

United States
Department of
Agriculture

Research, Education &
Economics

Agricultural Research
Service

National Program Staff

January, 2006

Version 1.0

Sclerotinia Research Initiative Annual Report January 1 - December 31, 2005

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



Sclerotinia Initiative Annual Report for January 1 thru December 31, 2005

Strategic Goal 1: Develop novel germplasm and varieties with field resistance to Sclerotinia sclerotiorum

Performance Measure (PM) 1.1.1: Identify new sources of resistance in Brassica germplasm. Commercially available canola cultivars are not resistant to *S. sclerotiorum*, although some differences in susceptibility exist. The U.S. collections of *B. napus*, *B. rapa*, and *B. juncea* have not been fully evaluated for resistance to *S. sclerotiorum*.

Baseline: Some Chinese winter accessions with partial resistance are available, but B. napus, B. rapa, and B. juncea U.S. collections have not been fully evaluated.

Target 2005: Fully evaluate Brassica napus and B. rapa collections.

Accomplishment: Over twenty canola cultivars, representing current production varieties and private breeding lines, were evaluated for Sclerotinia stem rot resistance in the field each year at Carrington, ND and Red Lake Falls, MN from 2001 to 2004. Flowering plants were inoculated with ascospores and misted to provide a favorable environment for disease development. Disease incidence and severity were evaluated each year. A selection of these cultivars was also evaluated in multiple greenhouse and growth chamber tests at North Dakota State University in Fargo using a petiole inoculation technique. Although no cultivars with complete resistance were identified, differences in susceptibility among the cultivars were observed.

Over 500 Brassica napus accessions have been evaluated for Sclerotinia stem rot resistance in multiple greenhouse tests at North Dakota State University using a petiole inoculation technique. A selection of these accessions were also evaluated in field trial micro-plots in 2004 and 2005. Plants in the field trials were inoculated with Sclerotinia – infested millet seed and mist-irrigated to provide a favorable environment for disease development. All accessions were obtained from the USDA Plant Introduction Station at Ames, IA, which is part of the National Plant Germplasm System. Few accessions appeared to have resistance levels in both greenhouse and field tests; however, some accessions did have significantly equal or better resistance levels than the partially-resistant check cultivar Hyola 357 in greenhouse and/or field trials.

PM 1.1.2: Improve methods to identify resistant canola germplasm. Numerous methods have been reported in the literature to screen for resistance with varying results. A standardized, efficient and accurate method of screening for *S. sclerotiorum* – resistant lines needs to be developed. Inoculation techniques that have been used in canola and other crops will be compared to identify the best technique.

Baseline: Screening methods are not optimal.

Target 2005: Develop an efficient and accurate screening method to be used under greenhouse and/or field conditions.

Accomplishment: A petiole inoculation technique, a detached leaf assay, and an oxalic acid assay were used to differentiate Sclerotinia – resistance levels in a set of canola cultivars under controlled conditions at North Dakota State University. Reactions of the cultivars to these systems were also compared to Sclerotinia stem rot reactions obtained under field conditions. Although none of the inoculation systems in controlled conditions significantly correlated to disease reactions in the field, the petiole inoculation technique was able to differentiate cultivars in the greenhouse. A similar petiole inoculation technique was successful in differentiating canola cultivars in the greenhouse at the University of Wisconsin as well.

PM 1.1.3: Develop canola germplasm with improved Sclerotinia – resistance using novel methods. Novel methods of developing Sclerotinia – resistant canola germplasm include mutagenesis and transformation. These novel approaches may provide alternative methods to traditional disease-screening and breeding that could be used to develop Sclerotinia – resistant canola germplasm.

Baseline: Canola germplasm with resistance to Sclerotinia has not been developed using novel methods.

Target 2005: Develop and test doubled haploid lines from elite lines that have gone through mutagenesis.

Accomplishment: A total of 169 doubled haploid (DH) lines of spring-type canola, developed following mutagenesis and oxalic acid resistance screening at the Univ. of Guelph, have been tested for resistance to Sclerotinia by petiole inoculation in the greenhouse. Sixty of these DH lines were previously identified as being tolerant to high levels of oxalic acid in laboratory tests. A total of 160 DH lines are being field tested for resistance in hill plots under mist irrigation at Fargo, ND, and 80 DH lines are being field tested in row plots under mist irrigation near Crookston, MN. Lines that do poorly in these field tests will be eliminated and the others will be tested further in the greenhouse and in a third field trial planted in October in Georgia. Both the laboratory and greenhouse tests indicate that about 1/3 of the DH lines generated from the 10 spring-type canola cultivars could have increased resistance to Sclerotinia. This however, cannot be confirmed until the field test data are available. The 3% of inoculated plants that developed stem lesions but survived and produced seed will be tested to see if they were escapes or have greatly improved ability to arrest lesion development.

PM 1.1.4: Identify quantitative trait loci (QTL) that confer resistance to Sclerotinia in canola. Identification of QTLs will help in the development of *S. sclerotiorum* – resistant canola cultivars.

Baseline: QTLs not identified.

Target 2005: Develop populations of elite canola lines to characterize resistance genes.

Accomplishment: QTL involved in the resistance to Sclerotinia sclerotiorum in two segregating populations of canola DH lines: the HUA population, derived from a cross between a partially resistant Chinese winter line (Hua db12) and a susceptible European spring line (P1804); and

the MS population, derived from a partially resistant French winter cultivar (Major) and a susceptible Canadian spring cultivar (Stellar), were identified. A total of eight QTL affecting resistance were detected, seven in the HUA population and one in the MS population. At five of the QTL from both populations, alleles from the resistant parent contributed to the resistance. Individual QTL explained 6 – 22% of the variance. QTL with smaller effects were detected only in single evaluations, although the QTL on N2 from the HUA population was detected only in the first evaluation and it had the highest LOD score and R² value. The N12 resistance allele in Hua db12 was detected in a region containing a homoeologous non-reciprocal transposition (HNRT) from the resistance-containing portion of N2. This result suggests that the N12.n2 HNRT enhance resistance of Hua db12 by increasing the dosage of resistance genes.

PM 1.1.5: Release canola germplasm/cultivars with improved resistance. Release of cultivars or germplasm with improved resistance to *S. sclerotiorum* is a long-term performance measure. Any releases made would ultimately benefit U.S. canola growers.

Baseline: No resistant spring-type germplasm or cultivars are available.

Target 2005: Evaluate breeding lines for their reaction to S. sclerotiorum in partnership with seed industry and start efforts towards production of breeding populations from elite B. napus and B. rapa accessions identified for their high levels of resistance in previous screenings.

PM 1.2.1: Genetic analysis for resistance in scarlet runner bean. Within the genus *Phaseolus*, the scarlet runner bean (*P. coccineus*) has highest levels of white mold resistance. This species is in the secondary gene pool for common bean, and it is possible to introgress genes without using extraordinary measures, but sterility and recombination barriers do hamper transfer of complex traits. Previously, researchers have partially transferred resistance (PM1.2.3), but there is a need to map QTL for resistance within *P. coccineus* so that complete resistance can be transferred to common bean.

Baseline: Segregating recombinant inbred line population developed and characterized for reaction to white mold under greenhouse conditions. Molecular marker data initiated and putative QTL identified.

Target 2005: Continue mapping of PI 255956 x Wolven Pole F₂ population. Add AFLPs and SSRs to existing RAPD map to obtain approximately 300 markers and map QTLs for resistance. Continue to screen potentially useful related Phaseolus species as accessions become available.

Accomplishment: Additional AFLP markers were screened to add to the P. coccineus map. From 64 primer combinations, the four with the greatest polymorphism were chosen for characterization of the full mapping population. A screen of subpopulation of 14 lines and the two parents revealed 88 polymorphic markers. Characterization of the full population for the four AFLP primers is underway.

PM 1.2.2: Transfer resistance from scarlet runner bean to dry and snap bean via interspecific hybridization. Some accessions of scarlet runner bean are known to possess high levels of white mold resistance (PM1.2.1). In the past, only moderate levels of resistance were

inadvertently transferred into dry bean (PM1.2.3). It is therefore essential to analyze inheritance and introgress high levels of white mold resistance from recently identified scarlet runner (*P. coccineus* L.) into dry bean.

Baseline: Interspecific hybridization conducted and inheritance analysis and development of breeding lines by backcrossing and selfing are progressing.

Target 2005: Complete inheritance of white mold resistance between interspecific crosses of common (G 122, 91G, UI-320, Othello, MO 162) and runner beans. Continue development of interspecific breeding lines and inbred backcross lines. Complete development of backcross inbred populations. Collect DNA and conduct greenhouse and field tests for white mold resistance. Begin map construction and identification of QTL associated with resistance. Begin determination of whether QTL identified in PM1.2.1 are associated with resistance in backcross inbred populations.

*Accomplishment: Inheritance of white mold resistance studied in the greenhouse in Colorado using the straw test and in Idaho using the modified petiole test in crosses of pinto Othello and UI 320 with *P. coccineus* PI 433246 and PI 439534, respectively, was controlled by a single dominant gene. Also, 562 F3 from two single-crosses, 502 F2 from two first backcrosses, and 290 F3 from two second backcrosses of Othello and UI 320 with PI 433246 and PI 439534 were produced in the greenhouse in Idaho.*

*Additional backcross inbred populations were developed. 91G x PI433251B, MO162 x PI433251B, and G122 x PI433251B were advanced to form BC2F1 populations. DNA was extracted from 399, 222, and 167 individuals, respectively. 91G x PI433251B is intended to integrate a second source of resistance from *P. coccineus* into susceptible common beans. In may provide additional validation for identified QTL for resistance found in the 91G x PI255956 population. MO162 x PI433251B and G122 x PI433251B pyramid putatively different sources of resistance from *P. vulgaris* and *P. coccineus* into a single package.*

One hundred ninety two polymorphic markers were identified in the Colorado RIL population. 124 markers were used to generate a molecular marker map and determine if regions of the genome were related to resistance to white mold. The map covered 10 of the 11 linkage groups with a total map distance of 1370 cM. Variation at 25 marker loci was significantly associated with ASI values. Many of these markers clustered around four loci, and the analysis focused on the most significant marker from each cluster. Relationships between markers and reactions to white mold ranged from 13.6 to 8.3% using single factor analysis of variance. Composite interval mapping identified specific regions or putative QTLs associated with resistance in the population. Five regions had LOD scores greater than 3.2. The R² values for relationships to each ranged from 6.9 to 16.5%. Four of the five QTLs were derived from G122 and one from CO 72458.

