United States Department of Agriculture

Research, Education & Economics

Agricultural Research Service

National Program Staff

January, 2006

Version 1.0

National Program Strategic Plan for the Sclerotinia Research Initiative

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

Table of Contents

Strategic Goals, Performance Measures & Milestones

Executive Summary

Vision Statement: An integrated research approach is needed to guide the effective development of diagnostic technologies, disease management systems, genomic resources, and soybean germplasm exhibiting durable resistance to Asian soybean rust. Strategic deployment and use of these resources will help ensure the competitiveness of U.S. soybean producers in domestic and global markets.

Process & Development of the Sclerotinia Initiative: On January 20-22, 2004, approximately 80 scientists and related stakeholders with knowledge of the fungal disease, *Sclerotinia sclerotiorum* (white mold) participated in an annual meeting/workshop hosted by the United States Department of Agriculture's Agricultural Research Service (ARS) in Minneapolis, MN. ARS, the National Sunflower Association, the U.S. Canola Association, the USA Dry Pea and Lentil Council, the U.S. Dry Bean Council, and the American Soybean Association co-organized this program. The participants reviewed the current status of research targeted at improved understanding and management of white mold in canola, dry edible beans, peas & lentils, soybean, and sunflower. A strategic plan was developed that outlined four strategic goals with related overarching research objectives. This plan provided program and scientific focus to ensure that the *Sclerotinia sclerotiorum* research community working in canola, dry edible beans, peas & lentils, soybean & sunflower under the auspices of ARS and the National Sclerotinia Research Initiative attained planned results in an effective & timely manner.

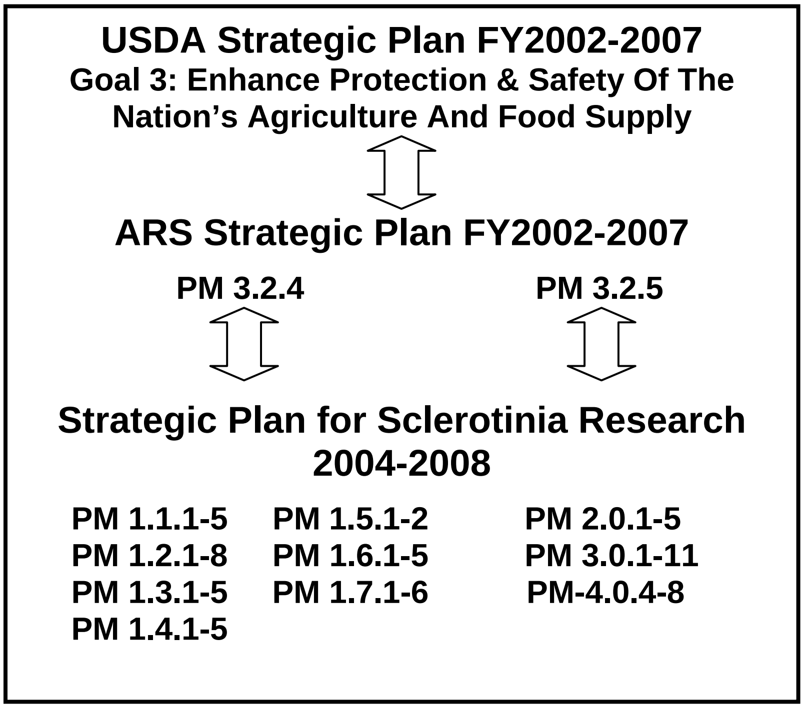
STRATEGIC GOALS OF THE SCLEROTINIA RESEARCH INITIATIVE

1. Develop novel germplasm and varieties with field resistance to *Sclerotinia sclerotiorum.*
2. Improve understanding of *Sclerotinia sclerotiorum* biology and development.
3. Improve knowledge of *Sclerotinia sclerotiorum* and host genomics.
4. Broaden knowledge of *Sclerotinia sclerotiorum* epidemiology & improve disease management strategies.

These strategic goals encompass the breadth of research disciplines necessary to better understand the disease and to provide significant management options for the affected producers across the U.S. To achieve these strategic goals and research objectives, this plan emphasizes achievements that hinge on teamwork throughout the Sclerotinia research community. All actions and results will be attained in a manner that is both inclusive and open to public scrutiny as this Initiative is implemented.

Relation of Performance Measures to the ARS and USDA Strategic Plans: Expected outputs of the *National Strategic Plan for the Sclerotinia Research Initiative* were developed in support of "Actionable Strategies" associated with performance measures 3.2.4 and 3.2.5 of the 2003-2007 *ARS Strategic Plan, under* Objective 3.2: *Develop and Deliver Science-Based Information and Technologies to Reduce the Number and Severity of Agricultural Pest, Insect, Weed, and Disease Outbreaks.*

Performance Measure 3.2.4: Develop and release to potential users varieties and/or germplasm of agriculturally important plants that are new or provide significantly improved (either through traditional breeding or biotechnology) characteristics enhancing pest or disease resistance.



USDA Strategic Plan FY2002-2007

Goal 3: Enhance Protection & Safety Of The Nation's Agriculture And Food Supply

ARS Strategic Plan FY2002-2007

PM 3.2.4 **PM 3.2.5**

Strategic Plan for Sclerotinia Research 2004-2008

PM 1.1.1-5 **PM 1.5.1-2** **PM 2.0.1-5**
PM 1.2.1-8 **PM 1.6.1-5** **PM 3.0.1-11**
PM 1.3.1-5 **PM 1.7.1-6** **PM-4.0.4-8**
PM 1.4.1-5

Performance Measure 3.2.5: Provide fundamental and applied information and technology to protect agriculturally important plants from pests and diseases.

