sclerotinia head rot relative to the non-inoculated control; inoculations conducted after bloom did not. In the trial conducted in Carrington in 2012, inoculations conducted at the R5 (bloom) and R6 (flowering complete, ray flowers wilted) growth stages resulted in significant increases in Sclerotinia head rot relative to the control ($P < 0.05$), but disease in the resistant variety was significantly higher when inoculations were conducted at the R5 growth stage than at the R6 growth stage. The resistance reaction of sunflower hybrids (resistant vs. susceptible) was consistent irrespective of whether disease developed at the R5 or R6 growth stage. These results are similar to findings from 2011 and suggest that obtaining replicable results in disease screening nurseries requires that inoculations be conducted over multiple days such that all plants in all entries are inoculated at the same growth stage.

- *Identify crop Germplasm with partial resistance to virulent isolates.*

1.7415 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University identified a set of 18 lines as moderately to highly resistant, and have been seasonally tested the past three years. Most lines show stable resistance although a few have reverted back to a susceptible state (ie WMG836). In general, ranking of lines has remained the same. Crosses were made to 'Spinel' great northern and OSU5613 BBL during the spring of 2011 with 13 interspecific lines possessing markers for identified interspecific QTL to produce 55 cross combinations (including crosses to sublines within an interspecific line type). These numbers were reduced by discarding crosses with apparent revertants. In 2012, 24 populations (12 to each susceptible parent) were advanced to the F_3 by single seed descent. The populations will be used to fine map and validate the QTL.

1.7420 Scientists in the Department of Plant, Soil and Microbial Sciences, Michigan State University. East Lansing, MI 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 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.

Pathogen Biology & Mechanisms of Disease Resistance

PM 2.1: Population structure & dynamics

- *Standardized genotypic characterization on wild and cultivated crops*

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

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

- *Geographical inventory of US populations*

2.1419 Scientists in the Crop & Soil Sciences Dept, Michigan State University, USDA ARS, Prosser WA; BioAg Science & Pest Management Dept, Colorado State University, Ft. Collins CO; University of Idaho, Kemperly ID; Cornell University, Geneva NY; NDSU Carrington Res Ext Center, Carrington ND; Seminis Seeds, DeForest WI; Dept Plant Pathology University of Nebraska, Lincoln NE; and Dept Horticulture, Oregon State University, Corvallis OR collected 245 isolates collected from grower fields and screening nurseries. These isolates were tested for clonality. MI had the most shared MCGs at 75% while OR had 11% and WA had 18%. There is evidence that supports pathogen variation influencing resistance screening results and grower field isolates differing from screening nurseries. More grower isolates are needed to have confidence in the comparisons. A summary of MCG and aggressiveness characterization of 157 screening isolates collected across the USA was published recently (Otto-Hanson et al., 2011).

PM 2.2: Durable host resistance

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

2.2119 Scientists in the Crop & Soil Sciences Dept, Michigan State University, USDA ARS, Prosser WA; BioAg Science & Pest Management Dept, Colorado State University, Ft. Collins CO; University of Idaho, Kemperly ID; Cornell University, Geneva NY; NDSU Carrington Res Ext Center, Carrington ND; Seminis Seeds, DeForest WI; Dept Plant Pathology University of Nebraska, Lincoln NE; and Dept Horticulture, Oregon State University, Corvallis OR tested isolates for aggressiveness using the straw test under greenhouse conditions and were found to have varying degrees of aggressiveness (2.8 to 7.9) based on a CIAT scale of 1 = no disease and to 9 = death of the plant. Significant differences in isolate aggressiveness were found among the isolates while isolates that are clones had similar aggressiveness. Clonality varied from 8 clones from 62 MI isolates to 27 from 59 isolates from WA. Shared clones also varied from seven to one across screening sites.