*To date 87 BC1F1 plants have been developed. The BC1F1 plants are anticipated to be self fertile and development of a BIL population will commence. The BIL population will be used to pyramid resistance from both *P. vulgaris* and *P. coccineus*. Marker screening for polymorphism between the parents of the BIL population has started.*

PM 1.2.3: Determine durability and genetics of resistance among interspecific dry bean breeding lines. Of the three species, namely *Phaseolus coccineus*, *P. costaricensis*, and *P. dumosus* (synonymous with *P. polyanthus*) in the secondary gene pool of the common bean only low to intermediate levels of resistance to white mold have been inadvertently introgressed into common bean despite the fact that it is well known since 1960's that some accessions of *P. coccineus* possess the highest levels of resistance (PM1.2.1). It should be worth determining if white mold resistance also exists in the other two species. Inter-specific crosses of the common bean with the three species in the secondary gene pool, although not specifically made for white mold resistance, were therefore introduced from CIAT, Cali, Colombia to determine their reaction to white mold. Similarly derived lines from interspecific populations will be evaluated, with QTL and inheritance examined for those lines possessing resistance

Baseline: Approximately 450 interspecific breeding lines derived from 10 crosses between a tropical small-seeded black bean 'ICA Pijao' and the three species in the secondary gene pool, namely P. coccineus, P. costaricensis, and P. polyanthus (synonymous with P. dumosus) were introduced from CIAT, Cali, Colombia. These were screened under the field and greenhouse conditions for reaction to white mold for three years. However, further evaluation of 102 of 450 breeding lines is continuing. Segregating recombinant inbred line populations have been developed for three dry bean I9365-25, I9365-31, and VA19 which derive moderate levels of resistance to white mold from interspecific hybridization. These populations will be used to estimate inheritance of resistance and to develop genetic linkage maps for QTL analysis.

Target 2005: Evaluate 102 interspecific breeding lines for white mold resistance in the field in Idaho and in the greenhouse in Colorado and Idaho. Test for white mold resistance in multi-location field and greenhouse conditions 17 of 102 interspecific breeding lines of which three will also be tested in the National White Mold Nursery. Evaluate the Lamprecht P. coccineus interspecific lines with straw and field tests to identify additional sources of white mold resistance. For the three RIL populations, collection of disease reaction data from multiple field environments and greenhouse tests will be initiated and the data compiled for determining inheritance of resistance.

Accomplishment: Approximately 400 interspecific breeding lines derived from crosses of dry bean "ICA Pijao" with P. coccineus, P. costaricensis, and P. polyanthus (synonymous with P. dumosus) were evaluated in the greenhouse in Idaho using the petiole and branch inoculation techniques. Subsequently, approximately 150 breeding lines were evaluated in the greenhouse in Colorado using the straw test and 19 were tested in a replicated trial in the field at Paterson, WA. Thus, 102 interspecific breeding lines were selected for the field and greenhouse testing in Idaho and Colorado in 2005 and 2006. Of these 17 are being tested in Colorado, Idaho, North Dakota, and Washington, and 3 are also in the National White Mold Nursery.

91G x PI255956 BC2F2 population (a cross between a white mold susceptible P. vulgaris cultivar and a resistant P. coccineus accession) was subjected to a straw test in the greenhouse and advanced to the BC2F4. DNA was extracted from 141 individuals selected for pod quality and agronomic traits and grown in the field at the Oregon State Univ. vegetable farm. A straw test of BC2F2 individuals revealed a bimodal distribution for resistance to white mold, where resistance or susceptibility was defined as whether the infection passed through the first node. A

portion of the population was retested with the straw testing the BC2F4 population. Two families scored better than MO162 and similar to G122, a definite improvement over the recurrent parental line's performance. The other families ranged from slightly less resistant to more susceptible than the recurrent parent, providing an excellent range from which to map resistance QTL.

Forty-six accessions of "MO" lines were from the UDSA NPGS collection were subject to a straw test in a greenhouse to screen for white mold resistance. Most were susceptible, but several, including MO048, MO056, MO070, MO169, and MO186 outperformed G122 and MO162. These may provide additional sources of *P. coccineus* derived resistance already in a stabilized *P. vulgaris* background.

Field disease reaction data from two environments were obtained for Benton x VA19 RIL population and three greenhouse straw tests consisting of three replications each were conducted. DNA of this population has been extracted for generation of molecular marker data. Six QTL were identified in the Raven x I93265-31 RIL population. Two QTL, each explaining ~25% of the phenotypic variation, were expressed across five straw tests. Four QTL were associated with field avoidance traits, plant height and plant canopy, in two separate field tests.

PM 1.2.4: Pyramid white mold resistance in dry bean. Partial resistance to white mold exists in a few snap bean, small-seeded Middle American and larger-seeded Andean dry bean, and in interspecific breeding lines derived from the scarlet runner bean. Resistance is quantitatively inherited with >10 QTL distributed across the genome. Breeding methods used thus far, with or without the reliance on molecular markers, have been inadequate and improved breeding lines and cultivars have moderate resistance that may not hold under severe white mold pressure. The use of multiple-parent crosses combined with multi-location field and greenhouse testing and gamete and recurrent selection methods should permit introgression and pyramiding of high levels of resistance from across different germplasm sources.

Baseline: Four multiple-parent populations involving seven white mold resistant genotypes have been developed. Progeny lines using single seed descent, pedigree selection, and other breeding methods have been developed.

Target 2005: Two hundred RSC₀S₀ plants screened in the greenhouse from each of four multiple-parent populations for the recurrent selection cycle 1 (RSC₁S₀) using the petiole and branch inoculation methods. Screen progeny lines for disease reaction in field and greenhouse environments.

Accomplishment: Twenty-five advance lines across four bean market classes (pinto, great northern, pink, and small red) were selected for partial resistance to white mold from a field test conducted in WA. These lines combine partial physiological resistance with disease avoidance traits from multiple sources including AN-37 pinto, G99750 great northern, I9365-25 pink, and G 122 cranberry.

PM 1.2.5: Use marker-assisted selection for dry bean resistance to white mold. There is valid interest in using marker-assisted selection to expedite development of germplasm with

improved resistance to white mold. Marker-assisted selection has been successfully used to augment and increase efficiency of conventional breeding for complex resistance to other bean diseases. The primary focus of this study is to determine if marker-assisted backcrossing can be used effectively to transfer resistance from unadapted sources into pinto bean. Marker-assisted selection for two QTL that derive from different sources, namely G122 and NY6020-4, will be investigated. These two QTL were chosen for marker-assisted selection in this study because they condition physiological resistance, are expressed in the field, have large effects with stable expression across environments, and are tightly linked with sequence characterized amplified region (SCAR) markers.

Baseline: QTL for marker assisted backcrossing have been identified in G122 and NY6020-4 sources of partial white mold resistance, on linkage groups B7 and B8, respectively. Technique requires validation in additional populations.

Target 2005: Disease reaction data from multiple field and greenhouse environments will be obtained for the advanced backcross populations. Success of MAS for both QTL will be determined. Backcross the G122 QTL into bush blue lake (BBL) snap beans using T-phaseolin as a selectable marker. Validate presence of NY6020-4 QTL in resistant BBL snap bean lines. Develop a more complete molecular map for the G122 X Pinto bean RIL population.

Accomplishment: Marker-assisted selection was effectively used to transfer partial resistance to Sclerotinia white mold into pinto bean. The B7 QTL from G122 explained 42% of the variation in the straw test and 17% in the field in a pinto bean background. The B8 QTL explained 35% and 27% in the straw test and 17% and 27% variation in pinto and great northern background, respectively.

Validation completed of the T. phaseolin SCAR marker lined to the resistant QTL located on B7. The B7 QTL was related to improved resistance from data collected in an infected field nursery grown in North Dakota. Mapping of 122 polymorphic molecular markers in the RIL population was completed. Using single factor regression analysis, the B7 QTL accounted for 8.8% and 9.4% of the variation for resistance based on the straw test and field reactions, respectively.

PM 1.2.6: Characterize and transfer resistance from Bunsí into pinto bean and other susceptible market classes. ICA Bunsí (synonymous with Ex Rico 23) from the tropical race Mesoamerica of the Middle American gene pool is a well known source of resistance for enhancing white mold resistance in navy and black beans. There are no previous reports of the exploitation of ICA Bunsí-derived resistance to white mold in pinto bean. We will determine the heritability of ICA Bunsí-derived resistance to white mold in pinto bean, identify new QTL, validate importance of previously identified QTL from this source, and develop the 1st generation of pinto bean lines with partial resistance.

Baseline: Several QTL identified for Bunsí source of resistance.

Target 2005: Identify and verify QTL from ICA Bunsí in a pinto bean background. Determine association of QTL with those found in other populations by integrating linkage maps. Select advanced pinto bean breeding lines with resistance for advanced testing (PM1.2.9).

Accomplishment: A QTL from Bunsí on linkage group B2 was verified in a pinto bean background. Another QTL in the Aztec x ND99106-06 population mapped to B3. The first pinto bean USPT-WM-1 with partial resistance to white mold derived from Bunsí navy bean was officially released in 2005.

PM 1.2.7: Identify candidate genes contributing to resistance. Although QTL have been identified in several dry bean mapping populations little is understood concerning the genes that underlie these QTL. The development of such an understanding may aid breeders in selecting resistance QTL to introgress via marker-assisted selection. In the absence of sequence data in *Phaseolus*, the utilization of a cDNA-AFLP approach is an efficient and cost-effective method to identify candidate resistance genes in dry bean.

Baseline: We have developed two inbred backcross mapping populations with a common recurrent parent (black bean cultivar 'Tacana') to discover novel QTL from unadapted germplasm and identify candidate genes underlying the QTL.

Target 2005: Complete molecular mapping, phenotypic data collection, and QTL analysis on these populations in greenhouse and field trials.

*Genotyped and a preliminary QTL analysis has been conducted on a mapping population generated through backcrossing with wild *Phaseolus vulgaris* accession. One QTL associated with white mold resistance in the greenhouse straw test was identified on common bean linkage group B9 and two additional QTL associated with resistance in the field were identified but have still to be assigned to linkage groups. Phenotypic data has been collected from both the greenhouse straw test and field white mold nurseries on a second mapping population and genotyping of the population is underway.*

PM 1.2.8: Transform beans with Germin oxalate oxidase gene. The wheat germin, *gf-2.8*, gene encodes an oxalate oxidase that breaks down oxalic acid into water and hydrogen peroxide. The potential of dry beans engineered to express the wheat germin gene may provide an opportunity to control the oxalic acid generated by the white mold pathogen upon infection. The pBKSbar/*gf-2.8* transformation plasmid was constructed to contain the wheat germin, *gf-2.8*, which encodes oxalate oxidase and the reporter gene, *bar* which confers tolerance to herbicide glufosinate ammonium. This plasmid was used to transform two white mold susceptible dry bean cultivars Olathe and Matterhorn using an electrotransformation system developed at Michigan State University.