Introduction:

Sclerotinia sclerotiorum is a plant pathogenic fungus that causes important diseases known as white mold, Sclerotinia stem rot, wilt or stalk rot, or Sclerotinia head rot on a wide variety of broadleaf crops. It is commonly found damaging dry edible beans, sunflowers, soybean, canola, peas, and lentils. There are many other crops that are susceptible such as alfalfa, potato, peanut, mustard, safflower, flax, borage, crambe, buckwheat, chickpea, lupine, faba bean and numerous vegetables such as lettuce and carrots. Some of these crops are rarely damaged by the fungus, while others are quite susceptible. This pathogen is known to infect about 400 species of plants. Numerous weeds such as marsh elder, lambsquarters, pigweed, Canada thistle, sow thistle, and wild mustard are also hosts and can play a role in disease cycles.

Sclerotinia causes serious economic loss by negatively impacting crop quality and yields. The collective annual losses for the five crops participating in the ARS National Sclerotinia Research Initiative have been as high as $482 million. Specifically, annual losses for each of the crops have been as high as $100 million for sunflowers; $300 million for soybean; $46 for dry edible beans; $24 million for canola; and $12 million for pulse crops. The disease is a serious threat to the future of the confection sunflower, where quality is a significant concern. Diseased seeds cannot always be separated in cleaning and processing resulting in bitter tasting seeds which are rejected by consumers.

The primary survival (overwintering) structure of *S. sclerotiorum* is the sclerotium. A sclerotium is a hard resting structure consisting of a light colored interior portion called a medulla and an exterior black protective covering called the rind. The rind contains melanin pigments which are highly resistant to degradation, while the medulla consists of fungal cells rich in beta glucans and proteins. The shape and size of sclerotia depend on the host and where they are produced in or on infected plants.

The Sclerotinia disease cycle begins when sclerotia are germinate after overwintering in soil. Sclerotia may undergo carpogenic germination which results in the production of a small mushroom called an apothecium and ascospores which are ejected into the environment. The pathogen produces oxalic acid and numerous enzymes that break down and degrade plant tissue. Disease development is favored by moisture and moderate temperatures of 15 to 25 C.

Another method of germination is myceliogenic, where sclerotium produce mycelium. This is common in the disease cycle in Sclerotinia wilt of sunflower. Most other Sclerotinia or white mold diseases of dry edible beans, soybean, canola and sunflower head rot are initiated by carpogenic germination and infection of above ground plant parts by ascospores. The primary methods for field infestation are: 1) incoming crops or weeds infected by ascospores or sclerotia from adjacent infested fields; 2) via surface irrigation water or rain water; and 3) contaminated seed.

Few studies have quantified sclerotia survival in the field. Microbial degradation is the principal reason for a decline in populations of sclerotia. Many fungi, bacteria and other soil organisms parasitize or utilize sclerotia as carbon sources. Crop rotations allow the natural microbial population to degrade sclerotia. Two important fungal parasites involved in the natural degradation of sclerotia are *Coniothyrium minitans* and *Sporidesmium sclerotivorum.* Both may become biocontrol agents for sclerotia. The effect of tillage on survival of sclerotia is poorly understood.

Fungicides have been used with some success in dry edible bean and canola. Crop rotation continues to be used for certain crops such as sunflower where inoculum densities in the soil play a major role in disease development. Host resistance has been an elusive goal of many control programs. Most Sclerotinia diseases are not controlled by host resistance. However, moderate levels of host resistance in dry edible beans and soybean have been used in integrated control programs.

The *Strategic Plan for the Peanut Genomics 2004-2008* provides the peanut research community with a foundation for a comprehensive and integrated research approach toward this problem. Performance measures that have need for immediate implementation are documented in this Action Plan. In accord with the *President's Management Agenda*, this plan also defines the actions that will be taken to solve the problem, describes what is promised or will be produced, assigns accountability for the work to be accomplished, and provides a mechanism for peer review and assessment of research progress.

Strategic Plan for Sclerotinia Research

Crop Germplasm Resources & Genetics

Index – (Goal#).(Commodity#) where, 1= canola; 2 = common bean; 3 = pea; 4 = lentil; 5 = chickpea; 6 = soybean; 7 = sunflower; 0 = all

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

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*, *B. carinata*, 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, B. carinata, and B. juncea U.S. collections have not been fully evaluated.

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

Target 2007: Confirm potential resistant sources in field and controlled studies.

Target 2009: Initiate evaluations of B. carinata and B. juncea collections.

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.

Target 2007: Validate developed screening method.

Target 2009: Continue validation and improvement of screening methods.

PM 1.1.3: Develop canola germplasm with improved 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: Doubled haploid lines with resistance to S. sclerotiorum are not available.

Target 2005: Develop and test doubled haploid lines for resistance.

Target 2007: Identify lines with best resistance and test in multiple environments.

Target 2009: Continue field and greenhouse evaluations of lines, concentrating on those showing the highest levels of resistance.

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.

Target 2007: Identify QTLs conferring resistance and markers linked to the QTLs.

Target 2009: Continue genetic mapping and identification of QTLs.

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.

Target 2007: This is beyond 2007.

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 F_2 *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.*

Target 2007: Complete mapping in PI 255956 x Wolven Pole population. Validate QTLs identified in F_2 *population in interspecific backcross-inbred populations.*

Target 2009: *Begin determination of QTLs as they relate to the physiological basis of resistance.*

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.

Target 2007: Complete development of breeding lines and inbred backcross lines derived from interspecific crosses of common and runner beans. Complete mapping studies of recombinant inbred and backcross inbred populations. Use QTL in selection of the most resistant individuals with acceptable agronomic characteristics during inbreeding process and advance to BC_2F_6 *using winter nurseries. Cross best sources of resistance to additional market classes*

Target 2009: Complete partial screening for white mold resistance of breeding lines and inbred backcross lines derived from interspecific crosses of common and runner beans. Release QTL assisted selection germplasm with high levels of resistance and acceptable agronomic performance.

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.

Target 2007: Submit a few additional interspecific breeding lines for advanced testing in the National White Mold Nursery (PM 1.2.9). For the RIL populations, collection of disease reaction data will be completed. Molecular markers will be generated and genetic linkage maps constructed for performance of QTL analysis. QTL will be identified and compared across populations.

Target 2009: Release white mold resistant interspecific breeding lines and initiate crosses to study inheritance and map QTL. For the three RIL populations, QTL conditioning resistance with major effect and stable expression across environments will be targeted for marker-assisted selection.