- *Pathogen population dynamics on partially resistant* crops

- *Pathogen x environmental interaction*

2.2311 USDA-ARS scientists in the Dept. of Crop Sciences, University of Illinois, Urbana IL determined that growth rates on agar of isolates from the USA and Brazil differed when grown at 22 °C and 28 °C indicating the possible presence of ecotypes. Experiments to verify aggressiveness of these and other isolates indicated a wide range of aggressiveness with isolates from the USA and Brazil based on inoculation of three different soybean cultivars. Sclerotia of isolates from different geographic regions were tested for apothecia production at 20°C and 30°C, and after vernalization at 4°C for 60 days before be transferred to 20°C and 30°C. A new set of isolates from Brazil and the USA will be tested for growth, aggressiveness (soybean dry bean and canola), and apothecia production at different temperature extremes.

- *Knowledge of plant x pathogen x environmental interactions*

- *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 sclerotia-sphere microbes on germination and dormancy*

- *Effect of sclerotia-sphere 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*

2.4219 Scientists in the Crop & Soil Sciences Dept, Michigan State University, USDA ARS, Prosser WA; BioAg Science & Pest Management Dept, Colorado State University, Ft. Collins CO; University of Idaho, Kemperly ID; Cornell University, Geneva NY; NDSU Carrington Res Ext Center, Carrington ND; Seminis Seeds, DeForest WI; Dept Plant Pathology University of Nebraska, Lincoln NE; and Dept Horticulture, Oregon State University, Corvallis OR identified 18 polymorphic microsatellite markers for analysis of isolate populations collected from 2003-2012. A preliminary study of 239 isolates that were sequenced formed 63 microsatellite haplotypes which did not always associate with the MCGs. A database will be developed to recommend and supply characterized isolates that breeders/pathologists can use to screen for white mold resistance in local areas or across regions while also selecting for high, moderate or low levels of resistance.

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

PM 2.5: EST libraries from pathogen stains

- *Useful cDNA libraries from pathogen expressed genes*

2.5106 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND and the Discovery DuPont Crop Protection Stine Haskell Research Center, Newark DE constructed cDNA libraries from *S. sclerotiorum* inoculated canola petioles, and leaves. Libraries were generated from S. sclerotiorum mycelium grown on culture. Samples were sent to the University of Minnesota for Illumina-based sequencing.

- *Useful genome sequence information*

- *Full length, normalized cDNA libraries*

PM 2.6: Candidate genes for pathogenicity

- *Large ATMT collections for phenotypic screens*

- *Transcriptome profiles and high through put functional analyses*

2.6201 USDA-ARS scientists at the Grain Legume Genetics and Physiology Research, Pullman WA used RNA-Seq technology to study at genomic level the interactions between *S. sclerotiorum* and host plants chickpea and lentil to capture and investigate the genes that are specifically or differentially expressed by the pathogen and the host plants during interactions between the pathogen and the hosts. The specifically expressed genes will be employed to gain mechanistic understanding of Sclerotinia and chickpea/lentil interactions to devise effective and efficient strategies for managing Sclerotinia white mold.

- *Promoters for RNAi constructs during infection*

- *Catalog genes from ATMT random mutagenesis*

- *Discovery of candidate gene function*

2.6506 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND and the Discovery DuPont Crop Protection Stine Haskell Research Center, Newark DE analyzed the expression of selected S. *sclerotiorum* genes (oxaloacetate acetylhydrolase, metallothionein, and cellobiohydrolase I) which were involved with virulence or pathogenicity, 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.

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*

3.1108 USDA-ARS scientists in the Department of Crop Sciences, University of Illinois used 384 GoldenGate SNP markers to analysis over 3000 lines via genotyping-by-sequencing (GBS). Loci were evaluated in F2 populations, which produced the first genetic maps for any perennial Glycine species and showed that most *G. max* and *G. latifolia* chromosomes were collinear. Non-destructive methods were compared for the phenotypic evaluation of *G. latifolia* for response to Sclerotinia stem rot, and showed that cut- stem assays using mycelial agar plugs or dilute solutions of oxalic acid produced the best discrimination between susceptible and resistant *G. latifolia* accessions. Preliminary mapping studies in an F2 population identified a QTL (LOD=3.1; R2=0.18) for resistance to Sclerotinia stem rot on the *G. latifolia* homeologue of soybean chromosome 17. RIL population development was continued. A draft genome sequence was assembled for *G. latifolia*. A manuscript describing the production and mapping of SNPs in *G. latifolia* was accepted and is now being revised for publication in Theoretical and Applied Genetics.