*Baseline: To date, 1250 Matterhorn and Olathe plants have been transformed through the electrotransformation protocol. Six thousand thirty eight T₁ plants were screened for the integration of the bar gene by spraying with the herbicide glufosinate ammonium. The screen produced 104 putative herbicide resistant plants in which the integration of the germin gene (*gf-2.8*) was confirmed in 18 Matterhorn and 11 Olathe T₁ plants through PCR analysis.*

Target 2005: Conduct molecular confirmation of integration of gf-2.8 with Southern and/or Northern Hybridizations on selected T₁ and T₂ plants. Evaluate potential resistance to white mold through Fungal Bioassay, Oxalic Acid Assay and H₂O₂ assay.

Accomplishment: The pBKSbar/gf-2.8 transformation plasmid was constructed to contain the wheat germin, gf-2.8, which encodes oxalate oxidase and the reporter gene, bar which confers tolerance to herbicide glufosinate ammonium. Two transformation approaches, electrotransformation and particle bombardment, were carried out using pBKSbar/gf-2.8 to transform the dry bean cultivars Matterhorn and Olathe. Using the electrotransformation procedure, nineteen different apical meristem pretreatments were tested on the two cultivars. 1160 Matterhorn and Olathe plants have been transformed using this method. Southern hybridization to verify gene integration and oxalic acid and fungal bioassays has yet to be performed.

PM 1.2.9: Determine genotype x environment effects on performance of resistant dry bean germplasm. Useful screening methods are needed to identify sources of resistance in adapted common bean lines. To gain confidence in resistance sources they must be tested at multiple sites located in most of the major bean production areas of the USA. In addition, direct and indirect screening methods will be used to evaluate the disease reaction of the putative resistance sources. Breeders can use identified lines to improve white mold resistance.

Baseline: Tested 12 lines for disease reaction across five states (CO, ID, MN, ND, NE, WA).

Target 2005: Continue multistate testing of germplasm lines developed with improved resistance, and including control lines/cultivars for comparison. Add more lines for preliminary greenhouse screening at multiple sites.

Accomplishment: A white mold resistant snap bean breeding line, Cornell 501, was released. The multi-site test system provided data that verified the putative white mold resistance in the line. Further germplasm releases in various bean seed classes of white mold resistant lines occurred (pinto USPT-WM-1). Substantial progress in developing white mold resistant bean breeding lines has been made: 22 breeding lines in 9 seed classes were entered in the 2005 nursery. Significant differences in pathogen virulence were found between isolates from different geographical regions. Also, each of nine isolates tested from greenhouse/lab screening locations were unique when mycelial compatibility grouping was used to test clonality.

PM 1.3.1: Identify sources of resistance in pea germplasm and wild species. The U.S. World Collection of pea germplasm is maintained at the Western Regional Plant Introduction Station located at Pullman, Washington. That collection includes accessions from throughout the world and also numerous wild species including *Pisum elatius*, *P. humile*, *P. fulvum* and several other species. These accessions represent an untapped resource of genes that have potential for providing defense genes against SWM.

Baseline: Screening of germplasm and breeding lines has been underway with the intermediate goal of identifying resistant or tolerant lines that might be used as parents in a breeding program and also for use in determining the genetics of resistance/tolerance to the disease.

Target 2005: Germplasm lines identified with a useful degree of resistance to SWM and are shown to significantly reduce damage from the disease. These lines will be used in crosses to susceptible parents for the purpose of transferring the resistance to improved varieties and also to develop genetically defined populations that will be used to determine the genetics of resistance. Determining the genetics of resistance will enable the formation of an effective selection strategy for resistance to SWM in peas.

Accomplishment: Partial resistance has been identified in pea and lentil cultivars and breeding lines. The partially resistant lines have been added to the crossing program to transfer the resistance to improved germplasm.

PM 1.3.2: Transfer resistance to improved pea varieties through crossing and selection.

Baseline: Crosses of purportedly resistant germplasm lines with adapted pea germplasm have been made and are being advanced toward homozygosity.

Target 2005: Additional crosses made using more recently identified germplasm sources. Hybrid material screened using inoculations in controlled environmental conditions.

Accomplishment: Breeding lines from crosses of partially resistant germplasm have been established and are being advanced.

PM 1.3.3: Develop mapping populations for inheritance and to genomic analysis of resistance genes in pea.

Baseline: Two genetic mapping populations of recombinant inbred lines (RILs) are being developed in pea. Current plans are to phenotype these RIL populations for reaction to SWM and to use that data in concert with molecular marker data to determine the genetic map positions of the genes for resistance.

Target 2005: Mapping populations (RILs) advanced to the F7 and available for phenotyping for resistance to SWM. RIL populations will have been genotyped for markers from the consensus map of pea and the resulting map from the RIL population used for a quantitative trait loci analysis to locate the regions of the genome where genes for resistance to SWM are located.

Accomplishment: Mapping populations in pea are available for phenotyping.

PM 1.3.4: Use DNA markers for resistance genes in pea for marker-assisted selection.

Baseline: No markers are currently available for use in marker-assisted selection. Mapping of the SWM resistance genes and associated molecular markers will form the basis for candidate markers that may be linked to the resistance genes. Success in mapping the genes for resistance to SWM will determine whether closely linked markers will be identified.

Target 2005: Development of a genetic map that includes the genes for resistance to SWM and closely linked markers. Progress in this area will depend on a critical mapping population that has been phenotyped for reaction to SWM and also where a sufficient number of molecular markers have been mapped. This target will necessarily depend on the success of PM 1.3.1.

Accomplishment: No research in this area is currently underway; however, plans are being made to identify appropriate pea mapping populations among existing material. If none of those populations are considered to be appropriate, new mapping populations will be developed using the germplasm identified under PM 1.3.1.

PM 1.3.5: Introduce resistance or anti-fungal genes in pea germplasm by genetic modification. Of particular interest is the oxalate oxidase gene that reportedly has some effect on reducing damage from *Sclerotinia*. Germplasm shown to have improved resistance to SWM will be released to bona fide plant breeders and registered in Crop Science. The material will also be added to the pea germplasm collection.

*Baseline: While genetic modification holds promise for control of SWM, no attempts have been made toward modifying pea germplasm for any candidate disease resistance genes. Promising fungal resistance genes such as oxalate oxidase may be effective against *Sclerotinia sclerotiorum* and this prospect should be investigated for potential control of SWM.*

Target 2005: Establish protocols and constructs for introduction of the oxalate oxidase gene and other defense related genes into pea germplasm. Develop an inventory of candidate disease resistance genes, promoters, and constructs for eventual transformation into pea germplasm.

Accomplishment: Genetic transformation protocols have been established for peas. Development of appropriate constructs for use in transforming peas for the oxalate oxidase gene is underway. Pathogen isolates have been transformed and tested for virulence.

PM 1.4.1: Identify sources of resistance in lentil germplasm and wild species. The Western Regional Plant Introduction Station located at Pullman, Washington maintains the world collection of lentil germplasm that numbers in excess of 3200 accessions including a substantial number of wild species accessions. Of particular interest is *Lens orientalis* and *L. odemensis*; both species are crossable to the cultivated *L. culinaris*.

Baseline: Screening of U.S. germplasm collection and available breeding lines has been underway with the intermediate goal of identifying resistant or tolerant accessions and breeding lines that might be used as parents in a breeding program designed to develop material resistant or tolerant to SWM. Determination of the genetics of resistance to SWM is an intermediate goal that is needed to formulate an effective screening procedure for identification of resistant germplasm.

Target 2005: Identify germplasm with resistance to SWM using controlled screening procedures. Resistance to SWM was found in lentil germplasm and shown to significantly reduce damage from the disease. These lines will be used in crosses to susceptible parents for the purpose of transferring the resistance to improved varieties and also to develop genetically defined

populations that will be used to determine the genetics of resistance. Determining the genetics of resistance will enable the formation of an effective selection strategy for resistance to SWM in lentils.

Accomplishment: Partial resistance has been identified in lentil cultivars and breeding lines. The identified germplasm has been added to the crossing program and the resulting hybrids have been advanced for resistance evaluations. Screening of the core collection of lentil germplasm is planned.

PM 1.4.2: Transfer resistance to improved lentil varieties through crossing and selection.

Baseline: Crosses between germplasm lines showing resistance to SWM and adapted lentil cultivars of all the major types of lentil have been made and are currently being advanced toward homozygosity in the lentil breeding program.

Target 2005: Additional crosses are being planned as new resistance sources are identified. Hybrid material screened under controlled conditions using mycelium and ascospores of the pathogen as inoculum. Field disease nurseries established for direct screening of progenies under field conditions. Improvement in the procedures for direct screening under field conditions and refinement of the procedures for screening in controlled environments.

Accomplishment: Breeding lines of lentil from crosses using partially resistant lines have been established and are being advanced for further testing.

PM 1.4.3: Develop mapping populations for inheritance of resistance and genomic analysis of resistance genes in lentil. Knowledge of the genetics of resistance to SWM is essential to formulation of effective screening strategies for SWM resistance in lentil. Mapping of the lentil genome and determining the genomic location of the genes for resistance is a necessary first step in understanding the nature of resistance in lentil and will provide information on the number of genes involved in resistance and also their genomic locations. The mapping of the resistance genes will also provide information on closely linked molecular markers that could possibly be used in a marker assisted selection program for SWM resistance in lentil.

Baseline: Several genetically defined populations of recombinant inbred lines (RILs) are under development for use in mapping the genes for resistance to SWM in lentil. The key cross of Pennell, shown to have a high degree of resistance to SWM, and Pardina, shown to be highly susceptible to SWM, is currently in the F₂ and will be further advanced to the F₆. A genetic map based on this F₂ population is currently being developed. Other RIL populations are available for phenotyping; however, those populations are not expected to significantly segregate for SWM resistance. Current plans are to phenotype available RIL populations for reaction to SWM and to use that data in concert with molecular marker data to determine the genetic map positions of the genes for resistance. It is anticipated that a quantitative trait loci analysis (QTL) will be needed to determine the map locations of the genes for resistance in these populations.

