National Strategic Plan for the Sclerotinia Research Initiative

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

2017 to 2021

Table of Contents

Executive Summary

Vision Statement: An integrated research approach is needed to guide effective development of diagnostic technologies, disease management systems, genomic resources, and crop germplasm exhibiting durable resistance to *Sclerotinia sclerotiorum*. Strategic deployment of these resources will help sustain global food security through increased competitiveness of U.S. canola, pea, lentil, chickpea, common bean, soybean, and sunflower producers.

Process & Development of the Strategic Plan for the National Sclerotinia Research Initiative – 2017-2021: On January 20-22, 2016, approximately 60 scientists and stakeholders with knowledge of the fungal pathogen, *Sclerotinia sclerotiorum* participated in an annual 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 United Soybean Board co-organized this program. Participants reviewed annual research accomplishments and peer assessment of program performance toward that targeted improved understanding and management of Sclerotinia disease in canola, dry edible beans, peas & lentils, soybean, and sunflower. A summary (pending addition of 2015 data) of program performance against priorities of the USDA ARS National Sclerotinia Research Initiative (NSI) strategic plan for 2013 to 2017 follows:

NSI Program Performance 2013-2017

Sclerotinia Initiative Research Progress Evaluation

	2013	2014	2015	2016	2017
	number of accomplishment citations				
Total Accomlishments	43	58			
Total Milestones	79	79			
Achievement Rating (%)	54.4	73.4			
Total Projects	21	22			
Accomplishments / project	2.0	2.6			
Total Publications	266	283			
Germplasm/Varieties released	41	9			

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

ARS leadership considered stakeholder input in the overall assessment of the NSI program performance and in determining the research needs of industry. Reviews of ARS projects associated with NSI also were conducted by the Office of Scientific Quality Review to ensure relevance, quality and performance in meeting goals of ARS national programs. USDA-ARS leadership of NSI included:

Dr. Roy Scott, National Program Leader, Office of National Programs, Beltsville MD
Dr. John McMurtry, Area Director, Northern Plains Area, Ft. Collins, CO
Dr. William Kemp, Agricultural Administrator, Red River Agricultural Research Center, Fargo ND

Stakeholder and scientists worked in concert during the 2015 calendar year to gather and develop input for highest research priorities for the next 5-years. These data were compiled for final edits by stakeholders and scientists during break-out sessions the annual NSI meeting in January, 2016.

There was consensus that genetic as well as management solutions to the Sclerotinia problem were attainable. This optimism was largely due to NSI scientist applications of advances in molecular biology to characterize genes involved in Sclerotinia resistance genomic data of soybean, dry bean, *Medicago truncatula*, the pathogen and other plant genomes. Implementation of molecular tools in breeding programs and the availability of genome sequence resources has greatly enhanced gene discovery and characterization of NSI data through on-line genomic research tools.

Three goals plus performance measures and milestones were agreed upon for the NSI Strategic Plan 2017 to 2021. The following individuals lead teams that developed and edited this Strategic Plan:

Goal 1: Germplasm Resources & Translational Genomics--Phil Miklas, USDA ARS, Prosser WA
Goal 2: Pathogen Biology & Mechanisms of Resistance----Jim Steadman, Univ. Nebraska, Lincoln, NE
Goal 3 Disease Management & Crop Production-------------Michael Wunsch, ND State Univ., Fargo ND

This strategic plan encompasses 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 the 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.

Background

Sclerotinia sclerotiorum, the most important species of *Sclerotinia,* has an unusually large host range of over 400 plant species in numerous families. This fungus causes diseases known as white mold, Sclerotinia stem rot, wilt or stalk rot, or Sclerotinia head rot on a wide variety of broadleaf crops. It commonly causes economic yield loss in dry edible beans, sunflower, soybean, canola, pea and lentils. Many other crops also 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. The pathogen is found in diverse environments from southern to northern climates and in different agricultural systems under both dryland 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. Improved knowledge of population structure, ecological types, virulence diversity, germination factors, pathogenicity factors, and advances in molecular biology are needed to develop effective control methods for the numerous diseases caused by this pathogen.