3.1120 Scientists in the Department of Plant, Soil and Microbial Sciences, Michigan State University. East Lansing MI evaluated over 900 advanced breeding lines with acceptable agronomic traits for Sclerotinia stem rot resistance using the drop-mycelium method in the greenhouse. A subset of these lines were also evaluated in the field arterially inoculated with the pathogen during flowering time in 2012. These lines were also

evaluated with over 52,000 SNP DNA markers in an attempt to identify DNA markers closely linked to the disease resistance genes.

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

3.1203 Scientists at Michigan State University, North Dakota State University and Dow AgroSciences, LLC prepared manuscripts describing the transcriptome characterization and gene expression profiling in pea with partial resistance to *S. sclerotiorum* and the expression profiling of the pathogen during infection of susceptible and partially resistant pea lines. Data generated from the initial EST analysis is available for use by the Sclerotinia and pea community and data from the current RNAseq efforts will be available shortly. The project has already developed 37 SSR markers which have been incorporated into mapping populations by Dr. McPhee and an additional 541 SSRs have been made available.

3.1208 USDA-ARS scientists in the Department of Crop Sciences, University of Illinois sequenced reduced representation libraries of genomic DNAs of *G. latifolia* resistant and susceptible accessions. Over 9,000 SNPs were identified that mapped to unique positions in the soybean genome. Nine *G. latifolia* SNPs that aligned to two soybean chromosomes mapped in similar orders in *G. latifolia* and *G. max*, which confirmed the utility of the markers.

PM 3.2: Structure of resistance gene enriched genomic regions

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

3.2103 Scientists at Michigan State University, North Dakota State University and Dow AgroSciences, LLC have utilized next generation sequencing technologies to investigate the host-pathogen interaction between *P. sativum* and *S. sclerotiorum*. In initial experiments a large expressed sequence tag (EST) data set was developed 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 published in BMC Genomics 13:668. In addition, scientists identified SSR (microsatellite) markers in the 454 sequence data from pea and screened the markers for polymorphism across pea parents from 4 recombinant inbred lines developed by Dr. McPhee. A manuscript describing these novel SSR- EST markers has also been accepted by the American Journal of Botany - Primer Notes & Protocols.

3.2106 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND and the Discovery DuPont Crop Protection Stine Haskell Research Center, Newark DE completed RNA extraction, mRNA purification, and cDNA library preparation for DNA Sequencing and Analysis Facility, BioMedical Genomics Center at the University of Minnesota via Illumina GA IIx. After passing an initial quality control KAPA qPCR assay, a 76 cycle run was completed. This initial sequencing run was performed using the pooled samples in a single lane to validate the library preparation process and sequence data analysis. This initial run generated more than two million sequence reads. After validating the library preparation protocol, large scale experiments involving more time points were conducted using two different inoculation methods, the petiole inoculation method conducted previously and a leaf based inoculation method. Libraries also were generated libraries from inoculated canola leaf material for Illumina sequencing analysis. Germinated canola seedlings were grown in a growth chamber for 19 days with the same environmental conditions. Leaves were inoculated with PDA plugs (0.5 cm diameter) containing actively growing *S. sclerotiorum* mycelia from a two day old culture. PDA plugs with no mycelia were placed on non-inoculated control leaves. To maintain sufficient humidity to encourage *S. sclerotiorum* infection, clear, plastic bags were used to cover each canola plant. There were six leaves per treatment/control and the same four time points used during the petiole inoculations were used (i.e., 8, 16, 24, and 48 hours post inoculation). At each designated time point post inoculation, leaves were harvested, flash frozen in liquid nitrogen, and stored at -80°C. Similar to the inoculated petioles, there were no readily apparent differences in disease symptoms on leaves between NEP32 and NEP63 canola lines. However, a water-soaked, brown lesion expanded from the inoculation point, with considerable wilting of leaves observed at 48 hours post inoculation. cDNA libraries were prepared as described.