Target 2005: Mapping populations (RILs) advanced to the F₄ and available for further advancement to the F₆ for phenotyping for resistance to SWM. RIL populations genotyped for

markers from the consensus map of lentil and the resulting map based on a RIL population segregating for SWM resistance made available for quantitative trait locus analysis to locate the regions of the genome important for resistance to SWM.

Accomplishment: Mapping populations are well along in development and some phenotyping is currently underway. A skeletal genetic linkage map of lentil has been developed. Further population of the map with co-dominant markers (SSRs) and also dominant markers (AFLP, RAPD) is underway.

PM 1.4.4: Use DNA markers for resistance genes in lentil for marker-assisted selection.

Baseline: No markers are currently available for use in marker-assisted selection. The QTL analysis of SWM resistance in the lentil should identify candidate molecular markers for the genes. Success in QTL mapping of SWM resistance will determine where the genes are located and closely linked markers.

Target 2005: Development of a consensus genetic linkage map of lentil that includes the genes for resistance to SWM and closely linked markers. Mapping populations are currently available and are being used to develop a co-dominant molecular marker map of the lentil genome.

Accomplishment: A genetic linkage map of lentil has been developed and is being populated with additional markers. An improved genetic map is needed to be able to identify markers for potential use in marker assisted selection.

PM 1.4.5: Introduce resistance or anti-fungal genes in lentil germplasm by genetic modification. Of particular interest is the oxalate oxidase gene that reportedly has some effect on reducing damage from *Sclerotinia sclerotiorum*. Germplasm shown to have improved resistance to SWM will be released to bona fide plant breeders and registered in Crop Science. The material will also be added to the lentil germplasm collection.

*Baseline: While genetic modification holds promise for control of SWM, no attempts have been made toward modifying lentil germplasm for any candidate disease resistance genes. Promising fungal resistance genes such as oxalate oxidase may be effective against *Sclerotinia sclerotiorum* and this prospect should be investigated for potential control of SWM.*

Target 2005: Establish protocols and constructs for introduction of the oxalate oxidase gene and other defense related genes into lentil germplasm. Develop an inventory of candidate disease resistance genes, promoters, and constructs for eventual transformation into lentil germplasm.

Accomplishment: No work is currently underway in this area. Transformation protocols developed for peas can be adapted for lentil. Gene constructs with the oxalate oxidase gene made for pea transformation will be available for use in lentil.

PM 1.5.1: Identify sources of resistance in chickpea germplasm and wild species.

Baseline: No information is known concerning the genetic control of SWM in chickpea. However, there are substantial germplasm collections that include the wild species progenitors that are available for screening for resistance. Breeding populations are also available that can be evaluated for resistance to SWM.

Targets for 2005: Define the nature of SWM in chickpea and determine the scope for development of resistant germplasm.

Accomplishment: The nature of the disease on chickpea has been defined and includes both foliar and collar rot phases. Inoculation procedures are being developed.

PM 1.5.2: Transfer resistance to chickpea through hybridization and selection.

Baseline: Identification of the collar rot phase of SWM has been made. However, no resistance has been identified or used in crossing programs.

Targets for 2005: Plan for evaluations of chickpea germplasm in Sclerotinia infested nurseries.

Accomplishment: Some screening of chickpea cultivars has been attempted but results have been inconclusive.

PM 1.6.1: Release soybean germplasm and cultivars with resistance to Sclerotinia stem rot.

Differential reaction among soybean cultivars to sclerotinia stem rot exists and has been documented in the literature. There is no documented complete resistance to sclerotinia stem rot in soybean. Crosses among existing cultivars, as well as cultivar x PI crosses have been made to develop populations segregating for level of resistance to sclerotinia. Selections from those populations are being evaluated in both regional field disease nurseries and controlled-environment tests to assay their reaction to the fungus.

Baseline: Advanced lines are being tested in a cooperative field testing program, as well as a cooperative controlled-environment testing program utilizing a cut-stem protocol.

Target 2005: Release at least one germplasm lines or cultivars with an improved level of resistance compared with current cultivars.

Accomplishment: Two convenient greenhouse methods for large scale evaluation of soybean breeding materials for resistance to Sclerotinia stem rot were developed. A new soybean variety, named Skylla, with partial resistance to Sclerotinia stem rot was released. A soybean germplasm, A x N-1-55, with partial resistance to Sclerotinia stem rot was jointly released between the Michigan State University and the Illinois Agricultural Experiment Station.

PM 1.6.2: Combine resistance genes from different sources of Glycine. A total of 6,415 accessions of Maturity Group 0 to IV from the USDA Soybean Germplasm Collection were screened in the field in multiple environments for reaction to sclerotinia stem rot. Results were compared with check cultivars grown in the same tests. No complete resistance was identified, but several PI accessions showed high levels of resistance. If different genes are involved in the

various phenotypic responses, then combining genes from different sources should result in enhanced levels of resistance.

Baseline: Populations that combine genes from different resistance sources are being developed.

Target 2005: Identify lines with greater resistance than the most resistant checks.

Accomplishment: Characterized the infection of a partially resistant and a susceptible cultivar with light and scanning electron microscopy. No differences between the partially resistant and susceptible cultivars were found for the presence of fungal hyphae growth in the plants up to 110 hours post inoculation. However, the infected petioles on the partially resistant genotype dropped off the stem earlier than those on the susceptible, which may be the most plausible mechanism for partial resistance.

In a comparison of six soybean genotypes across eight greenhouse environments that differed for photosynthetically active radiation (PAR), scientists identified some genotypes that had resistance reactions that were sensitive to PAR, while others were not sensitive. Different levels of sensitivity should have different resistant genes.

PM 1.6.3: Use DNA markers for resistance genes in soybean for marker-assisted selection.

Markers associated with resistance to sclerotinia stem rot have been identified in multiple soybean populations, including resistant x susceptible and resistant x resistant and susceptible x susceptible crosses. Potential sources of resistance that have not yet been characterized with regard to DNA markers and identification of QTL are the target of this performance measure to further expand the range of resistance sources.

Baseline: Develop and initiate the testing of populations from crosses between new resistant and susceptible genotypes not yet characterized in soybean.

Target 2005: Identify markers associated with resistance in the new populations. This includes resistance QTL identified from genotypes with an otherwise “susceptible” phenotype.

Accomplishment: Twenty-four primers were initially developed from ESTs of 12 selected genes to evaluate DNA molecular polymorphisms among eight soybean parental cultivars. The molecular markers were used to genotype F4:5 RILs of the Merit x PI194639 population. Although several target region amplification polymorphism markers were mapped to different molecular linkage groups, none were associated with known QTL regions. Additional analysis was implemented to search for significantly expressed genes between genotypes at 18 hour post-inoculation. Development of additional PCR-based primers used for QTL analysis is in progress.

A soybean population developed from a cross between NKs19-90, a partially resistant cultivar, and PI 153282, a partially resistant accession, was tested for resistance in six field locations. Sufficient disease that allowed rating resistance was observed in only two environments. The populations were tested with genetic markers and four QTL controlling resistance were mapped. Three of these QTL were not associated with disease escape mechanisms, suggesting that they

control physiological resistance. In addition, a second population developed by crossing Kottman x PI91589A was developed and tested for resistance through the petiole inoculation method.

PM 1.6.4: Pyramid QTL for resistance genes in soybean germplasm. The resistance QTL reported to date occur on multiple linkage groups, and some are associated with regions containing other fungal resistance genes or resistance gene analogs. Some of the favorable QTL alleles that were identified in different populations were contributed by the susceptible parent, indicating that some of the genes in some more susceptible genotypes may also contribute to enhanced resistance. None of the original parental lines used in development of the recombinant inbred line (RIL) populations for QTL analysis possessed all of the identified favorable QTL alleles for reaction to sclerotinia. Because the RIL populations are from bi-parental crosses, neither do the resulting inbred lines contain favorable QTL alleles from multiple sources. The goal of this work is to combine QTL from multiple sources into single lines to enhance overall resistance.

Baseline: F2-derived F3 lines (F2:3) from four different populations were grown and over 1200 individual F3 plants were screened for 21 microsatellite (SSR) markers to identify genotypes at up to 10 QTL.

Target 2005: Obtain F4:5 lines homozygous for multiple desirable QTL alleles for resistance to Sclerotinia based on marker genotypes. Evaluate the multiple-marker lines, parents, and check cultivars in controlled-environment experiments. Obtain seed increase of lines to move to regional disease nurseries.

*Accomplishment: Crosses were made to combine independent QTL into single soybean lines using SSR primers to mark the QTL regions. Three different populations were developed that combine resistance QTL from different sources. After screening over 4,000 plants through the F2, F3, and F4 generations during 2003 and 2004, currently there are F4-derived soybean lines that are homozygous for the desired marker alleles for the 8 QTL on 7 different linkage groups. A seed increase of the lines was conducted during the 2004-05 winter season and 60 selected F4-derived lines and their parents were planted in May 2005 and grown in replicated tests to evaluate resistance to *S. sclerotiorum*. A detached leaf assay using a mycelium plug was conducted in 12 replications of the recombinant inbred lines and parents and checks to evaluate their reaction to the disease.*

A study to identify QTL for Sclerotinia resistance in soybean was completed. Two major QTL, which were detected from a genetic mapping population derived from the Merit x PI194639 cross, were mapped to linkage groups A2 and B2.

PM 1.6.5: Evaluate transgenic approaches for control of *Sclerotinia sclerotiorum* in crops. In addition to accumulation of endogenous plant genes to enhance Sclerotinia resistance in crops, introduction of specific single genes through genetic transformation may provide complementary and useful approaches for effective control of white mold in soybean and other species.

Baseline: Feasible transformation protocols are currently being used in public labs to stably insert genes of interest into crops genomes using either Agrobacterium or biolistics. Examples of genes that have been transformed into some crops with the intent of providing enhanced control of Sclerotinia diseases include: Perlka-resistance, peptide D4E1, and wheat germin oxalate oxidase. Perlka is a chemical that is inhibitory to Sclerotinia, D4E1 is an antifungal peptide, and wheat germin gene encodes an oxalate oxidase (OxO) that breaks down oxalic acid into water and hydrogen peroxide. All three of these genes have been transformed into soybean and show promising effectiveness. In dry bean, 29 T1 plants that have gone through antibiotic selection and OxO expression has been detected via RT-PCR.

Target 2005: Evaluate Sclerotinia resistance and agronomic traits of current transgenic lines. Know results of yield tests on Perlka-resistant soybean lines and effects of Perlka on germination of Sclerotia and development of apothecia. Conduct controlled-environment and field tests on transgenic soybean plants homozygous for the antifungal peptide D4E1 gene. Conduct molecular confirmation of integration of OxO gene gf-2.8 with Southern and northern hybridizations on selected T₁ and T₂ dry bean plants. Evaluate potential resistance to white mold through fungal bioassay as well as oxalic acid and hydrogen peroxide assays.