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 RSC_0S_0 *plants screened in the greenhouse from each of four multiple-parent populations for the recurrent selection cycle 1 (*RSC_1S_0*) using the petiole and branch inoculation methods. Screen progeny lines for disease reaction in field and greenhouse environments.*

Target 2007: A few highly white mold resistant breeding lines from the recurrent selection cycle 1 are available for further testing. Also, the recurrent selection cycle 2 has been initiated. Advanced lines from other selection programs evaluated for field reaction to white mold.

Target 2009: A few highly white mold resistant breeding lines from the recurrent selection cycle 1 available for advanced testing in the National White Mold Nursery (PM 1.2.9). Some resistant breeding lines may be released to the public for breeding and genetic research. Also, a new set of highly white mold resistant breeding lines from the recurrent selection cycle 2 may be available for multi-location *field and greenhouse testing. A few advanced lines from other selection programs evaluated for field reaction to white mold.*

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.

Target 2007: New populations will be generated to study the potential of MAS for combining (pyramiding) QTL to achieve higher levels of resistance in pinto bean and snap bean. Complete three backcrosses of the G122 QTL into BBL lines. Begin pyramiding the two QTL into pinto and BBL materials.

Target 2009: Disease reaction date from multiple field environments and greenhouse tests will be obtained for advanced RIL populations possessing different combinations of QTL. Formulate strategies for effective use of MAS to increase resistance levels via gene (QTL) pyramiding. Complete pyramiding project in BBL lines; test in greenhouse and multiple field environments to determine the economic value of each QTL separately and in combination. Begin pyramiding additional QTL from P. coccineus identified in PM 1.2.1 and PM 1.2.2 and Bunsi identified in PM 1.2.6.

PM 1.2.6: Characterize and transfer resistance from Bunsi into pinto bean and other susceptible market classes. ICA Bunsi (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 Bunsi-derived resistance to white mold in pinto bean. We will determine the heritability of ICA Bunsi-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 Bunsi source of resistance.

Target 2005: Identify and verify QTL from ICA Bunsi 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).

Target 2007: Develop MAS capability for the most important QTL derived from ICA Bunsi. Release the most resistant lines with the best agronomic attributes (high yield, adequate seed size, etc.) to breeding programs nationwide for use in improving resistance of pinto bean and related market classes (great northern, pink, small red)

Target 2009: Initiate transfer of the ICA-Bunsi derived resistance into other susceptible market classes.

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.

Target 2007: Complete cDNA-AFLP analysis to identify candidate genes in each population. Identify homologus genes in other species, and map all possible candidates.

Target 2009: Develop markers to aid in marker-assisted selection of most valuable QTLs and underlying candidate genes into susceptible bean germplasm.

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 T_1 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 T_1 plants through PCR analysis.

Target 2005: Conduct molecular confirmation of integration of gf-2.8 with Southern and/or Northern Hybridizations on selected T_1 and T_2 plants. Evaluate potential resistance to white mold through Fungal Bioassay, Oxalic Acid Assay and H_2O_2 assay.

Target 2007: Conduct inheritance studies and field evaluation of the putative transgenic lines. Perform crosses with other bean genotypes and look at the heritability of the transgene in dry beans. Expand screening of putative transgenic plants to the field.

Target 2009: Further field evaluation of potentially useful plants demonstrated previous resistance to white mold in the field. Quantify enzyme/gene activity in the same plants to identify source and mode of action of resistance and continue genetic studies of resistance sources in segregating populations.

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.

Target 2007: Continue multistate testing and greenhouse screening. Release germplasm lines and cultivars with resistance.

Target 2009: Test and release germplasm with pyramided QTL for WM resistance.

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.

Targets for 2005: Identify germplasm lines 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.

Targets for 2007: Germplasm with resistance to SWM characterized and made available to the user community as breeding lines. Germplasm registered in suitable outlets to inform potential users of availability of the identified material. Resistant germplasm used widely in breeding programs designed to develop resistant varieties.

Target 2009: Genetic populations of recombinant inbred lines (RILs) developed and used to determine the genetics of resistance and genomic locations of the important genes. Molecular markers for resistance genes identified.

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.

Targets for 2005: Additional crosses made using more recently identified germplasm sources. Hybrid material screened for resistance.

Targets for 2007: White mold resistant selections of pea evaluated for adaptation and other agronomic traits. Promising resistant selections have been increased and available for release as improved cultivars.

Target 2009: Cultivars with improved resistance released and made available to producers. Validation of markers for use in indirect selection of germplasm for improved resistance to SWM.

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.

Targets for 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.

Targets for 2007: High density genetic map of the pea genome and potential for map based cloning of genes for resistance to SWM.

Target 2009: Validation of molecular markers and verification of their efficacy in selection for resistance to SWM in peas.

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.

Targets for 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 1.3.3.

Targets for 2007: Marker-assisted selection protocol established for resistance to SWM in peas. Markers converted to easily assayed sequence characterized amplified regions (SCARs) for more efficient genotyping and use in marker-assisted selection.

Target 2009: Fully established marker assisted selection procedures for SWM in peas.

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.

Targets for 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.

Targets for 2007: Determine efficacy of defense related genes for control of SWM in peas. Prove or disprove the concept that the oxalate oxidase gene has efficacy against Sclerotinia sclerotiorum when the gene is present in the genome of pea.

Target 2009: Introduce anti-fungal genes into a wide range of pea germplasm and demonstrate the efficacy of the technology for reducing damage from SWM.

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 and needed to formulate an effective screening procedure for identification of resistant lines.

Targets for 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.

Targets for 2007: Germplasm with resistance to SWM characterized and used by breeding programs to incorporate the resistance to adapted varieties for economical control of the disease. Germplasm *registered in suitable outlets to inform potential users of availability of the identified material. Broad use of the resistant germplasm in breeding programs designed to develop resistant cultivars.*

Target 2009: Resistance to SWM incorporated into germplasm of the major types of lentil. Evaluations prove or disprove the effectiveness of resistance in controlling the disease over a wide area.