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

3.2203 Scientists at Michigan State University, North Dakota State University and Dow AgroSciences, LLC used the 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 by Dr. Lyndon Porter at Washington State. mRNA was extracted and converted to cDNA prior to massively parallel sequencing. Around 300 million pairs of reads were produced with a 75 bp paired-end sequencing method. As a reference genome is not available for pea we are utilizing software called Trans-ABySS and Trinity which allows de novo assembly and analysis of RNA-seq data without a reference genome.

3.2205 Scientists in the Department of Plant Pathology, Department of Plant Sciences, Langdon Research Extension Center, at North Dakota State University detected eighteen DArT markers associated with resistance to *S. sclerotiorum* in *B. napus* and the *B. rapa* genome. Some markers were also detected in the *Arabidopsis thaliana* and *B. oleracea* genomes.

3.2215 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University mapped WM8.3 QTL. SNPs are anchored onto the genomic sequence of common bean. This QTL spans a large portion of the genome, while only covering a small genetic region. The reason for this is that the markers mostly map to the low recombination region of the genome that was defined by the sequencing project. The next question is whether scientists can discover genes associated with this QTL.

- *Physical map of genomic regions containing resistance genes*

3.2315 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University fine mapped QTL WM8.3. The availability of an assembled genome now enables QTL mapping in the realm of a physical map. To understand the introgression events associated with the backcross program, scientists performed introgression mapping using the RNA-seq data. They mapped all of the RNA-seq reads onto the reference genome and looked for those regions with an excess of SNPs. Any region in the genome with an extensive number of SNPs would represent the introgression regions that accompanied the movement of the WM8.3 QTL into the susceptible background. The typical view is that because the scientists were selecting for the QTL, only chromosome Pv08 would show an excess of SNPs. They discovered a total of 48,852 SNPs between the resistant and susceptible lines used in the RNA-seq analysis. Scientists next looked at 500kb sliding windows, of 50kb increments, across the genome for a total of 10,193 windows, studied the distribution, and did a bootstrap analysis to set the confidence interval and define the upper 5% cutoff of the distribution. Any window with greater than 19 SNPs was considered significant. This analysis revealed that at this cutoff level, 53.4 Mb (out of a genome size of 600 Mb) was introgressed into the susceptible genotype. Of this, 17.8% (9.6 Mb) was introgressed into chromosome Pv08 which represented the chromosome with the largest introgressed block. Smaller blocks were introgressed into each of the other ten chromosomes. Given that these data were actually pooled over four susceptible and four resistant lines, these introgression regions are actually a mixture of the introgression events from multiple lines. Buried within these data, though, should be those regions most closely associated with the WM8.3 QTL on Pv08. To discover potential regions, scientists set a cutoff at the upper 0.1% level and discovered three regions: 0.20-0.70 Mb, 7.55-8.05 Mb, and 57.30-57.90 Mb, and consider these to be the top candidates for regions containing those genes associated with WM8.3. Scientists also generated a physical map of QTL WM7.1. As with the WM8.3 analysis, they have begun a reanalysis of the WM7.1 data set that was collected in 2012. Scientists anticipate these data to be completely reevaluated in the manner described above during 2013.

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

3.2410 Scientists at the USDA-ARS Sunflower & Plant Biology Research Unit, Fargo ND; the Ag Research Center, Central Lakes College, Staples MN, the Panhandle Res. & Exten. Center, University of Nebraska, Scottsbluff NE; the NDSU Res. & Exten. Center, Carrington ND; and Plant Pathology Department, University of Nebraska-Lincoln have genotyped the entire set of PIs with 8,700 SNP markers and re-sequenced resistance gene candidates from the PIs. These data will be used to map stalk rot resistance and can now be applied to head rot.