Accomplishment: The T1 herbicide-tolerant individuals derived from the transgenic soybean lines carrying the antifungal peptide D4E1 were evaluated from greenhouse-grown plants during the winter of 2003-04 using a detached leaf assay. Plants were harvested from the greenhouse in July 2004. The T2 plants were grown in the greenhouse for generation advance to the T4 generation before the end of May 2005. A field increase of seed of selected lines occurred and phenotypic evaluation for resistance to Sclerotinia stem rot initiated.

Transgenic soybeans that are tolerant of Ca-cyanamide application using the cah gene from Myrothecium verrucaria were developed. Yield evaluation of Perlka-treated plots in two disease and on non-disease environment were conducted.

The pBKSbar/gf-2.8 transformation plasmid was constructed to contain the wheat germin, gf-2.8, which encodes oxalate oxidase and the reporter gene, bar which confers tolerance to herbicide glufosinate ammonium. Two transformation approaches, electrotransformation and particle bombardment, were carried out using pBKSbar/gf-2.8 to transform the dry bean cultivars Matterhorn and Olathe. Using the electrotransformation procedure, nineteen different apical meristem pretreatments were tested on the two cultivars. 1160 Matterhorn and Olathe plants have been transformed using this method. Southern hybridization to verify gene integration and oxalic acid and fungal bioassays has yet to be performed.

PM 1.7.1: Develop inoculation methods for field and greenhouse to assess head rot and stalk rot resistance in sunflower. The occurrence of both head rot and stalk rot is very weather dependent, and the diseases do not occur with enough predictability to allow breeders to make selections every year in any location. To assure accurate evaluations of head rot and stalk rot resistance on adult plants in field nurseries, large quantities of inoculum are needed (mycelium for stalk rot and ascospores for head rot). Additionally, a mist irrigation system is needed to insure that sunflower heads remain wet allowing Sclerotinia infection to occur. Large scale testing for stalk rot (thousands of rows at multiple locations) will necessitate a mechanized

inoculation method. Wild sunflowers, because their growth habit (multiple, small heads; perennials which do not flower the first year) will require a modified inoculation procedure to accurately assess head rot resistance. To permit multiple stalk rot evaluations per year, it will be necessary to develop a greenhouse inoculation method, suitable for both cultivated and wild sunflower.

Baseline: Sclerotinia researchers across the U.S. have each developed different protocols for mass-producing either Sclerotinia mycelium or ascospores.

Target 2005: Adapt methodology to mass produce Sclerotinia mycelium in hundred pound quantities, and construct a mechanized device to facilitate inoculating thousands of rows at multiple test sites. Modify apothecial production method, if necessary, to maximize spore production with sunflower Sclerotinia isolate.

Accomplishment: Sclerotinia mycelium, grown on cooked millet seed, is mass produced for field stalk rot evaluations. In 2005 we produced 300 kg of inoculum, and, using a tractor-mounted inoculator, were able to inoculate 2500 rows at four locations. We have also succeeded in mass producing apothecia and ascospores in the winter months, for use in our head rot nurseries at multiple sites.

PM 1.7.2: Use marker-assisted selection approaches for Sclerotinia resistance in sunflower.

Determination of Sclerotinia resistance to date requires inoculation of adult sunflower plants in field nurseries. Total immunity has not been observed to date, and thus selection is for smaller lesion length or slower disease progress. Viable seeds may not be produced on these “more resistant” plants, and thus an alternative, non-destructive means of identifying partial resistance would be helpful. Another method is to identify genetic markers associated with *Sclerotinia* resistance. This method, once perfected, could be used with seedling plants, and thus resistant individuals could be identified prior to flowering, and susceptible plants discarded.

Baseline: Laboratory assessments using detached stems or petioles are currently used to identify bean and soybean germplasm with Sclerotinia resistance. No molecular markers are currently available in sunflower to identify Sclerotinia resistance.

Target 2005: Initiate field and laboratory work to identify markers in sunflower populations with known Sclerotinia head rot and stalk rot resistance.

Accomplishment: F₂ populations were developed from two hybrids with high levels of head rot resistance, and used to select markers for head rot resistance

PM 1.7.3: Evaluate cultivated sunflower germplasm for head rot and stalk rot resistance.

Public and private sunflower breeding programs in other countries concentrate mainly on head rot resistance and minimally on stalk rot resistance. Public breeding material and commercial hybrids should be evaluated regularly under U.S. environmental conditions to identify germplasm which could enhance existing levels of Sclerotinia resistance. Since Sclerotinia resistance, like yield, is a polygenic trait, it will be more efficient to identify Sclerotinia resistance in cultivated material already selected for high yield.

Baseline: Sources of stalk rot and head rot resistance have been identified in oilseed and confection germplasm, obtained from INRA Sunflower Research Project, France, and the INTA Sunflower Research Project, Argentina. Some U. S. based seed companies have improved the level of Sclerotinia resistance in their experimental hybrids.

Target 2005: Resistant germplasm from foreign research programs will be planted in evaluation and crossing block trials where resistance will be verified against U.S. isolates of Sclerotinia, and lines with resistance will be crossed into U.S. adapted germplasm. U.S. hybrids will be evaluated on an annual basis for their resistance to head rot and stalk rot at multiple locations.

Accomplishment: Seventy-six commercial hybrids were tested for resistance to Sclerotinia stalk rot at five locations in North Dakota and Minnesota using artificial inoculation. Stalk rot severity by hybrid ranged from 10 to 85%, with the four NuSun hybrids equal to or more resistant than the resistant check hybrid. Comparison of head rot (one location) and stalk rot ratings from inoculated trials show that high levels of resistance to both diseases is currently not found in any currently available commercial hybrids.

The inheritance of Sclerotinia head rot resistance in three maintainer and three restorer lines were studied, with General Combining Ability effects accounted for a greater proportion of variance than Specific Combining Ability effects. This indicated that additive gene effects were more important than dominance gene effects controlling resistance to Sclerotinia head rot.

In 2005 the Carrington, ND sunflower head rot screening nursery system was expanded to additional sites in Morden, Manitoba, Brookings, SD, and Langdon, ND. This expansion provided for a broader base of data on disease susceptibility. The objectives are to identify sunflower germplasm with improved stalk rot and head rot resistance. The best entries from the 2005 trials will be evaluated in 2006.

PM 1.7.4: Transfer resistance from cultivated to oilseed and confection sunflower germplasm. The USDA Sunflower Research Unit has had a continuous effort at improving resistance to Sclerotinia stalk rot for over twenty years. Our breeding efforts to incorporate head rot resistance are less than ten years in duration, spurred on by recent epidemics of head rot. While 78% of the sunflowers grown in the U.S. are oilseed types, the confection sunflower industry is much more impacted by Sclerotinia head rot, as the presence of any sclerotia in seed destined for human consumption renders the product unmarketable. Therefore we have added a separate breeding effort to develop confection germplasm with resistance to both Sclerotinia diseases. Germplasm to be released to the public, however, needs to embody not only the sought-after Sclerotinia resistance, but also the required yield, oil, herbicide tolerance and resistance to other diseases and insects that the consumer has come to expect.

Baseline: One oilseed maintainer female line, HA 441, and two oilseed restorer male lines, RHA 439 and RHA 440, were released by USDA-ARS to industry and public researchers to create new Sclerotinia resistant hybrids for the producer. Testcross hybrids utilizing these lines as parents were extremely tolerant to Sclerotinia head rot after artificial inoculation under the mist

irrigation system and under natural conditions of infection in two locations of the U.S. and one location in Argentina.

Target 2005: The genetic resistance contained in the germplasm lines, HA 441, RHA 439, and RHA 440, will be transferred to confection sunflower by crossing with confection sunflower maintainer and restorer lines. The resultant F_1 cross will be backcrossed to the confection lines with desired seed size and seed coat color.

Accomplishment: Large-seeded confection sunflower accessions were crossed with elite oilseed lines having head rot resistance in winter greenhouse, and field diseases evaluations were made in 2005. We anticipate that the initial germplasm with improved head rot resistance will be available for release in the fall of 2006.

PM 1.7.5: Evaluate wild *Helianthus* germplasm for head rot and stalk rot resistance. Over 60 species and subspecies of *Helianthus* exist in North America (primarily the United States). Seeds of many, but not all of these, are available in the USDA's germplasm collection, housed in Ames, IA. Only one species, *H. annuus*, has had any evaluations done for Sclerotinia stalk rot resistance. Tests on the other annual and perennial species have not been made, either in field or greenhouse trials. With such a diverse and untapped source of genes in wild sunflower available, high levels of Sclerotinia resistance may be found equal to that of transgenic resistance being explored in other crops.

*Baseline: Minimal information on stalk rot resistance of some wild *Helianthus annuus* accessions available.*

*Target 2005: Evaluate selected annual and perennial *Helianthus* species in greenhouse trials, and verify in field trials.*

*Accomplishment: Field and greenhouse evaluations of annual and perennial wild *Helianthus* species with artificial inoculation produced peduncle necrosis and mummified heads rather than the typical rot symptoms, which suggests that identification of head rot resistance in wild sunflower will need to be done with F_1 interspecific crosses with cultivated sunflower.*

The growth stage of early flowering was identified as the peak head rot infection period in wild sunflower.

*Eight *Helianthus nuttallii* accessions and six *H. maximiliani* accessions were identified over a three year period as having the highest level of head rot resistance and will be introgressed into cultivated sunflower.*

PM 1.7.6: Transfer resistance from wild *Helianthus* into adapted sunflower germplasm. The genus *Helianthus* consists of diploid, tetraploid and hexaploid species. Crosses between polyploid *Helianthus* species and diploid cultivated sunflower require special techniques. In attempts to transfer a polygenic trait from a wild species, extra effort is needed to retain the majority of the targeted genes during the backcross generations in which the agronomic

phenotype is recaptured. Additionally, released germplasm should contain other agronomically desired traits including high yield, high oleic acid content, and herbicide tolerance among others

Baseline: USDA-ARS released maintainer female line HA 441 is tolerant to head rot and HA 410 is tolerant to stem rot. Wild Helianthus perennial species H. maximiliani and H. nuttallii were shown to be highly resistant to head rot, and stem rot resistance was shown in recently collected perennial accessions of H. schweinitzii, H. californicus, and H. verticillatus.

