2018 NATIONAL SCLEROTINIA INITIATIVE MEETING

January 17-18, 2018

Crowne Plaza AiRE MSP Airport – Mall of America
Three Appletree Square, Bloomington, MN

AGENDA

2018 National Sclerotinia Initiative Meeting
January 17-18, 2018

Wednesday – January 17, 2018

11 am-1 pm	Registration & Poster Set-Up *(posters are displayed for the entire meeting)*	**AIRE Ballroom 4**
1:00 pm	Welcome & Introductions – **Mike Grusak, USDA-ARS, Fargo, ND**	**AIRE Ballroom 3**
1:10 pm	Welcome & Update from the Plains Area – **Bryan Kaphammer, USDA-ARS, Fort Collins, CO**	
1:20 pm	Welcome & Update from Office of National Programs – **Roy Scott, USDA-ARS, Beltsville, MD**	
1:30 pm	Introduction of *Keynote Speaker* **– Mike Grusak, USDA-ARS, Fargo, ND**	
	Using data driven results to develop integrated management strategies for Sclerotinia stem rot of soybean – **Damon Smith, University of Wisconsin, Madison, WI**	
2:30 pm	Break	**AIRE Ballroom 4**
	Sclerotinia Research Activities – Session 1 **AIRE Ballroom 3** **Moderator – Shin-Yi Marzano, South Dakota State University, Brookings, SD**	
3:00 pm	Identification of *Sclerotinia sclerotiorum* virulence determinants relevant to infection of multiple host plants by association mapping (Abstract p. 11) – **William Underwood, USDA-ARS, Fargo, ND**	
3:15 pm	Characterizing pathogenicity effectors of Sclerotinia sclerotiorum preferentially expressed under acidic conditions and during plant infection (Abstracts p. 20, 22; Poster #9) **– Weidong Chen, USDA-ARS, Pullman, WA**	
3:30 pm	Expression profiling of the Pea-Sclerotinia sclerotiorum interaction for genomics assisted breeding (Abstracts p. 16, 17; Poster #6) **– Martin Chilvers, Michigan State University, East Lansing, MI**	
3:45 pm	Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas (Abstracts p. 19, 27, 29; Posters #12, 14, 18) **– James Steadman and Sydney Everhart, University of Nebraska, Lincoln, NE**	

4:00 pm	Improving stalk rot phenotyping by evaluation of environment, pathogen, and host factors for S. sclerotiorum infection in sunflower disease nurseries (Abstract p. 24) – **William Underwood, USDA-ARS, Fargo, ND**	
4:10 pm	Break	**AIRE Ballroom 4**
4:30-5:30 pm	Poster Session	**AIRE Ballroom 4**
5:30-7:30 pm	Dinner & Posters	**AIRE Ballroom 4**

Thursday – January 18, 2018

7:00-8:00 am	Steering Committee Breakfast Meeting	**Control Tower**
7:00 am	Continental Breakfast	**AIRE Ballroom 4**
	Sclerotinia Research Activities – Session 2 **AIRE Ballroom 3** **Moderator – Sydney Everhart, University of Nebraska, Lincoln, NE**	
8:15 am	Enhancing soybean for resistance to Sclerotinia stem rot (Abstract p. 15; Poster #16) – **Dechun Wang, Michigan State University, East Lansing, MI**	
8:30 am	Fine mapping of loci for resistance to Sclerotinia stem rot in Glycine latifolia (Abstract p. 18; Poster #7) – **Leslie Domier, USDA-ARS, Urbana, IL**	
8:45 am	Inheritance of Sclerotinia basal stalk rot resistance derived from sunflower wild species (Abstract p 25; Poster #17) – **Lili Qi, USDA-ARS, Fargo, ND**	
9:00 am	Using genomic selection to optimize prediction of Sclerotinia and agronomic phenptypes for more efficient breeding (Abstract p. 32; Poster #2) – **Brent Hulke, USDA-ARS, Fargo, ND**	
9:15 am	Improving resistance to Sclerotinia sclerotiorum in spring canola (Abstracts p. 9, 10; Posters #15, 16) – **Luis del Rio Mendoza, North Dakota State University, Fargo, ND**	
9:30 am	White mold resistance QTL: identification, interactions, and fine mapping in common bean (Abstracts p. 21, 23; Poster #8) – **Phil Miklas, USDA-ARS, Prosser, WA; Jim Myers, Oregon State University, Corvallis, OR; Phil McClean, North Dakota State University, Fargo, ND**	
10:15 am	Break & Poster Session	**AIRE Ballroom 4**