3.2415 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University initiated a physical map of QTL WM2.2. They are in the process collecting samples for the WM2.2 region. This will follow the same process described for the other two regions.

PM 3.3: Function of candidate resistance genes

- *Microarrays for high throughput gene screening*

3.3104 Scientists at USDA-ARS, Urbana IL and Agriculture and Agri-Food Canada, Ottawa, Ontario identified and verified candidate defense genes playing an active role in defense through specific functional studies involving transgenics, and mapping of defense genes to known QTL. Microarray gene expression studies compared the partially resistant PI194639 to susceptible Williams 82, a resistant oxalate oxidase (OxO) transgenic to susceptible control, and infiltrated OA to water/acid controls (Calla et al., 2013, submitted). 60 candidate defense-related genes were selected based on their response to OA and Sclerotinia in the transgenic OXO line 80(3d0)-1.

3.3106 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND and the Discovery DuPont Crop Protection Stine Haskell Research Center, Newark DE found that analysis of the petiole inoculation libraries revealed a total of 4,350 differentially expressed ESTs, of which 1,946 are up-regulated. More than 400 ESTs appear to be turned on exclusively in resistant line, with 436 ESTs being up-regulated by 10 fold. Functional categorization of these differentially expressed genes is essential to understand the mechanism underlying the differences in resistance response in the two canola lines. Initial searches suggest that many of the ESTs have no known function, and thus may be considered as potential candidates for identification of novel resistance genes. However, 1,013 of the up-regulated ESTs have known role in biological processes among which 107 ESTs were involved in defense response. Other important functional classes up-regulated in resistant line were signal transduction (82), and innate immune response (42). Comparison of gene expression between inoculated and non- inoculated conditions will provide an insight into the various responses of the host to pathogen infection.

- *Sequenced cDNA libraries from infected host tissue*

3.3203 Scientists at Michigan State University, North Dakota State University and Dow AgroSciences, LLC used a RNA-seq approach to generate a large amount of transcript sequence data for each pea parent, which will be valuable for the development of additional gene-linked markers such as simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). The markers were utilized in Luminex xMAP technology (or similar technologies) to map resistance to Sclerotinia in pea.

3.3206 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND and the Discovery DuPont Crop Protection Stine Haskell Research Center, Newark DE generated from sequencing runs, a total of 153,851,612 and 109,899,384 quality filter passed 76 bp short reads from petiole inoculated and leaf inoculated libraries respectively. Reads from fungal origin (pathogen) were filtered out by aligning to *S. sclerotiorum* genome using software program Tophat. Initially it was proposed to use *A. thaliana* genome as a reference for aligning short reads generated from sequencing, but from the preliminary analysis it was found that only very low number of reads from the libraries had matches with *A. thaliana*. Hence, Brassica EST assembly, 95k unigene set from the Brassica genome gateway (http://brassica.bbsrc.ac.uk/) which represents all the publicly available ESTs from A, B, and C genomes of *Brassica spp.* was used as reference to map the reads. Read counts that are uniquely aligned (only one best match in the reference) were obtained; Read count data obtained from the alignment of libraries was used to determine differential expression patterns using software package DESeq. Analysis of the massive data generated from sequencing has been initiated and is in progress. So far, comparisons have been made to determine genes or ESTs differentially expressed in resistant line NEP63 compared to the susceptible line NEP32 during infection process. From the preliminary analysis of 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 were identified in the initial analysis. Functional categorization of the up-regulated gene set was performed using Blast2Go. Of the 320 ESTs only 163 were found to have a known role in biological processes; 24 of the 163 ESTs appeared to be involved in defense response. Response to stress, hormone mediated signaling pathways, transcription factors were other categories of ESTs identified that may also be involved in the regulation of a resistant response.