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 have been made and are currently being advanced toward homozygosity in the lentil breeding program.

Targets for 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.

Targets for 2007: Partial resistance to SWM incorporated into lentil selections that have suitable agronomic traits for adaptation and end-use. Promising selections with resistance to SWM have been increased and are available for release as improved cultivars.

Target 2009: Breeding lines identified with improved resistance to SWM and data for release of promising lines is available. Releases to the producer community are made and the material is shown to have an impact on reducing the severity of the disease.

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 F_2 *and will be further advanced to the* F_6*. A genetic map based on this* F_2 *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.*

Targets for 2005: Mapping populations (RILs) advanced to the F_4 *and available for further advancement to the* F_6 *for phenotyping for resistance to SWM. RIL populations genotyped for markers from the consensus map of lentil and the resulting map used for a quantitative trait locus analysis to locate the region of the genome where genes for resistance to SWM are located.*

Targets for 2007: Completion of the phenotyping of the mapping populations for SWM resistance. Completed development of a high density genetic map of the lentil genome and a QTL analysis of SWM in lentil. Identification of genomic regions that confer resistance to SWM in lentil. Verification of closely linked molecular markers and their association with resistance to SWM.

Target 2009: Verification of closely linked molecular markers and their association with resistance to SWM.

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.

Targets for 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.

Targets for 2007: Validation of the identified markers for their effectiveness in marker-assisted selection will be completed. Establishment of a marker-assisted selection protocol for resistance to SWM in lentils. Markers converted to easily assayed sequence characterized amplified regions (SCARs) for more efficient genotyping and use in marker-assisted selection.

Target 2009: Use of the identified markers in a marker assisted selection procedure for SWM resistance in lentil.

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.

Targets for 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.

Targets for 2007: Determine efficacy of defense related genes for control of SWM in lentils. Prove or disprove the concept that the oxalate oxidase gene has efficacy against Sclerotinia sclerotiorum when the gene is present in the lentil genome.

Target 2009: Introduce anti-fungal genes into a wide range of lentil germplasm and demonstrate the efficacy of the technology for reducing damage from SWM.

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.

Targets for 2007: Germplasm with resistance to the collar rot phase of SWM made available to the user community. Germplasm registered in suitable outlets to inform potential users of availability of the identified material. Resistant germplasm used widely in breeding programs designed to develop resistant varieties.

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.

Targets for 2007: Chickpea lines with tolerance to the collar rot phase of the disease identified and used extensively in chickpea breeding programs.

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 line or cultivar with an improved level of resistance compared with current cultivars.

Target 2007: Release at least one germplasm line or cultivar with improved resistance and yield combination. Evaluate Sclerotinia resistance in regional nurseries. Know reaction to BSR, SCN, and Phytophthora for the lines that are being developed and released for improved Sclerotinia resistance. Initiate investigations for interaction among resistance genes for Sclerotinia, SCN, BSR, and Phytophthora. Initiate development of elite lines with multiple pest resistance, including Sclerotinia stem rot.

Target 2009: Know initial results of some resistance gene interactions. Advance populations and begin evaluation of lines from crosses to develop Sclerotinia resistant lines with other fungal and pest resistances.

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.

Target 2007: Release at least one line with a different source of improved resistance than is available in previous releases. Evaluate field resistance in regional Sclerotinia disease nurseries and evaluation programs. Use improved resistance sources in new soybean cultivar development programs.

Target 2009: Develop breeding populations to incorporate the confirmed resistance QTL into elite germplasm. Initiate development of near-isogenic lines for specific QTL and candidate genes. These materials will be useful for investigations to understand mechanisms related to specific QTL/resistance genes (See PM1.6.4) as well as genotype x genotype and genotype x environment interactions (See PM1.6.3).

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 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.

Target 2007: Develop breeding populations to incorporate the confirmed resistance into elite germplasm. Evaluate the resistance phenotype of lines in regional disease nurseries. Also begin to understand the role of environment in characterizing resistance phenotypes – light, temperature, moisture, nutrition – and interactions with genotype – both the plant and the pathogen.

Target 2009: Initiate studies to understand interaction among genotypes (both plant and pathogen) and environmental factors for resistance to Sclerotinia in soybean.

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.

Target 2007: Release at least one breeding line with pyramided resistance QTL. Evaluate yield and agronomic traits of multi-QTL lines in multiple environments. Initiate crosses to incorporate enhanced resistance into elite cultivars. Evaluate Sclerotinia resistance in regional disease nurseries. Begin to pyramid additional new QTL from other sources into a new set of multi-QTL lines.

Target 2009: Know preliminary results of studies to identify mechanisms involved in reaction to S. sclerotiorum for some of the identified QTL/resistance genes. Initiate crosses to combine complimentary QTL from the best multi-QTL lines identified to date.

PM 1.6.5: Evaluate transgenic approaches for control of *Sclerotinia sclerotiorum* in soybean. In addition to accumulation of endogenous plant genes to enhance Sclerotinia resistance in soybean, introduction of genes from unrelated plants or other sources may provide complementary and useful approaches for effective control of white mold in soybean and other species.

Baseline: Developed Perlka-resistant soybean lines; initiated yield tests in field. Identified T1 plants possessing an antifungal peptide, D4E1.

Target 2005: 1. Know results of yield tests on Perlka-resistant lines and effects of Perlka on germination of sclerotia and development of apothecia. 2. Conduct controlled-environment and field tests on transgenic soybean plants homozygous for the antifungal peptide gene. Evaluate Sclerotinia resistance and agronomic traits.

Target 2007: 1. Possible release of Perlka-resistant soybean lines as a management tool for S. sclerotiorum and possible regional testing of resistant lines, depending on research results and regulatory approvals. 2. Know results of field and controlled-environment tests on transgenic soybean lines containing the antifungal peptide. Initiate seed increase of the best lines for tests and regulatory approval. Possible regional testing of lines. Initiate crosses with elite soybean germplasm if effectiveness and regulatory approval look promising. Investigate nematicidal effects of Perlka on soybean cyst nematode (Heterodera glycines Ichinohe).