Target 2005: Further confirm head rot resistance in H. maximiliani and H. nuttallii. Establish interspecific F₁ hybrids between tolerant HA 441 and HA 410 with respective resistant accessions of wild Helianthus species. Evaluate interspecific amphiploids for their resistance to both head and stem rot.

Accomplishment: Interspecific crosses between hexaploid perennial H. californicus and H. schweinitzii using embryo culture were successful in obtaining F₁ plants.

Greenhouse and field screening of F₁ progeny of interspecific crosses of H. californicus and H. schweinitzii showed good tolerance to sclerotinia stem rot. Also, good head rot and stem rot tolerance was observed in an amphiploid of H. nuttallii x P21, with other amphiploids also showing promise.

Strategic Goal 2: Improve the understanding of Sclerotinia sclerotiorum biology and development

Sclerotinia sclerotiorum has an unusually large host range of over 400 plant species in numerous families. The pathogen is found in diverse environments from southern to northern climates and in different agricultural systems under both dry land and irrigated conditions. Although found primarily as a pathogen in the field, it can also be a problem under storage conditions for some crops. The success of this pathogen has demonstrated its ability to adapt to a wide range of conditions. There are many aspects of the biology of this pathogen that are not understood. An improved knowledge of the biology of *S. sclerotiorum* would aid in the development of controls for the numerous diseases caused by this fungus.

PM 2.0.1: Characterize migration/population structure and ecological variability of genotypes. We know there is genetic variability in *S. sclerotiorum* and that clonal and sexual processes are involved, but the true genotype structure of the population within North America is not well characterized. More research in this area on expanded collections of genotypes from a wide variety of economic and wild hosts is necessary. Identifying ecological types within the population will provide an understanding of how disease develops in agro-ecosystems and provide insight into pathogen survival. There is little information on ecotypes associated with certain hosts and agroecosystems. There is still limited knowledge on the virulence range of isolates. Furthermore, certain ecological traits, such as fungicide resistance, are highly important to control of this pathogen.

Baseline: Characterization of genotypes on pea, lentil, bean and soybean have been initiated.

Target 2005: Characterize genotypes on at least one of the crops and initiate studies on clonal groups and evidence for outbreeding.

*Accomplishment: A characterization of the population of *S. sclerotiorum* from lentil fields in the Pacific Northwest has shown a high degree of genetic diversity. Up to 34 mycelial compatibility groups (MCG's) were identified among 37 sclerotial isolates from a single field. A characterization of the pathogen in pea fields using AFLP's also indicated high genetic diversity. These results along with other studies by researchers on populations from the western USA have indicated that the western populations of *S. sclerotiorum* are more genetically diverse, most likely from sexual recombination, compared to the midwestern and eastern US population on canola and soybean which are primarily clonal in most fields and show less diversity. Additional studies with isolates from lentil in the Pacific Northwest have identified double stranded RNA mycoviruses of various sizes within the population. Their role in biology of the pathogen remains to be determined.*

*Nine dry bean isolates of *S. sclerotiorum* from 9 different states were tested for MCG's. Isolates from 4 states were found to be two clones while all the other 5 isolates were unique MCG's, indicating significant genetic variation between isolates from different states. In addition, DNA profiles using microsatellite markers also indicated genetic variation among isolates.*

PM 2.0.2: Characterize virulence/aggressiveness within the population and monitor durability of host resistance. We know there are differences in virulence within the population, but the extent of the variation and how it relates to pathogen genotype and host range is still poorly understood. Variation may also be related to key physiological characteristics which are important to disease development. Identifying certain virulence types would be valuable tools for understanding pathogenesis. In addition, standard methods to describe virulence/aggressiveness in this pathogen are needed. The range of virulence/aggressiveness of collections from different hosts and environments will be tested. There still is a question of whether *S. sclerotiorum* shows some level of host specificity. These studies will require cooperation among groups of scientists. Partial resistance is becoming an important control for Sclerotinia diseases. It is imperative to understand how the variability in virulence/aggressiveness impacts partial resistance. Also, new forms of resistance such as the oxalate oxidase are under development and must be evaluated against a range of virulent types.

Baseline: Limited knowledge on variation in virulence/aggressiveness is available. Some baseline studies on clones have been conducted on certain crops. Partial resistance in certain crops, such as soybean and dry bean, has been identified while in other crops the research is currently in progress.

Target 2005: Collect isolates from widely different geographic areas on a range of crops and wild hosts and characterize isolates.

*Accomplishment: Researchers on soybean, canola, lentils and beans have collected, tested and selected virulent isolates of *S. sclerotiorum* for screening germplasm for resistance. These isolates are currently being used in resistance screening programs. Nine isolates from dry bean*

from nine different states were shown to vary in virulence and there was a significant host genotype x isolate interaction when tested on two partially resistant bean lines.

PM 2.0.3: Identify factors involved in myceliogenic and carpogenic germination of sclerotia. Germination of sclerotia is a critical event in disease development. Although we know that certain environmental factors are involved in the germination process, we do not precisely understand how they work or the interactions of all these factors with host genotype. In addition, we know little about the role of soil microorganisms, other than mycoparasites, in the sclerotiasphere on the germination process. Are microbes involved in the dormancy observed in populations of sclerotia in the field? A better understanding of these factors will aid in the prediction of disease and may identify points in the cycle where germination can be disrupted.

Baseline: Some environmental factors affecting sclerotia germination have been identified and quantified. However, a more precise knowledge of how those factors and their interactions affect germination is needed. The role of most soil microorganisms in germination of sclerotia remains unknown.

Target 2005: Identify other factors besides temperature and moisture that can foster or inhibit germination of sclerotia. Initiate studies to more precisely measure environmental parameters and soil types favoring germination.

Accomplishment: The effects of varying sand and moisture contents of soil on carpogenic germination of sclerotia was investigated. A significant negative linear relationship between sand content and percent germination was detected when soils were at 25% field capacity (FC). However, when soils were at 50% or 100% FC the association between percent germination and sand content was positive. These results indicate the association between soil moisture and carpogenic germination may depend on soil texture. The results have direct implications on predicting apothecia formation for modeling white mold outbreaks.

PM 2.0.4: Develop genetic markers and other types of molecular tools to study the biology of the pathogen. To study many aspects of the biology of this pathogen, we need molecular tools. Genotyping for example, will require an array of molecular tools such as microsattellites, ALFPs and others. New techniques such as SNPs should also be developed. The use of reporter genes such as the green fluorescent will also be useful in ecological studies and in studies on the interactions of plant host and pathogen. These techniques may be extremely useful when studying host resistance. They can also be used for studies on the interactions of *S. sclerotiorum* with other microorganisms.

Baseline: Some genetic markers are available for genotyping but we need to expand the available tools. Research has been initiated on transforming isolates of the pathogen with reporter genes.

Target 2005: Create stable transformed isolates with reporter genes.

Accomplishment: An effective transformation system was developed using Agrobacterium tumefaciens – mediated transformation to generate tagged mutation to identify genetic factors

for virulence of Sclerotinia sclerotiorum from lentil. Factors important to the transformation protocol such as the promoters and cell type were investigated and the protocol improved. More than 130 transformants have been characterized for hygromycin B resistance and for presence of T-DNA, and most of the transformants have been tested for virulence on lentil. Sixteen transformants showed significantly lower levels of virulence, or lost virulence. Additional transformants are still being analyzed. Agrobacterium tumefaciens-mediated transformation was also used with an isolate from dry bean to produce 1,200 random insertional mutants. In addition, 6 targeted gene deletion mutants were also produced with this isolate. Mutants from all these studies are a valuable library of pathogen variability which can be used to study virulence and other traits important to understanding basic biology of the pathogen.

Two isolates of Sclerotinia sclerotiorum (ND21 and ND30) were successfully transformed with the green fluorescent protein gene (gfp) using two different constructs (pCT74 and gGFP) and protoplast-PEG technology. The transformed isolates were shown to be pathogenic on dry bean, canola, soybean, and sunflower. These isolates expressed GFP in vitro and in the tissue of the four crops. The fluorescing hyphae were readily distinguished from plant tissue allowing the process of infection and tissue colonization to be observed. A protocol for the extraction and quantification of the green fluorescent protein from plants infected with GFP transformants was developed.

Strategic Goal 3: Improve the knowledge of Sclerotinia sclerotiorum and host genomics

PM 3.0.1: Accumulate EST sequence information from major crops susceptible to Sclerotinia. Sequence data from host crops is essential to identify common defense mechanisms across hosts. In addition to ESTs, sequence information from non-coding regions of the genome would aid mapping efforts and provide further information on gene regulation by detailing upstream and downstream regulatory regions.

Baseline: Currently there are major sequencing efforts initiated for soybean, canola, and sunflower including the production of over 330,000, 150,000, and 60,000 ESTs, respectively, being funded by a variety of agencies. However, there is limited sequence information from the other major crops susceptible to Sclerotinia: chick pea, common bean, lentil, and pea. Several efforts are focused on soybean genome sequencing (e.g., large BAC clones), as well as improvements to the soybean genetic and physical maps. A whole genome sequencing project for soybean will be initiated in 2006.

Target 2005: Initiate construction of cDNA libraries developed from host tissue at different stages of infection.

Accomplishment: Currently, cDNA libraries from S. sclerotiorum-infected canola and soybean are available, approximately 1,500 EST (primarily representing host genes) have been established from these libraries.

PM 3.0.2: Use transcriptomics to identify candidate genes involved in Sclerotinia resistance. Gene expression underlies much of the physiological change in a tissue in response to pathogen attack. Identifying key genes specific to defense against *Sclerotinia sclerotiorum* as

well as those which allow susceptibility will allow breeders to follow these favorable and unfavorable genes during cultivar development and will present molecular plant biologists with strategies for genetic manipulations that could enhance protection to this pathogen. One of the most powerful means to identify genes involved in any physiology is the use of gene microarrays. These arrays contain thousands of gene representatives within a space small enough to fit on a standard glass microscope slide (1x3 inches) or glass chips less than 1 inch squared. Microarray hybridization analyses will allow one to survey pathogen-challenged tissue for changes in gene expression patterns of thousands of genes simultaneously.

Baseline: Microarrays representing high numbers of genes are currently available for soybean (Affymetrix: 38,000 soybean genes represented; U of Illinois: 36,000 soybean genes represented; USDA: 8,000 soybean genes represented from pathogen-challenged tissues), and canola (Genome Canada: 10,000 unique genes), but need further development for chick pea, common bean, lentil, pea, and sunflower.

Target 2005: Assess the quality of the various microarrays available. Cross compare available array platforms for same species (i.e.: determine if Affymetrix soybean chip produces results similar to that obtained from soybean cDNA microarrays).