3.3215 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University performed RNA-seq analysis to evaluate the response of WM8.3 resistant and susceptible line to pathogen

infection. They evaluated the 130,414,421 RNA-seq by first mapping the reads from each library (representing the different experiment treatments) to the reference set of common bean genes using the SOAP software. Only unique reads specific for a gene were maintained. Scientists then looked for those genes that exhibited at least a two-fold difference in expression, and exhibited a false discovery rate (to account for Type I errors that could possibly occur by repeated mapping of the reads onto the complete gene set) of < 0.05. They used the multiple experimental treatments to account for genotype, wound, and agar effects to arrive at the distribution of genes up regulated at 24 and 48 hr post treatment. These genes were then annotated using a blast analysis against the soybean version 1.0 and Arabidopsis version 1.0, and allowed two questions to be addressed: 1) what is the relative overall pattern of observed expression? and 2) are any differentially expressed genes located in the highly conserved introgressed regions? Only three genes (Phvul.003G173300, Phvul.005G137700, Phvul.006G141200) showed differential expression at both time points; two of these are transcription factors and one is of unknown function. Scientists next evaluated the GO terms associated with the differentially expressed data sets. This provides valuable information regarding the types of genes that are activated in response to the pathogen. At 24 h, the genes in the GO:0007096 biological process, involved in "regulation of exit from mitosis", were over-expressed relative to their abundance in the genome. While at 48 h, those genes associated with GO:0031072, a "heat shock protein binding" molecular function, were over-expressed.

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

. 3.3304 Scientists at USDA-ARS, Urbana IL and Agriculture and Agri-Food Canada, Ottawa, Ontario identified 105 genes (19 of which are located within 500 kb of a known white-mold resistance QTL) as being significantly differentially expressed between resistant and susceptible lines in a previously funded NSI project on soybean stem response to Sclerotinia.

3.3306 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND and the Discovery DuPont Crop Protection Stine Haskell Research Center, Newark DE compared 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 differentially expressed genes of *S. sclerotiorum* during the infection process of both resistant and susceptible lines was performed using the software programs Cufflinks and Cuffdiff. From the petiole inoculation, 2057 and 1843 fungal genes were found to be differentially expressed inplanta within 16 and 48 hpi respectively during the interaction with susceptible line NEP32. Of the differentially expressed genes, 552 and 710 genes were up-regulated during pathogenesis. During the interaction with the resistant line NEP63, 2588 and 1870 genes were differentially expressed of which 299 and 788 genes were up-regulated within 16 and 48 hpi respectively. Within the up-regulated gene set, 7 genes were found to be exclusively expressed inplanta indicating these genes could be very important for infection process. Six of the seven genes are common during interaction of both susceptible and resistant lines; whereas one gene (SS 1G_11865) was found to be expressed only during the interaction with protein kinase (MAPK) gene BMP 1 of closely related plant pathogenic fungus Botrytis cinerea, protein kinase (MAPK) gene BMP 1 of closely related plant pathogenic fungus Botrytis cinerea, which is characterized as essential for plant infection.

3.3315 Scientists at USDA-ARS, Prosser WA, Oregon State University, and North Dakota State University merged SNP and gene expression data with QTL mapping of the introgressed regions and the differential gene expression data. Three regions were discovered that had a statistically significant overabundance of SNPs at the 0.0001 level. These regions were investigated to determine if any genes in those regions were being over-expressed. The most intriguing result was the over-expression of the disease resistance gene in the 7.55-8.05 Mb interval at 48 h. This gene, Phvul.008G0797000, is a receptor-like protein with a leucine rich domain known to interact with pathogen factors to regulate the downstream proteins involved in the actual disease resistance response. This protein was significantly over-expressed in the susceptible genotypes. It is also typical of other resistance type genes, in that it is expressed at low, but differentially expressed, levels.

** Function of candidate genes using gene silencing methods*

3.3404 Scientists at USDA-ARS, Urbana IL and Agriculture and Agri-Food Canada, Ottawa, Ontario functionally characterized three defense- related candidate genes using the soybean stable transformation system from

Simmond's lab. Scientists also tested Arabidopsis T-DNA insertion lines for candidate genes responding to Sclerotinia infection. Results showed that silencing expression of a G-protein coupled receptor (GPCR) enhanced susceptibility (Calla et al., 2013, in preparation); indicating that G-protein signaling is an important component of Sclerotinia defense.