Target 2009: Know effects of new antifungal peptide transgenic event for resistance to S. sclerotiorum. Also evaluate resistance to other pathogens, including soybean rust. Begin evaluation of yield and agronomic performance in multiple environments.

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.

Target 2007: Develop greenhouse inoculation procedure for stalk rot, and verify results in field trials, with both cultivated and wild sunflowers. Evaluate head rot inoculation procedures on wild Helianthus in field nurseries.

Target 2009: Develop greenhouse inoculation procedure for head rot, evaluate practicality, and verify results with field trials.

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: *No molecular markers are currently available in sunflower to identify Sclerotinia resistance.*

Target 2005: Identify markers in sunflower populations with known Sclerotinia head rot and stalk rot resistance.

Target 2007: Verify ability of markers to consistently identify plants with high levels of stalk rot and/or head rot resistance by greenhouse and/or field tests. Identify major QTL (quantitative trait loci) controlling stalk rot and/or head rot resistance from different sources.

Target 2009: Field verify that germplasm identified with detached stem test are resistant. Perform marker-assisted selection to combine resistance genes from different sources into elite lines.

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.

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.

Target 2007: Lines combining genes from the new sources of resistance with known genes already in U.S. adapted germplasms will be advanced to the F_4 *stage utilizing the pedigree breeding method. Testcrosses will be made with each* F_4 *line and hybrids evaluated under the mist irrigation system and artificial inoculation with ascospores.*

Target 2009: After three years of testing in hybrid testcrosses under the mist irrigation system and artificial inoculation with ascospores and for stalk rot resistance, F_6*-derived* F_7 *germplasm lines will be evaluated for yield, oil content, and superior agronomic characteristics. The lines with highest potential will be released by USDA-ARS as new germplasm lines for use by industry and public researchers to create new Sclerotinia resistant hybrids for the producer.*

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.*

Target 2007: Depending on whether the backcross populations are maintainer or restorer based, two different testers will be utilized to create testcross hybrids. The testcross hybrids will then be initially screened for Sclerotinia head rot resistance utilizing the mist irrigation system and for Sclerotinia stalk rot resistance utilizing infected media.

Target 2009: BC_1F_6*-derived* BC_1F_7 *germplasm lines will be testcrossed and the resultant hybrid seed will be evaluated for resistance to both Sclerotinia head rot and stalk rot, using artificial inoculations at multiple locations. Lines will be evaluated for proper agronomic characteristics and seed quality factors, and those with the highest potential will be released by USDA-ARS for use by industry and public confection sunflower researchers to create new Sclerotinia resistant hybrids for the producer.*

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.

Target 2007: Complete evaluation of 20+ annual Helianthus taxa (because of ease of crossing annual species with cultivated sunflower) for resistance to stalk rot in greenhouse tests and head rot in field trials. Commence testing of 39 available perennial Helianthus taxa (with different levels of ploidy) for resistance to stalk rot and head rot. Organize exploration trips to obtain seeds of Helianthus species currently not in the USDA germplasm collection, which will take an additional five years.

Target 2009: Continue field and greenhouse evaluations of perennial Helianthus species for resistance to stalk rot and head rot, concentrating on species showing the highest levels of resistance.

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 F_1 *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.*

Target 2007: Continue backcrossing and sib-crossing of progenies from new interspecific crosses, and select for plants with higher level of resistance than HA 410 and HA 441. Complete evaluation of amphiploids to Sclerotinia and release as genetic stocks if found resistant. Cross resistant amphiploids with HA 410 and HA 441, and continue backcrossing to incorporate more resistance genes into HA 410 and HA 441 while reducing chromosome number to 2n=34.

Target 2009: Identify backcrossed progenies with higher resistance than HA 410 and HA 441, and release germplasm. Confirm all resistance selections with replicated field plot evaluation.

Pathogen Biology & Development

Goal 2: Understanding 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 and its demonstrated ability to adapt to a wide range of conditions can be largely attributed to its aggressive mode of pathogenesis and to the production of specialized multicellular developmental structures for survival and dispersal. There are many aspects of the biology of this biology that are not understood. An improved knowledge of population structure, ecological types, virulence diversity, germination factors, pathogenicity factors, and improved molecular methods for studying the biology, 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 agro-ecosystems. 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.

Target 2007: Characterize genotypes on wild plants and additional crops. Utilize molecular tools to characterize genotypes. Begin discussions among researchers on ways to standardize genotype characterization. Begin studies to determine if biotypes have specific environmental requirements for germination and disease development. This requires cooperation among scientists and sharing of isolates and data. Determine if outcrossing is a significant factor in population variability.

Target 2009: Determine if there are ecological types in the population and specifically determine if there are biotypes in the population with fungicide resistance. Compare populations across the US. Organize a multistate approach to undertake the comparisons. Make data available to those scientists involved in identifying gene activity in Sclerotinia so associations can be made between gene data and specific phenotypic characteristics of isolates.

PM 2.0.2: Characterize virulence/aggressiveness within the population, identify isolates for use in screening, 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. Identification of virulence types would be a valuable tool 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 in currently in progress.

Target 2005: Collect isolates from widely different geographic areas on a range of crops and wild hosts, characterize isolates using various methods and select virulent isolates for screening for resistance in multiple crops.

Target 2007: Determine the variability in virulence/aggressiveness of isolates from widely different geographic areas on a range of hosts. Propose methods to characterize virulence/aggressiveness on specific hosts. Initiate testing of virulent types on sources of partial resistance in various crops. Virulent types should be made available to any scientist who needs a variety of isolates to test on certain plant lines.

Target in 2009: Determine environmental parameters that affect virulence/aggressiveness of isolates. Determine if there is a virulent isolate by partially resistant host interaction. Determine if the host source of a virulent isolate is a factor in the virulence on other hosts. Continue testing partially resistant crop lines to a variety of virulent isolates. Publish or put data on the web so scientists working with gene identification in Sclerotinia can utilize these virulent/avirulent types to test hypotheses on gene action. Establish criteria for testing virulence/aggressiveness on specific hosts.

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.