Accomplishment: Studies have been initiated to examine gene expression responses to Sclerotinia sclerotiorum infection in soybean and canola. Soybean studies include the comparison of a highly resistant transgenic soybean line 80(30)-1 carrying the OxO gene and its susceptible parental/isogenic line (AC Colibri) as well as susceptible Williams 82 versus PII94639. Two or more replicate hybridizations to soybean cDNA microarrays consisting of approximately 38,000 genes, have been completed with final replicates to be performed during the winter/spring 2006. In canola, gene expression changes associated with Sclerotinia sclerotiorum resistance in two genotypes of were investigated using spotted 70-mer oligo-gene microarray representing 26,000 annotated genes in Arabidopsis, a close relative of canola. A total of 2,587 genes were detected to be differentially expressed at three time points after S. sclerotiorum infection. Fewer genes changed their expression levels significantly at the early time point; however, the number of differentially expressed genes increased over time. The majority of genes were up-regulated in both genotypes. Results indicate that responses to S. sclerotiorum are complex, and may involve multiple signaling pathways. Results support that transcription factors and genes involved in the plant cell wall enforcement play an important role in S. sclerotiorum disease response.

PM 3.0.3: Develop new DNA markers for QTL identification and marker assisted selection.

Genetic resistance is often classified as single gene resistance or resistance controlled by multiple genes [quantitative trait loci (QTL)]. Defense to *Sclerotinia* has been characterized as a quantitative trait in all hosts (i.e.: single gene resistance is not known to exist). Defense controlled by QTLs might not be 100% effective, but because many genes are playing a role in defense, it is much more difficult for the fungus to evade the multiple defense mechanisms. Sequence data from cDNA clones (ESTs) can be used to design gene-specific primers which can be screened for polymorphisms and to map gene locations. In addition, cDNA clones and ESTs may be used as RFLP or AFLP mapping markers. cDNA clones derived from *Sclerotinia*-challenged tissue will provide candidate defense-related genes ideal to screen for polymorphisms

as there is a high likelihood that defense-related genes will be activated or repressed by the pathogen and therefore differentially expressed compared to disease-free plants. Gene expression survey techniques such as microarrays will identify genes differentially expressed in resistant versus susceptible material and has the potential to rapidly identify new molecular markers to assist breeding efforts.

Baseline: Extensive cDNA libraries and EST databases have been established for soybean, canola, and Arabidopsis. Efforts to use cDNA and EST for mapping are being initiated and techniques refined. Microarrays are available for some Sclerotinia hosts such as canola (using Arabidopsis arrays) and soybean.

Target 2005: Conduct expression studies of Sclerotinia infected hosts and develop molecular markers based on EST sequences of differentially expressed genes.

Accomplishment: Based on differential expression after inoculation with Sclerotinia, expressed sequence tags (ESTs) of 24 selected genes from the soybean genome database were employed to design primers, consisting of 24 forward and 24 reverse oligos. Target region amplification polymorphism (TRAP) technique, in which EST-based oligos were used as fixed primers and combined with arbitrary primers labeled with fluorescent dyes (6-FAM, HEX, and NED) was employed. The markers detected in parental screening were utilized to genotype 155 F4:5 RILs of the Merit x P1194639 population. QTL analysis indicated that although no TRAP markers were mapped to previously identified QTL, several markers were mapped to new genomic regions in MLG L, E and B1.

PM 3.0.4: Identify mechanisms/genes of resistance in the model plant Arabidopsis using high throughput genome-scale screens. Genetic and molecular tools are most proficient for the model plant *Arabidopsis thaliana*. *S. sclerotiorum* causes lethal rot on all ecotypes tested to date. However, sensitivity to the virulence factor oxalate varies and the rate of disease progress may differ among ecotypes. Traits related to *Sclerotinia* infection can be used effectively to identify and characterize genes that confer partial resistance. *Arabidopsis* offers additional resources that are related to resistance or defense genes and an easy genetic system to functionally validate the roles of any given gene. *Arabidopsis* genetics can provide a means to identify genes that mediate oxalate toxicity and thus susceptibility to the pathogen.

Baseline: Very limited screening of Arabidopsis has been done thus far.

Target 2005: Develop effective inoculation and disease assay methods.

*Accomplishment: 28,000 EMS Arabidopsis mutants have been screened for oxalate sensitivity. More than 90 mutants have been subjected to secondary screening and two lines performed differently to parental ecotype Columbia in terms of oxalate sensitivity or susceptibility to oxalate-deficient *S. sclerotiorum*. Ten ecotypes have been screened for oxalate sensitivity. Ecotypes Landsberg and Columbia differ in oxalate sensitivity and susceptibility to oxalate-deficient *S. sclerotiorum*.*

PM 3.0.5: Target candidate genes by RNAi to screen for susceptibility and resistance.

Universal mechanisms exist in organisms to inactivate target genes using interfering RNA molecules. These methods are collectively termed RNAi. Applicability of RNAi approaches in several plant species has been documented, as has the use of RNAi to target vital genes of pathogens. Plant transformation procedures are becoming more standardized and are more widely available.

Baseline: Efficient transformation is not currently available for all hosts. Several plant-based RNAi cloning vectors are commercially or publicly available. Promising reports have appeared on the use of viral induced gene silencing in soybean (VIGS).

Target 2005: Test existing RNAi systems currently being used in plants.

Accomplishment: Proposals related to RNAi have not been awarded by NSI.

PM 3.0.6: Develop EST libraries and microarrays from differing physiological, pathological, and developmental stages of the pathogen. To rapidly screen pathogen genes for importance in disease, the sequence identity of fungal transcripts needs to be available. Expressed sequence tags (ESTs) are the most effective means of collecting gene sequence information as this method depends on the selection of the RNA transcripts, thereby selecting only actively transcribed regions of the genome. To obtain a collection of genes related to the pathogen's interaction with the host, one needs to collect transcripts from infected tissue in addition to fungal cultures. Development of microarrays from *Sclerotinia* would allow for microarray studies of pathogen gene expression during pathogen attack and will provide further clues as to key factors for pathogenicity and defense. Other techniques that survey gene expression, such as RT-PCR and analysis of EST sequences, will also be useful in this endeavor.

Baseline: A publicly-funded Sclerotinia sclerotiorum whole genome sequencing project has been initiated with the goal of producing a draft sequence of the nuclear and mitochondrial genomes at an 8X sequence depth and to provide a computer-based annotation of the coding sequences within the genome. This project is expected to be completed by fall 2005. Although gene discovery in Sclerotinia will be achieved at an unprecedented rate through this whole genome sequencing approach, the computer-based gene identification and annotation methods require significant amounts of sequence data from transcribed genes to achieve high confidence in the annotation. A number of cDNA libraries and ESTs have also been established by the scientific community at large from a variety of physiological and developmental stages of Sclerotinia. These include (i) sclerotial initials (2,000 ESTs), (ii) polygalacturonic acid-grown mycelia (1,500 ESTs), (iii) low pH vegetative mycelia (iv) agar grown mycelia (900 ESTs), and (v) infection cushions (800 ESTs). As a component of the full genome sequencing project for S. sclerotiorum, we anticipate the EST resource will grow significantly in the near future.

Target 2005: Complete whole genome draft sequence of Sclerotinia sclerotiorum with an 8X sequence depth and provide a computer-based annotation of the coding sequences. Initiate construction of cDNA libraries from fungal tissue grown in culture under different growth conditions and stresses.

Accomplishment: Two new cDNA libraries were constructed from wild type S. sclerotiorum isolate '1980'. The first library ('mycleia_pH7') was from vegetative mycelia that had been transferred from acid pH to neutral pH conditions and cultured for 4 hours at neutral pH. The second ('stipe_55h') was from stipe tissue undergoing morphological transition from a dark-etiolated stage to developing-apothecial disc stage. 5,345 independent clones were single pass sequenced from the 'mycleia_pH7' library, and 5,056 clones from the 'stipe_55h' library. A 90% pass rate occurred with 1.3% vector-only occurrence with the first library and an 80% pass rate occurred with 0.9% vector-only occurrence with the second library resulting in 4,728 and 4,031 usable sequence reads, respectively.

PM 3.0.7: Develop random tagged mutagenesis and targeted gene inactivation techniques to identify genes required for pathogenicity and virulence.

Baseline: Agrobacterium tumefaciens mediated transformation (ATMT) has been developed as a reliable method for transforming fungi. This techniques offers an opportunity to create mutations in Sclerotinia that affect pathogenicity and identify the gene responsible for the trait. Several labs have unpublished reports of transforming Sclerotinia with Agrobacterium. Targeted gene disruption and RNAi methods of gene inactivation have been reported in the literature.

Target 2005: Develop ATMT for routine insertional mutagenesis and tagged-gene recovery.

Accomplishment: 135 ATMT have been characterized for hygromycin B resistance and for presence of T-DNA, and most of the transformants have been tested for virulence on lentil. Sixteen transformants showed lower levels of virulence. One transformant was identified with significantly reduced virulence and normal growth and sclerotial development.

PM 3.0.8: Develop bioinformatics resources to provide genomic information to the Sclerotinia community. Genetic information is being generated at a very rapid pace. The amount of data is so great that the traditional journal-based system for accessing this data is becoming ineffective, especially in providing plant breeders and fungal biologists with easy, logical access to sequence and genomic data in a format that they can readily assimilate.

Baseline: The Sclerotinia Genome Sequence is freely available in a user-friendly format on the web-site of the BROAD Institute (www.broad.mit.edu/annotation/fungi/sclerotinia_sclerotiorum/).

Target 2005: Provide links from the Sclerotinia Initiative home page to host and pathogen genome databases.

Accomplishment: Links have been established from the Sclerotinia Initiative home page to host and pathogen databases. A genetic screen for oxalate sensitivity in yeast was successful. A total of 105 genes were discovered which confer oxalate sensitivity in yeast. Genes from Arabidopsis and Sclerotinia that are homologous to these yeast genes have been identified and this information will be available upon request.

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

PM 4.0.1: Optimize fungicide application programs. Efforts will be concentrated in identification of fungicides (or mixes of), rates, timing and adjuvants that provide best management of the disease in the different crops that conform to the Sclerotinia Initiative Program.

Base line: Chemical management is not optimized. Field studies have been conducted mostly for canola and dry beans, but not for pulse crops or sunflower. Sensitivity baselines for benzimidazoles and newer fungicides (e.g., strobilurins) do not exist.