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*

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*

Disease Management & Pathogen Epidemiology

PM 4.1: Optimized fungicide application programs

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

4.1107 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND, the NDSU-Langdon REC, Langdon ND and University of Minnesota, Northwest Research and Outreach Center, Crookston MN investigated the efficacy of control of isolates insensitive to thiophanate methyl in greenhouse trials using a pair of isolates for each of the following categories: sensitive, moderately insensitive, and insensitive to thiophanate methyl. These six isolates were inoculated on sunflower plants that had been protected with the recommended doses of Topsin (20 fl oz/A= x), or with 0.25 x, 0.5x, and 2x of the recommended doses. Insensitive isolates caused similar levels of disease severity than sensitive ones on plants protected by the full recommended doses. However, when disease severity was considered across all concentrations used, isolates insensitive to the fungicide produced statistically higher disease levels.

- *Efficacy of new chemistries*

4.1207 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND, the NDSU-Langdon REC, Langdon ND and University of Minnesota, Northwest Research and Outreach Center, Crookston MN evaluated six fungicides Topsin, Endura, Praline, Quash, Omega, and Switch alone or in tank combinations with one other compound, for control of SSR in canola. The trial was conducted in Langdon and had a total of 18 treatments. The SSR epidemic developed to a moderate level with observed incidences ranging from 11 to 36% only. This range was not significantly different among treatments. These low levels were observed with the misting system to promote disease development.

- *Updated management guides for disease management*

4.1307 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND, the NDSU-Langdon REC, Langdon ND and University of Minnesota, Northwest Research and Outreach Center, Crookston MN characterized the fitness of isolates to determine whether resistance imposed a penalty on their ability to affect plants. Estimates of the effect of currently recommended field doses on disease caused by these isolates were determined. Since these fungicides also are used in dry bean and soybean for control of *S. sclerotiorum*, these data will have immediate impact on the way growers of these crops manage the disease.

- *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*

4.3407 Scientists in the Department of Plant Pathology, North Dakota State University, Fargo ND, the NDSU-Langdon REC, Langdon ND and University of Minnesota, Northwest Research and Outreach Center, Crookston MN estimated sensitivity to metconazole for 85 isolates collected from states across North Central US. Calculation of the 50% effective concentration (EC_{50}) was obtained by growing the isolates in metconazole-amended potato dextrose agar. The isolates evaluated were skewed towards sensitivity since metconazole has not been extensively used for control of S. *sclerotiorum;* however, a few isolates were moderately insensitive to the fungicide at $EC_{50} < 1$ µg/mr. Greenhouse trials were conducted to determine the effect these levels of resistance to metconazole have on the efficacy of the recommended doses for commercial applications. The threshold used to identify these isolates as insensitive needs to be reevaluated since EC_{50} values for other fungi are markedly different that those observed in our trials.

PM 4.4: Optimized cultural practices for disease management

- *Improved variety selection criteria*

4.4121 Scientists at the NDSU - Carrington Research Extension Center, Carrington ND; NDSU - Langdon Research Extension Center, Langdon ND; NDSU - Carrington REC Oakes Irrigation Research Site, Oakes ND; and University of Nebraska Panhandle Research and Extension Center, Scottsbluff, NE demonstrated fungicide efficacy against Sclerotinia head rot in a field trial conducted in Scottsbluff NE but not in Langdon ND in 2012. In Scottsbluff, three fungicides significantly reduced Sclerotinia head rot incidence and severity and four significantly reduced Sclerotinia stalk rot. In Langdon, none of the eight fungicides tested resulted in a significant reduction in Sclerotinia head rot relative to the inoculated control. Disease levels in the trial conducted in Carrington were too low to permit a rigorous assessment of fungicide efficacy.