The collective annual economic loss attributed to Sclerotinia damage in the five crops participating in the ARS National Sclerotinia Research Initiative has 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 can't 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 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 produces 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. 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. 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 *National Strategic Plan for the Sclerotinia Initiative 2017-2021* provides the research community with a foundation for a comprehensive and integrated research approach toward these problems. The performance measures outlined in this plan are relevant to the current needs of US agriculture. The plan defines the actions that will be taken to solve these problems, 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.

National Sclerotinia Research Initiative Strategic Plan (2017 to 2021)

Crop Germplasm Resources & Translational Genomics

Goal 1: Characterize genetic diversity and facilitate transfer of useful genes among germplasm resources to achieve higher levels of field resistance against a wide range of aggressive *Sclerotinia sclerotiorum* isolates.

PM 1.1: Identify new sources of resistance in plant germplasm. USDA & International Germplasm Collections are a valuable and virtually untapped source of genes that could mediate effective resistance to *S. sclerotiorum* in canola, pea, lentil, chickpea, common bean, soybean and sunflower cultivars.

Milestones & Deliverables:

- Improved phenotypic methods for identifying & validating DNA markers for *S. sclerotiorum* resistance in accessions in USDA & World germplasm collections.

- Molecular cytogenetic systems for developing comparative genomic hybridization and single nucleotide polymorphism (SNP) arrays to facilitate germplasm genotyping.

- A comprehensive association of genotypic x phenotypic features among germplasm accessions and wild crop relatives to identify useful sources of resistance to *S. sclerotiorum*.

PM 1.2: Use of interspecific resources to transfer resistance genes into cultivated plant germplasm. Transfer of resistance genes via interspecific and other wide crosses often is constrained by genetic incompatibilities or other problems resulting in non viable progeny. Undesirable genes may accompany the introgression of beneficial genetic variation for Sclerotinia resistance from unadapted and wild species in modern variety production.

Milestones & Deliverables:
- Ability to evaluate utility and track the chromosomal location and expression profile of DNA segments introgressed from interspecific crosses to related breeding lines.

- Improved genetic methods for combining useful genes for resistance to Sclerotinia stem rot from unadapted sources to agronomic lines of canola, chickpea, lentil, pea, soybean and/or sunflower

- Determine the utility of novel resources such as alien chromosome addition stocks for enhancing resistance to Sclerotinia stalk-rot & head-rot derived from wild annual and perennial species of sunflower.

PM 1.3: Generate high-density genetic maps with validated markers for quantitative trait loci (QTL) that confer resistance to Sclerotinia. Validated DNA markers from genome-wide-sequencing and/or exome-capture help annotate genetic maps of existing variation among recombinant-inbred lines or haplotypes for resistance genes. Micro-array technologies enable custom designed chips with marker sets that facilitate pre-breeding efficiency.

Milestones & Deliverables:

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

- High-resolution genetic and consensus maps of resistance QTL based on annotation with validated markers generated from genome-wide association (GWAS) mapping, exome maps, haplotype maps and/or linkage analysis.

- Transcriptomic, proteomic and metabolomic annotation of genomic sequences in QTL associated with resistance to Sclerotinia diseases

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

- A commodity-based gene atlas with a comprehensive list of all expressed genes, alternative splice products, identification of co-regulated genes and gene networks

- Discovery of transcription factors and elements of gene regulation that mediate expression of disease resistance genes.

- Effective use of genome editing technologies to genetically modify genomic regions in ways that enhance resistance to Sclerotinia diseases or determine candidate gene function

- Identification of allele-specific gene markers within QTL that influence Sclerotinia-host plant interactions

- Improved arrays of validated markers to facilitate screening germplasm resources and expedite marker-assisted-selection in canola, chickpea, common bean, lentil, pea, soybean, and sunflower breeding programs.