Target 2007: Determine host factors that foster myceliogenic germination of sclerotia. Are host exudates a critical factor in germination? Initiate studies to determine if the microbial population (other than mycoparasites) on the sclerotiasphere has a role in germination and dormancy. Determine if microbial populations on above or below ground plant parts affect growth of the mycelium.

Target 2009: Identify host factors that may enhance myceliogenic germination. Identify microbes that enhance or inhibit/retard germination of sclerotia in the soil. Identify microbes that inhibit/retard mycelial growth.

PM 2.0.4: Develop genetic markers and other 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 protein 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. The fungus is routinely transformed by integrative DNA techniques, but few reporter genes have been inserted and the number of useful promoters is limited.

Target 2005: Develop new molecular markers and new techniques for routine and stable integrative transformation. Initiate a library of mutants to study pathogen biology.

Target 2007: Develop reporter gene constructs with inducible promoters and organelle specific targets. Apply transformation techniques to the production of insertional mutant libraries. Demonstrate utility of transformed isolates in studying biology of pathogen. Expand molecular methods to genotype isolates.

Target 2009: Propose standard molecular protocols to genotype isolates. Use transformed isolates in studies on host/pathogen interactions, pathogen/microbe interactions and especially to study resistance. Provide a variety of transformed isolates of Sclerotinia to the scientific community for use in studying pathogen biology.

Pathogen and Host Genomics

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

PM 3.0.1: 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.

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.

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).

Target 2007: Develop and sequence cDNA libraries from infected host tissue of the 7 major crops susceptible to Sclerotinia (canola, chick pea, common bean, lentil, pea, soybean, and sunflower). Develop approximately 10,000 new ESTs from libraries of each of these crops. The completed genome sequence of S. sclerotiorum will allow identification of differentially expressed fungal genes in infected plant tissues. Perform microarray studies specific to host-Sclerotinia interactions to allow thorough comparative studies of various host responses to Sclerotinia infection to identify key defense- and resistance-related genes.

Target 2009: Incorporate new sequence data into microarrays to further enhance the gene coverage of current microarrays. Continue using microarrays and other gene expression techniques to identify genes responding during host-Sclerotinia interactions.

PM 3.0.2: Identify mechanisms/genes of resistance in the model plant Arabidopsis. 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. Very limited screening of Arabidopsis has been done thus far. 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.

Target 2007: Screen Arabidopsis thaliana ecotypes and well-characterized defense-related mutants for tolerance/resistance/increased susceptibility to Sclerotinia and oxalic acid. Begin the development of mapping populations of resistant and susceptible Sclerotinia hosts.

Target 2009: Develop molecular markers and maps of regions containing Sclerotinia defense-related genes. Begin isolating defense-related genes from Arabidopsis and the use of transgenic approaches to incorporate these genes into crops to determine if they provide possible resistance or enhanced defense to Sclerotinia.

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. Current research efforts are focusing on identifying molecular markers to identify QTLs for resistance. Genes identified in the EST projects of *Sclerotinia*-challenged tissue will provide candidate defense-related genes. As a partial sequence is known for every spotted gene on a microarray slide, spots that show expression patterns associated with defense can be used to generate sequenced-based molecular markers for breeders. Hybridization of DNA to microarrays has the potential to lead to rapid identification of hundreds of new markers and rapid mapping.

Baseline: Current research efforts are focusing on identifying molecular markers to identify QTLs for resistance. Genes identified in the EST projects of Sclerotinia-challenged tissue will provide candidate defense-related genes. As a partial sequence is known for every spotted gene on a microarray slide, spots that show expression patterns associated with defense can be used to generate sequenced-based molecular markers for breeders. Hybridization of DNA to microarrays has the potential to lead to rapid identification of hundreds of new markers and rapid mapping.

Target 2005: Conduct expression studies of Sclerotinia infected hosts.

Target 2007: Molecular markers should be developed from promising candidate genes identified by functional genomic approaches. These markers should be incorporated into marker assisted selection schemes to test their utility. Directed transcriptomic and DNA hybridization approaches should be applied to QTL populations to develop mapping markers.

Target 2009: Use molecular markers to define QTLs for Sclerotinia tolerance. Continue development of molecular markers from candidate genes and the use of microarrays to identify polymorphisms for marker development.

PM 3.0.4: Accumulate genome sequence information from major crops susceptible to Sclerotinia. 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. However, there is limited sequence information from the other major crops susceptible to *Sclerotinia*: chick pea, common bean, lentil, and pea. Sequence data from these other 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. 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.

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. 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.

Target 2007: EST sequencing of cDNA libraries developed from host tissue at different stages of infection.

Target 2009: Genome BAC sequencing should be initiated for each of the 7 major hosts of Sclerotinia with a focus on specific, gene-rich areas of the soybean genome.

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.

Target 2007: Functional genomic approaches will provide a variety of gene targets and RNAi should be used against those viewed to be most promising (e.g., those induced rapidly upon infection). Develop promoters useful for expressing RNAi constructs during infection (e.g., disease-inducible promoters). Improvements in transformation efficiency should be a continual goal for all crops.

Target 2009: Test additional candidate genes hopefully utilizing high throughput methods such as virus-induced gene silencing (VIGS) that may be available by this date. Integrate information from systems biology approach to refine targets for gene silencing.

PM 3.0.6: Develop EST libraries from stains of the pathogen. To rapidly screen pathogen genes for importance in disease, the sequence identity of fungal transcripts needs to be available. 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. 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. 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. A number of cDNA libraries and ESTs have also been established 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) neutral pH-shifted mycelia (5,000 ESTs), (iv) differentiating apothecia (5,000 ESTs), (v) low pH vegetative mycelia (vi) agar grown mycelia (900 ESTs), and (vii) 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.

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. 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. A number of cDNA libraries and ESTs have also been established 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) neutral pH-shifted mycelia (5,000 ESTs), (iv) differentiating apothecia (5,000 ESTs), (v) low pH vegetative mycelia (vi) agar grown mycelia (900 ESTs), and (vii) 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 form fungal tissue grown in culture under different growth conditions and stresses.