- *Management decision aids*
- *Precision agriculture program* ***(not funded)***

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268. Wunsch, M.J. 01/2012. Pulse disease update – lentils, chickpeas, and field peas. Northern Pulse Growers Association convention; Minot, ND. 110 people.

269. Wunsch, M.J. 02/2011. Managing root and foliar diseases in pulse crops. Mon-Dak Pulse Day, Montana State University and NDSU Extension Services; Williston, ND. 70 people.

270. Wunsch, M.J. 02/2012. Integrated management of lentil, field pea, and chickpea diseases. Western Crop and Pest Management School, NDSU Extension Service; Minot, ND. 110 people.

271. Wunsch, M.J. 02/2012. Integrated Management of white mold in soybeans and dry beans. International Crop Expo; Grand Forks, ND. 125 people.

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273. Wunsch, M.J. 12/2011. Integrated management of white mold in soybeans and other field crops. University of Minnesota Crop Pest Management Short Course; Minneapolis, MN. 320 people.

274. Wunsch, M.J. 01/2011. An update on pulse crop diseae management research. Northern Pulse Growers Convention, Minot, ND; 300 people.

275. Wunsch, M.J. 01/2011. Integrated management of soybean white mold. "Getting it right" soybean production meetings, Valley City and Cando, ND; 110 people.

276. Wunsch, M.J. 02/2011. Managing root and foliar diseases in pulse crops. Mon-Dak Pulse Day, Williston, ND; 70 people.

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2012 Sclerotinia Funding Matrix

Project	PI	2011	Cooperator	Commodity	2012
Genetics of Fungicide Resistance in *Sclerotinia sclerotiorum*	Chen	$0	ARS		$71,518
Expression profiling of the pea-Sclerotinia sclerotiorum interaction for genomics assisted breeding	Chilvers	$58,044	MI	Pea & Lentil	$55,824
Functional verification of candidate defense-related genes in *Sclerotinia sclerotiorum* in soybean and arabidopsis	Clough	$84,512	ARS	Soybean	$65,400
Identification of Resistance and Pathogenicity Genes Associated with Sclerotinia Sclerotiorum Infection using Next-generation Sequencing	del Rio	$60,306	ND	Canola	$58,000
Development of Canola Breeding Populations and Identification of Herbicide-tolerant Breeding Lines with Resistance to Sclerotinia Sclerotiorum	del Rio	$72,973	ND	Canola	$68,783
Optimizing Management of Sclerotinia Stem Rot of Canola Through Fungicide Use	del Rio	$37,370	ND	Canola	$35,941
Identification of novel loci for partial resistance to sclerotinia stem rot in perennial soybean accessions	Domier	$45,000	ARS	Soybean	$75,577
Discovery and use of novel sources of resistance to head rot and stalk rot in cultivated sunflower & wild *Helianthus*	Gulya	$78,699	ARS	Sunflower	$75,689
Characterization of growth, pathogenicity, and apothecial development of *Sclerotinia sclerotiorum* isolates from different geographic regions in contrasting temperature regimes	Hartman	$0	IL	Pathogen	$28,857
Transferring Sclerotinia resistance genes from wild *Helianthus* species into cultivated sunflower	Jan	$119,700	ARS	Sunflower	$115,123
Pyramiding QTL for White Mold Resistance into Mesoamerican Beans	Kelly	$0	MI		$34,667
White mold resistance-QTL: Identification, interactions & fine mapping in common bean	Miklas	$154,096	ARS	Dry Bean	$148,203
Deployment of novel sources of Sclerotinia resistance and tools for breeding resistance in sunflower	Qi	$109,250	ARS	Sunflower	$105,072
Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas	Steadman	$60,202	NE	Dry Bean	$57,900
Enhancing soybean for resistance to Sclerotinia stem rot	Wang	$0	MI	Soybean	$38,970
Evaluation of Sunflower Hybrids for Resistance to sclerotinia Head Rot	Wunsch	$70,856	ND	Sunflower	$65,880
Outreach (summit meeting, website, etc.)		$28,994	ARS	All	$29,000