	Group Discussion – Session 3 **Moderator – William Underwood, USDA-ARS, Fargo, ND**	**AIRE Ballroom 3**
10:45 am	Discussion Topic: *Efficient genetic mapping approaches and development/use of molecular markers*	
Noon	Lunch	**AIRE Ballroom 4**
	Sclerotinia Research Activities – Session 4 **Moderator – Brent Hulke, USDA-ARS, Fargo, ND**	**AIRE Ballroom 3**
1:00 pm	Optimizing fungicide application methods for improved Sclerotinia disease management in sunflowers, soybeans, and dry edible beans (Abstract p. 28; Posters #1, 2) – **Michael Wunsch, North Dakota State University, Carrington, ND**	
1:15 pm	Screening for resistance sources to Sclerotinia white mold in recently acquired germplasm of cool season grain legumes – **Weidong Chen, USDA-ARS, Pullman, WA**	
1:30 pm	Developing environmental friendly fungicides for managing white mold (Abstract p 13) – **Shin-Yi Marzano, South Dakota State University, Brookings, SD**	
1:45 pm	Characterizing resistance and pathogenicity genes associated with infection of B. napus by S. sclerotiorum (Abstract p. 12; Poster #5) – **Luis del Rio Mendoza, North Dakota State University, Fargo, ND**	
2:00 pm	Enhancing basal resistance to Sclerotinia sclerotiorum in Brassica (Abstract p. 31; Poster #19) – **Jeffrey Rollins and Zhonglin Mou, University of Florida, Gainesville, FL**	
2:15 pm	Leveraging Arabidopsis genomic resources to identify genes governing quantitative resistance to Sclerotinia – **William Underwood, USDA-ARS, Fargo, ND**	
2:30 pm	Break	**AIRE Ballroom 4**
	Group Discussion – Session 5 **Moderator – Phil Miklas, USDA-ARS, Prosser, WA**	**AIRE Ballroom 3**
3:00 pm	Discussion Topic: *Common threads across crop species in modes of resistance and management of Sclerotinia*	
4:15 pm	Meeting Wrap-Up & Future Plans	
4:45 pm	Safe Travels Home	

NATIONAL SCLEROTINIA INITIATIVE POSTER SESSION

January 17-18, 2018
AiRE Ballroom 4

Epidemiology & Disease Management		
Poster No.	Title	Author(s)
1	Impacts of row spacing & seeding rate on soybean agronomic performance under Sclerotinia disease pressure	M. Wunsch, B. Kraft, K. Cooper, V. Chapara, T. Tjelde, A. Kalil, L. Besemann, M. Schaefer, S. Kallis, H. Eslinger, A. Arens
2	Optimizing use of fungicides and the herbicide lactofen for management of Sclerotinia in soybeans	M. Wunsch, B. Kraft, K. Cooper, V. Chapara, T. Tjelde, L. Besemann, M. Schaefer, S. Kallis, H. Eslinger, A. Arens
3	Rescue of Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) for white mold control	P. Jadhav, J. Feng, S-Y. Marzano
4	Selective fungicides reduce Sclerotinia head rot and yield loss in sunflower	K. Rashid

Genomics		
Poster No.	Title	Author(s)
5	Characterizing resistance and pathogenicity genes associated with infection of B. napus by S. sclerotiorum	K. Chittem, L. del Rio Mendoza
6	Exploring the genetics of lesion and nodal resistance in pea (Pisum sativum) to Sclerotinia sclerotiorum using GWAS and RNA-seq	H-X Chang, H. Sang, J. Wang, K. McPhee, X. Zhuang, L. Porter, M. Chilvers
7	Fine mapping of loci for resistance to Sclerotinia stem rot in Glycine latifolia	L. Domier, N. McCoppin, T. Herman, Q. Liu, T. Thekke-Veetil, G. Hartman
8	Genome wide association study (GWAS) for white mold resistance in common bean	J. Myers, H.Arkwazee, J. Davis, P. Miklas, P. McClean, J. Hart, T. Porch, P. Griffiths

Pathogen Biology & Development		
Poster No.	Title	Author(s)
9	Characterizing pathogenicity effectors of Sclerotinia sclerotiorum	W. Wei, W. Chen
10	Engineering mycovirus to cause loss-of-function of sclerotium production in S. sclerotiorum	J. Feng, S-Y. Marzano
11	Exploring Sclerotinia sclerotiorum effectors during infection of pea	H. Sang, H-X. Chang, J. Wang, A. Rojas, K. McPhee, M. Chilvers
12	Fungicide sensitivity of 120 Sclerotinia sclerotiorum isolates determined with resistance factor analyses	T. Miorini, R. Higgins, J. Steadman, S. Everhart
13	Genetically defined oxalate-minus mutants of Sclerotinia sclerotiorum are pathogenic on many host plants	L. Xu, W. Chen
14	Population structure and phenotypic variation of Sclerotinia sclerotiorum from dry bean in the USA	Z. Kamvar, B. Sajeewa Amaradasa, R. Jhala, S. McCoy, J. Steadman, S. Everhart