Target 2007: Complete cDNA libraries from fungal tissue grown in culture under different growth conditions and stresses. Develop approximately 20,000 ESTs from fungal tissue and deposit in public databases.

Target 2009: Generate cDNA libraries from infected host tissue with an emphasis on identifying infection-related genes from both host and pathogen. Develop approximately 6,000 new ESTs from these libraries generated from 7 major crop hosts (canola, chick pea, common bean, lentil, pea, soybean, and sunflower) infected with Sclerotinia. The approach of collecting expressed sequences from infected tissue will identify both pathogen and host genes that were actively transcribed during infection. Independent sequence information derived from the Sclerotinia genome sequencing project as well as from various plant sequencing projects will facilitate differentiation of host and fungal genes. An emphasis should be placed on obtaining full length, normalized cDNA libraries to ensure a high rate of gene discovery.

PM 3.0.7: Use transcriptomics to identify candidate genes involved in Sclerotinia pathogenicity. 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: Currently there are no publicly available microarrays of Sclerotinia genes.

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

Target 2007: Generate large collections (>10,000) of ATMT for use in phenotypic screens. Transcriptome profiling approaches will provide a variety of gene targets and high through put functional analyses such as RNAi should be used against those viewed to be most promising (e.g., those induced rapidly upon infection). Develop promoters useful for expressing RNAi constructs during infection (e.g., plant-inducible promoters).

Target 2009: Identify genes involved in pathogenesis recovered from ATMT random mutagenesis. Test additional candidate genes utilizing high throughput gene knockout techniques that may be available by this date. Integrate information from genomic data to refine targets for functional gene analysis.

PM 3.0.8: Target candidate genes by RNAi to screen for pathogenicity and virulence. Universal mechanisms exist in organisms to inactivate target genes with interfering RNA molecules in order to prevent them from being translated into functional proteins. These methods are collectively termed RNAi. Applicability of RNAi approaches in several fungal species has been documented. *Sclerotinia* transformation procedures are becoming more standardized and are more widely available. Several fungal-based RNAi cloning vectors are commercially or publicly available.

Baseline: Applicability of RNAi approaches in several fungal species has been documented. Sclerotinia transformation procedures are becoming more standardized and are more widely available. Several fungal-based RNAi cloning vectors are commercially or publicly available.

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

Target 2007: Transcriptome profiling approaches will provide a variety of gene targets and high through put functional analyses such as RNAi should be used against those viewed to be most promising (e.g., those induced rapidly upon infection). Develop promoters useful for expressing RNAi constructs during infection (e.g., plant-inducible promoters).

Target 2009: Test additional candidate genes utilizing high throughput gene knockout techniques that may be available by this date. Integrate information from systems biology approach to refine targets for gene silencing.

PM 3.0.9: Develop bioinformatics resources to provide genomic information to the scientific 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. Data needs to be available in web-accessible, user friendly formats and the scientists need to be trained in use of these systems.

Baseline: Data needs to be available in web-accessible, user friendly formats and the scientists need to be trained in use of these systems.

Target 2005: A variety of formats (web sites, workshops, etc.) will be investigated to determine effectiveness to rapidly and accurately share current molecular and genomic information dealing with Sclerotinia to the plant and fungal research communities.

Target 2007: A mechanism will be in place to allow continuing communication between the fungal molecular and plant breeding communities.

Target 2009: Maintain a robust communication system between the fungal molecular and plant breeding communities. Continue efforts to target genomic approaches to important pathogenic and virulence traits and to move this information rapidly into field application.

Pathogen Epidemiology & Disease Management

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) and concentrations that provide best control of the disease in the different crops that conform to the Sclerotinia Initiative Program.

Base line: Chemical control 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 (strobilurines, etc) don’t exist.

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

Target 2007: Continue efforts to optimize use of fungicides in all crops. Evaluate new chemistries as they become available. Update management guides for growers on use of fungicides for disease management. Continue collection of S. sclerotiorum isolates and monitoring of fungicide sensitivity.

Target 2009: Evaluate new chemistries and fungicide application technologies as they become available. Continue efforts in monitoring fungicide sensitivity.

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.

Target 2005: Initiate studies to optimize the volume of water to be used as carrier in aerial and ground applications for all crops. Evaluate new spraying technologies that enhance canopy penetration, as they become available, like electrostatic and air-assisted blast for better canopy penetration.

Target 2007: Continue optimization of fungicide usage. Develop management guides for fungicide use for sunflowers, pulse crops, and update information available for dry beans and canola.

Target 2009: Continue optimization of fungicide usage. Update management guidelines for fungicide use for all 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 by L. del Rio (North Dakota State University, Fargo, ND) to identify new antagonists of *S. sclerotiorum*. Studies will include identification of optimal concentrations, tank mix compatibility, etc.

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

Target 2005: Optimize use of commercially available biocontrol agents that antagonize sclerotia of S. sclerotiorum (doses, time of application, tank mix compatibility, etc.). 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.

Target 2007: Evaluate Sporidesmium sclerotivorum as a potential new biocontrol agent. Continue efforts to optimize use of commercially available biofungicides. Evaluate new products as they become available. Update management guides for growers on use of biofungicides for disease management.

Target 2009: Continue screening of microorganisms for their potential as biocontrol agents. Continue evaluation of the potential use of Sporidesmium sclerotivorum as biocontrol agent and evaluate new products as they become available.

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, etc. The economics of the use of such compounds will be evaluated. The information generated will be presented to growers in adequate extension materials.

Base line: New chemistries that operate at biochemical levels in plants are being developed. None of them have been tested for S. sclerotiorum disease management on canola, dry bean, pulse crops or sunflower.

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

Target 2007: Continue screening compounds as they become available. Develop guidelines for growers on use of such compounds.

Target 2009: Continue screening compounds as they become available. Develop guidelines for growers on use of such compounds.

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 on canola.

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. 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 epidemics. Conduct field experiments to elucidate the impact of rain, temperature, inoculum density and solar radiation on disease development. Develop preliminary models with predictive capability that could be used in a disease warning system.