- Development and testing of agronomic crop germplasm transformed with putative anti-fungal genes or RNA interfering constructs for reaction to white mold.

- Centralized databases that connect DNA sequences to linkage groups, chromosomes, QTL, candidate genes, polymorphisms and phenotypic traits

PM 1.4: Pyramid white mold resistance in plant germplasm and release germplasm/cultivars with enhanced resistance. Germplasm resources seldom contain all identified favorable alleles for defense against Sclerotinia. QTL from multiple sources must be combined into single lines to enhance overall resistance. Translational genetics will help determine which of the marked genes for disease resistance are most important for use in breeding enhanced germplasm and cultivars.

Milestones & Deliverables:

- Use of allele specific markers and high-throughput phenotyping methods to facilitate pyramiding genes that mediate resistance to Sclerotinia diseases.

- Canola, chickpea, lentil, and pea lines with resistance to Sclerotinia and a broad

portfolio of desirable agronomic traits developed and released.

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

- Establish disease nurseries for characterizing field and greenhouse resistance to all pathogenic forms of Sclerotinia in common bean, soybean and sunflower.

- Soybean breeding lines with Sclerotinia resistance from multiple sources of resistance as verified by QTL-linked markers, including high yield, and resistance to other diseases or insects.

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

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

Pathogen Biology & Mechanisms of Resistance

Goal 2: Understand *Sclerotinia sclerotiorum* biology and development

PM 2.1: Characterize migration/population structure and ecological variability of genotypes. The genotypic basis for genetic variability in *S. sclerotiorum* populations within North America is not well characterized. Identifying ecological types within populations will provide an understanding of how disease develops and survives in agro-ecosystems.

   Milestones & Deliverables:

- Understanding the interaction of pathogen with environmental factors such as temperature and light.

- Identification of biotypes with resistance to new fungicide chemistry

- Characterization of the genetics of fungicide resistance

- Characterization of ecological types in the population.

- Associate traits in *Sclerotinia* with specific genetic markers.

PM 2.2: Characterize virulence/aggressiveness within the population, identify isolates for use in screening, and monitor durability of host resistance. Differences in virulence exist within pathogen populations, but relation of the variation to pathogen genotype and host range is poorly understood. Physiological characteristics may be important to disease development and pathogenesis. Standard methods will be developed to describe virulence/ aggressiveness in the pathogen. Host specificity and the range of virulence/ aggressiveness of collections from different hosts and environments will be tested to determine impact on partial resistance.

Milestones & Deliverables:

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

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

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

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

PM 2.3: Identify environmental and genetic factors involved in myceliogenic and carpogenic germination of sclerotia. Factors like plant exudates are involved in the myceliogenic germination, whereas moisture and temperature are important in carpogenic germination. The biological mechanisms and genetic control of sclerotial germination are not precisely understood. The role of soil microorganisms, other than mycoparasites, in the sclerotia-sphere also may impact the germination process and help identify points in the cycle where germination can be disrupted.

Milestones & Deliverables:

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

PM 2.4: Identify genes that are functional at specific growth and infection stages of Sclerotinia. The genome sequence of *Sclerotinia sclerotiorum* is now available. Gene discovery in *Sclerotinia* will be accelerated by effective means of studying functional genes at specific growth and infection stages, host-pathogen interactions, or under specific environmental conditions. Comparing *S. sclerotiorum* with related *Sclerotinia* spp. will provide insight into factors and mechanisms that limit host ranges of *S. minor* and *S. trifoliorum*, and will in turn help us better understand the mechanisms involved with the extremely wide host range of *S. sclerotiorum*.

Milestones & Deliverables:

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

- Improved gene annotation using transcriptomic data.

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

PM 2.5: Identification and verification of candidate genes involved in Sclerotinia pathogenicity. Profiling transcriptomes of *Sclerotinia* in interactions with various host plant tissues would allow identification of pathogen and host gene expression patterns and will provide further clues as to key factors for pathogenicity and defense. Universal mechanisms exist in organisms to inactivate target genes with interfering RNA molecules to prevent them from being translated into functional proteins. RNAi approaches in *Sclerotinia* will be standardized and widely available.