Target 2005: Optimize fungicide application programs for sunflower head rot and pulse crops. Develop fungicide application guidelines for disease management on pulse crops and sunflower headrot and update those for dry bean and canola. Create a region-wide collection of S. sclerotiorum isolates and use them to establish baseline of fungicide sensitivity.

Accomplishment: Topsin M, Endura, and Bayer Experimental JAU 6476 fungicides were evaluated for their efficacy on field pea for control of white mold disease on two cultivars with differing flowering durations at application timings of 10, 40, 100 and 10 + 100% flowering stage of growth at two locations in North Dakota. Significant disease differences were noted among cultivars and fungicides in some years. Bayer experimental fungicide JAU 6476 reduced Sclerotinia incidence and increased yield more than Endura or Topsin M.

Fungicide treatments were evaluated on sunflower at Carrington, ND, Langdon, ND, Fargo, ND, Morden, Manitoba, and Brookings, SD. A core set of treatments was developed based upon labeled products and results of Sclerotinia trials on other crops. Investigators at some sites included additional treatments to study the efficacy of other products and rates. Yield increases of up to 50% were observed when plots were protected with fungicides compared to unprotected plots. Adjuvant additions to the spray solutions appeared to show promise for improved fungicide efficacy and yield response.

Experimental plots were established in 12 canola fields across 6 North Dakota counties (2 fields per county) to evaluate the effectiveness of fungicide applications for Sclerotinia stem rot control. Disease incidence ranged from 12 to 68% for fungicide-protected plots with a mean of 29%, and from 14 to 76% for untreated plots with a mean of 41%. Fungicide-protected plots were treated with thiophanate methyl at a rate of 1 lb/A.

Field surveys were conducted in 60 canola fields across 6 North Dakota counties. Sclerotinia stem rot incidence was estimated and a collection of pathogen isolates made. These isolates will be used in future studies on fungicide sensitivity tests, pathogenicity tests, etc.

PM 4.0.2: Optimize fungicide delivery systems. Activities will be concentrated in improving penetration of chemicals into the canopy of crops and to ensure effective coverage of the canopies of canola, dry bean, pulse crops, sunflower, and soybean.

Base line: Use of low volumes of water that may not ensure adequate canopy coverage is common among growers. Dry bean growers have abandoned the use of dropped nozzles, which

enhance canopy penetration on ground applications; such technology is not in use on other crops. In many instances growers resort to aerial applications, which use even lower volumes of water, making canopy penetration even more difficult; or chemigation applications with higher volumes of water, which can lead to fungicide dilution and runoff.

Target 2005: Initiate studies to optimize the volume of water to be used as carrier in aerial, ground and chemigation applications for all crops, as well as cultural practices that may have an impact on fungicide penetration into the canopy. Evaluate new spraying technologies that enhance canopy penetration, as they become available, like electrostatic and air-assisted blast for better canopy penetration.

Accomplishment: A series of dry bean lab and greenhouse experiments demonstrated that the conventional fungicide, Topsin, applied in 5 to 250 of water per acre, provided very good control (84 to 96%) of white mold, even after 5 days of incubation. Optimum volumes for ground applications were around 25 gal per acre, while the higher level referred to chemigation. A newer fungicide, Endura, provided less control (39 to 93%) in this series of experiments with different spray volumes, but still offers a lot of potential for enhance fungicide management in dry bean and other susceptible crops.

PM 4.0.3: Develop bio-control alternatives for disease management. Initial activities will focus in the evaluation of already available commercial bio-control agents, like *Coniothyrium minitans*. Additional surveys and screening exercises will be conducted to identify new antagonists of *S. sclerotiorum*. Studies will include identification of optimal concentrations, tank mix compatibility, and synergy with other microorganisms.

Base line: Studies conducted to evaluate efficacy of sclerotial parasites have been inconclusive due to low and inconsistent disease pressure. A field survey evaluated the distribution and prevalence of fungal sclerotial antagonists. Information on impact of cultural practices on biocontrol activity and longevity in the soil is lacking. Guidelines for growers on the use of biocontrol agents for disease management are not available.

*Target 2005: Optimize use of commercially available biocontrol agents that antagonize sclerotia of *S. sclerotiorum* (e.g., dose, time of application, tank mix compatibility). Produce recommendations for growers on the use of commercially available sclerotial antagonists as control agents. Evaluate other commercially available microorganisms for their potential as biocontrol agents and possible synergy with different agents.*

*Accomplishment: Research completed in 2005 demonstrated that the strategic use of *Coniothyrium minitans* in canola produces comparable results to that of chemical fungicides applied at flowering time. Trials in snap beans showed that *C. minitans* could be an effective tool to reduce populations of sclerotia of *S. sclerotiorum* in fields in addition to reducing disease incidence. Another study showed that residual biological control activity by *C. minitans* in soils with high alkaline pH was at detectable levels even two years after a single application of *C. minitans* into soil.*

PM 4.0.4: Develop non-fungicidal chemical alternatives for disease management. A concerted effort will be dedicated to screen and identify compounds that can boost plant defense mechanisms, like harpin proteins, lactofen, and related compounds. The economics of the use of such compounds will be evaluated. The information generated will be presented to growers in extension bulletins, newsletters, and web sites.

Base line: New chemistries that operate at biochemical levels in plants are being developed. Few if any of them has been tested for S. sclerotiorum disease management on canola, dry bean, pulse crops or sunflower.

Target 2005: Screen commercially available non-fungicide compounds with plant protection properties on all crops. Optimize use of any identified compound to be applied alone or in combination with other chemical for disease control.

Accomplishment:

PM 4.0.5: Develop quantitative models that describe the role of weather variables on epidemic development. Research activities will be oriented towards the development of disease warning systems. Since the epidemiology of diseases caused by *S. sclerotiorum* in canola, dry bean, sunflower, soybean, and pulse crops is similar, most research will be conducted with the canola model.

Base line: Greenhouse experiments that evaluate the effect of constant temperature and continuous relative humidity conditions have been conducted on dry beans, but may have applications in other host crops. However, this information has not been incorporated into predictive models for other crops. Spatial distribution of epidemics in fields is not fully understood.

Target 2005: Elucidate the impact of interrupted leaf wetness periods on the onset of S. sclerotiorum epiphytotics. Initiate studies to characterize spatial attributes of S. sclerotiorum epiphytotics. Conduct field studies to elucidate the impact of rain, temperature, inoculum density and solar radiation on disease development and develop preliminary models.

Accomplishment: It was demonstrated that interrupted leaf wetness periods have a significant impact on disease development. Eight-hour intervals of dry and wet periods resulted in significant reduction in disease incidence compared to longer intervals, but even the combination of shortest wet (8 hours) and longest dry (24 hours) periods could not stop disease development completely. This information will be included in the development of algorithms for disease forecasting. Field studies in canola indicated that a 50% decrease in disease incidence occurs within 40 meters from the source of inoculum. This is an indication that 40 m could be used as an initial threshold for effective inoculum spread in canola fields. A significant interaction between soil texture and moisture was identified in relation to apothecia formation. In this study it was demonstrated that apothecia could be produced even when soils moisture contents were kept at 25% of their field capacity. The optimum moisture level for all soil textures tested in the study was 50% of field capacity. This information will have a direct application in models that predict apothecia formation.

PM 4.0.6: Develop quantitative models that describe the relationship between *S. sclerotiorum* diseases and yield for sunflower, canola, dry bean, and pulse crops. Yield loss is the ultimate reason why *S. sclerotiorum* is considered one of the most important limiting factors for production of canola, dry bean, sunflower, soybean, and pulse crops. However, the association between the disease and loss of yield and/or of quality of the final product has not been studied for all crops. This information will help growers as well as scientists to fine tune economical damage thresholds.

Base line: Models are available for dry bean and soybean but not for canola, sunflower or pulse crops. These models relate disease incidence to yield loss, but do not address impact of inoculum concentration and/or timing of inoculation on yield.

Target 2005: Determine effect of time of infection and inoculum concentration on yield and quality loss in canola, confection- and oilseed-sunflower, and pulses. Develop yield loss models based on data obtained.

Accomplishment: Field studies conducted in canola had to be abandoned to due hailstorm damage and poor plant emergency in two locations in 2005. Data analysis for other crops is still underway.

PM 4.0.7: Optimize cultural practices for disease management. The impact of common cultural practices on disease development will be evaluated through field experiments emphasizing selection of crops to be used in rotation schemes, variety/hybrid selection, planting patterns, and planting dates. Results of such experiments will be made available for growers through extension bulletins, newsletters, and websites.

Base line: Practices that promote higher yields, like high planting densities, narrow row plantings, and high fertilization levels also promote disease development on most crops. Impact of type of crop used in rotations is not known, but on-going studies are addressing this topic. Complete genetic resistance is not commercially available in any crop, but there may be differences in levels of genetic resistance, and/or plant tolerance architecture avoidance.

Target 2005: Characterize the impact of type of crop used in rotation on disease development. Characterize cultivars of all crops for yield, disease development, and the amount of sclerotia produced as selection criteria. Validate importance of planting dates and planting densities on different crops for their relation to disease development. Produce guidelines for growers.

Accomplishment: A field study involving 16 cropping-sequence treatments to better understand the effect of crop rotation and cover crop management on white mold development in canola was conducted (3rd year). Results indicate that planting sunflower every other year resulted in increased levels of Sclerotinia wilt compared to planting them in longer rotations. Planting wheat or sorghum for at least one season in the rotation contributed to reduce disease pressure. Tilling the ground reduced Sclerotinia wilt by almost 66% compared to no-till conditions. Ascospore and apothecial presence were recorded during the season, but data have not been analyzed yet. Use of rye as a cover crop to induce apothecia formation at a time when no canola

plant is flowering, reduced canola yields by an average of 13%, and did not affect disease incidence.

PM 4.0.8: Optimize disease management at field level by combining IPM practices with precision agriculture technologies. Integrating information generated by the above mentioned activities would optimize disease management practices. The use of precision agriculture technology would allow growers to only treat hot spots with fungicides instead of treating entire fields.

Base line: Precision agriculture technologies are not used to aid in the design and execution of Sclerotinia disease control programs.

Target 2005: Generate epidemiological information on disease development (spatial distribution, soil sampling to enumerate sclerotial populations, air sampling and enumeration of ascospores, remote sensing of infected plants) that could be used to support a precision agriculture program for canola, sunflower, dry bean, soybean, and pulse crops.

Accomplishment: Epidemiological data is being generated and highlights have been included in PM 4.0.5. Indices of risk to be used as help in decision making by growers of canola, dry bean, and sunflower have been included in the Sclerotinia Initiative website. These indices are based on current knowledge of epidemiology and cultural practices that influence disease onset and severity.