Target 2007: Continue development of models with predictive capability that could be used in a disease warning system in canola. Incorporate other cultural practices as factors in the predictive model. Continue evaluation of the association between weather variables and epiphytotics of S. sclerotiorum. Start field validation of models developed.

Target 2009: Continue testing and validation of predictive models in other crops. Continue exploring the impact of weather variables and cultural practices on disease onset.

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, etc.

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 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.

Target 2007: Update information generated using newer cultivars. Incorporate threshold levels obtained in yield loss experiments into disease management programs.

Target 2009: Update information using newer cultivars. Incorporate threshold levels into disease management programs.

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 in selection of crops to be used in rotation schemes, variety/hybrid selection, planting dates, etc. Results of such experiments will be made available for growers through extension bulletins.

Base line: Practices that promote higher yields, like high planting densities, narrow row plantings, high fertilization levels, etc. 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 horizontal resistance.

Target 2005: Characterize the impact of type of crop used in rotation on disease development. Characterize cultivars of all crops for yield 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.

Target 2007: Update information generated using newer cultivars. Integrate the generated information on disease management strategies and make them available to growers.

Target 2009: Integrate new information into disease management packages and make them available to growers.

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 treat hot spots with fungicides instead of broadcast them in entire fields.

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

Target 2005: Generate epidemiological information on disease development (spatial distribution, remote sensing, etc.) that could be used to support a precision agriculture program for canola, sunflower, dry bean, soybean, and pulses.

Target 2007: Continue generating and validating epidemiological data. Start integration of the information generated into disease management packets.

Target 2009: Validate disease management packets that include a precision technology portion.

Collaborators & Organizations

Steering Committee

Rick Bennett
Larry Chandler
Barry Coleman
Tim Courneya
Tom Grebb
William P. Kemp
Larry Kleingartner
Tim McGreevy
Stephen R. Muench
Beth C. W. Nelson
Ed Ready
Todd Scholz
Alicia Thompson
Dale Thorenson
Greg Varner
Brady Vick
Rich Wilson

Researchers

Alan Bakalinsky
Nikolay F. Balbyshev
Dwayne Beck
Greg J. Boland
Carl Bradley
Mark Brick
Lone Buchwaldt
Doug Buhler
Jim Busse
Fernando Daniel Castaño
Karen Century
Yiwu Chen
Weidong Chen
Wesley Chun
Steve Clough
Ann Cobb
Marc A. Cubeta
Luis del Rio
Erik DeWolf
Marty Dickman
Brian Diers
Helene Dillard
Anne Dorrance
Marty Draper
Dilantha Fernando
Russell Freed
Dale Gallenberg
Elizabeth Grabau
George L. Graef
Ken Grafton
Craig R. Grau
Phillip Griffiths
Rebecca Grube
Niklaus J. Grunwald
Thomas J. Gulya
Stephen Guy
Scott Halley
Ray Hammerschmidt
Bryan Hanson
Pat Hart
Glen L. Hartman
Dwayne D. Hegedus
Bob Henson
Jinguo Hu
Hung Chang Huang
Dave Huggins
Shelley Jansky
Brian Jenks
James D. Kelly
Kevin Kephart
Linda Kohn
Joseph M. Krupinsky
Jim Kurle
Randy Kutcher
Arthur Lamey
Ralph Lange
Christopher Lawrence
David G. LeGare
Gary Lemme
John Lukach
Phil McClean
Mary Ruth McDonald
Kent McKay
Kevin McPhee
Hassan A. Melouk
Phil Miklas

Jerry Miller
Fred Muehlbauer
Jim Myers
Randall Nelson
Berlin D. Nelson
Thomas C. Osborn
Suranjan Panigrahi
M. Pastor-Corrales
Wayne Pederson
Tobin Peever
Barb Pennypacker
Paul M. Porter
Lyndon Porter
Barry Pryor
Harikrishnan Ramasubramaniam
Khalid Y. Rashid
Jack Rasmussen
S. Roger Rimmer
Jeff Rollins
Blaine G. Schatz
Howard F. Schwartz
Gerald J. Seiler
Denis A. Shah
Barbara B. Shew
Daina Simmonds
Shree Singh
Ram Singh
David Spooner
Steven K. St. Martin
Glen Statler
James R. Steadman
Walt Stevenson
Henrik Stotz
Stephen Strelkov
Krishna V. Subbarao
Donald L. Tanaka
Frances Trail
Tri Vuong
Dechun Wang
Maury Weise
John M. Whipps
Bo-Ming Wu
X. B. Yang
Quingzhu Zhang
Jianwei Zhao
Steve Zwinger

AgriBusiness

Jesse Barthel
Mike Bodewitz
Joe Caroline
Christopher Clark
Jon Dockter
Pat Duhigg
Timothy Eschbach
Igor Falak
Radisa Gjuric
David Goulet
Tom Borgen
Bob Green
Sonia Hallier
Mike Hutter
Jim Johnson
Tom Johnson
Clinton Jurke
Ron Klinge
Mike Krueger
Jean Q. Liu
John Mayko
Daryn McBeth
Ottmar Philipp
Keith Reinholt
Robert R. Sutter
John Swanson
Kris Versdahl
Alan S. Wicks
Chunren Wu

Universities/Institutions

North Dakota State University
South Dakota State University
University of Minnesota, St. Paul
University of Nebraska, Lincoln
Michigan State University
Cornell University
University of Idaho
Ohio State University
Oregon State University
University of Florida
University of Georgia
University of Illinois
University of Wisconsin
USDA-ARS
RRV Ag Research Center

Agricultural Organizations

National Sunflower Association of Canada
USA Pea & Lentil Council
Northern Canola Growers
Northarvest Bean Growers Association
Central Bean Company
National Sunflower Association
United Soybean Board
Minnesota Canola Council
U.S. Canola Association

National Sclerotinia Initiative
Natural Resources Research Center
2150 Centre Avenue | Building D, Suite 300

Fort Collins, CO 80526-8119
Ph: 970-492-7058 | Fax: 970-492-7065
larry.chandler@ars.usda.gov