Milestones & Deliverables:

- Development and maintenance of relevant natural and derived culture collections for use in phenotypic association.

- Transcriptome profiling approaches for a variety of gene targets and high through put functional analyses.

- Promoters useful for expressing RNAi constructs during infection (e.g., plant-inducible promoters).

- Inventory of genes potentially involved in pathogenesis recovered from mutagenesis and transcriptome profiling.

- Functional verification of candidate genes using a systems biology approach to gene silencing and quantitative expression assays.

Disease Management & Crop Production

Goal 3: Broaden knowledge of *Sclerotinia sclerotiorum* epidemiology and improve disease management strategies

PM 3.1: Optimize fungicide application programs. Efforts will identify fungicides, concentrations and application methods that provide best control of *Sclerotinia* in canola, soybean, common bean, pea, lentil, chickpea and sunflower.

Milestones & Deliverables:

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

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

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

- New spraying technologies that improve fungicide performance by enhancing canopy penetration, plant coverage, and fungicide deposition

- Determine most effective timing of fungicide applications relative to canopy closure after blooming

PM 3.2: Develop bio-control alternatives for disease management. Activities will focus in the evaluation of already available commercial bio-control agents, like *Coniothyrium minitans*. Additional surveys and screening exercises will identify new antagonists of *S. sclerotiorum* and optimal application

> Milestones & Deliverables:

- Identification of application strategies that will maximize the efficacy of currently available biocontrol agents for control of *S. sclerotiorum*

- Identification of novel antagonists of *S. sclerotiorum* and assessment of their efficacy in field trials

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

PM 3.3: Develop disease-warning systems to optimize management of *S. sclerotiorum*. Disease-warning systems based on epidemiological associations between environmental conditions and cultural practices help optimize fungicide use for control of *S. sclerotiorum* in canola, dry bean, sunflower, soybean, and pulse crops.

> Milestones & Deliverables:

- Epidemiological information on disease development to support precision agriculture programs for disease control

- Models that calculate risk of disease development as functions of leaf wetness duration and temperature, and risk of apothecia formation as function of soil moisture conditions

- Effect of tillage practices on Sclerotinia survival

- Economic loss models based on plant density at time of disease onset

- Definition of risk levels to guide crop-specific fungicide selection decisions

PM 3.4: Optimize cultural practices for disease management.
The impact of common cultural practices on disease development will be evaluated through field experiments emphasizing crop rotation schemes, variety/hybrid selection, planting dates, etc. Use of precision agriculture technology will help optimize disease management.

> Milestones & Deliverables:

- Collated disease management information with distribution to growers through print media, internet postings and extension publications

- Quantified impact of irrigation scheduling on apothecia development and Sclerotinia disease dynamics with application to irrigation scheduling for optimized crop yields where Sclerotinia is an important limiting factor.

- Assessment of the relative importance of initial Sclerotinia infection from ascospores relative to secondary spread of Sclerotinia from diseased plants to adjacent healthy plants when stems of diseased plants are girdled by the disease, lodge, and become in direct contact with adjacent healthy plants.

Appendix

Collaborators & Organizations

Advisory Committee

Roy Scott
John McMurtry
Barry Coleman
Greg Varner
William P. Kemp

John Sandbakken
Tim McGreevy
Kelly Whiting
Todd Scholz
Rich Wilson

USDA Agricultural Research Service locations

Ft. Collins, Colorado
Pullman, Washington
Prosser, Washington

Fargo, North Dakota
Urbana, Illinois
Ames, IA

Universities/Institutions

North Dakota State University
University of Nebraska, Lincoln
Michigan State University
Oregon State University

University of Idaho
Ohio State University
Colorado State University
Agriculture & Agri-Food Canada

Commodity Organizations

US Dry Pea & Lentil Council
National Sunflower Association
United Soybean Board

US Dry Bean Council
U.S. Canola Association