Variety Development/Germplasm Enhancement		
Poster No.	Title	Author(s)
15	Association mapping for Sclerotinia stem rot disease in rapeseed/canola (Brassica napus L.)	J. Roy, L. del Rio Mendoza, K. Chittem, M. Rhaman
16	Association mapping for Sclerotinia stem rot disease in rapeseed/canola (Brassica napus L.)	J. Roy, L. del Rio Mendoza, K. Chittem, M. Rhaman
17	Enhancing soybean for resistance to Sclerotinia stem rot	Z. Wen, R. Tan, S. Zhang, P. Collins, S. Wani, C. Gu, M. Chilvers, D. Wang
18	Inheritance of Sclerotinia basal stalk rot resistance derived from sunflower wild species	Z. Talukder, W. Underwood, G. Seiler, L. Qi
19	New sources of white mold resistance derived from wide crosses in common bean and evaluated in the greenhouse and field using multi-site screening nurseries	R. Higgins, Z. Kamvar, S. Everhart, J. Steadman
20	Understanding and improving basal resistance to Sclerotinia sclerotiorum	C. Wang, J. Rollins, Z. Mou
21	Using genomic selection to optimize prediction of Sclerotinia and agronomic phenotypes for more efficient breeding	B. Hulke, Q. Gao, N. Kane

Association mapping for Sclerotinia stem rot disease in rapeseed/canola (*Brassica napus* L.)

Jayanta Roy[1], Luis E. del Río Mendoza[2], Kishore Chittem[2], and Mukhlesur Rahman[1]

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Funded Plan of Work: Improving resistance to *Sclerotinia sclerotiorum* in spring canola

ABSTRACT
One of the objectives of this project is to introduce NEP63-derived resistance into elite NDSU canola breeding lines (WC1417 and WC1421) using a backcross program and to characterize the reaction of *B. napus* germplasm to *S. sclerotiorum*. The resistant line NEP63 was planted in a greenhouse room for crossing and backcrossing with elite breeding lines (14B17 and 14B21) is in progress. A panel of 250 germplasm accessions originated from 27 countries are being screened in a controlled environment to identify resistant/tolerant germplasm. The germplasm is being evaluated using agar plug mycelial stem inoculation at flowering stage. For each accession, lesion length and width, a visual estimation of the percentage of the main stem that is girdled and the number of dead plants is being recorded for up to 15 days after inoculation. So far, a group of 90 spring and 26 winter-type canola accessions have been evaluated. Of this group, nine accessions, eight spring- and one winter-type, showed resistant reaction. These accessions have been genotyped using Ilumina genotyping-by-sequencing (GBS) platform at the Institute for Genomic Diversity at Cornell University, and 42,575 single nucleotide polymorphisms have been identified. A genome-wide association study will be conducted to identify the genomic region containing sclerotinia stem rot resistant genes once the entire set is phenotyped.

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Association mapping for Sclerotinia stem rot disease in rapeseed/canola (*Brassica napus* L.)

Jayanta Roy[1], Luis del Rio[2], Kishore Chittem[2], and Mukhlesur Rahman[1]

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Funded Plan of Work: Improving resistance to *Sclerotinia sclerotiorum* in spring canola. We proposed to introduce NEP63-derived resistance into elite NDSU canola breeding lines (WC1417 and WC1421) using a backcross program. The resistant line NEP63 was planted in a greenhouse room for crossing and backcrossing with elite breeding lines (14B17 and 14B21). The backcrossing program is in progress.

ABSTRACT

Sclerotinia stem rot is one of the most destructive fungal diseases of canola caused by *Sclerotinia sclerotiorum* (Lib) de Bary, that significantly reduce seed yield as well as oil content and quality. In North Dakota, average yield losses have been estimated 13 percent, and the loss can reach to 50 percent in some locations. The canola growers in Minnesota and North Dakota have seen economic losses of 17.3, 20.8, and 16.8 million dollars in 1999, 2000, and 2001, respectively due to this disease. Since, there is no completely resistant varieties available, identification of resistant genotypes and genes in diverged germplasm accessions is one of the best options to develop durable disease resistant cultivar for the growers. In the present study, a panel of 250 germplasm accessions originated from 27 countries will be screened in a controlled environment to identify potential resistant/tolerant germplasm. The germplasm will be evaluated using agar plug mycelial stem inoculation at flowering stage. For each accession, lesion length and width, along with a visual estimation of the percentage of the main stem that is girdled by the lesion and the number of dead plants will be recorded 3, 5, 7, 9, 11, 13 and 15 days after inoculation. The area under disease progress curve (AUDPC) will be calculated using lesion size and plant mortality. We have already screened about 90 spring and 26 winter type canola germplasm accessions. A total of eight spring and one winter type accessions showed resistance reaction during evaluation. All the accessions will also be evaluated using the petiole inoculation technique (PIT) at 4th to 5th leaf stage. The germplasm accessions have been genotyped using Ilumina genotyping-by-sequencing (GBS) platform at the Institute for Genomic Diversity at Cornell University, and 42,575 single nucleotide polymorphisms have been identified. Finally, a genome-wide association study will be conducted to identify the genomic region containing sclerotinia stem rot resistant genes in *Brassica napus.*

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Association mapping of *Sclerotinia* genes contributing to aggressiveness on sunflower

Kassaye Belay[1], Shyam Solanki[1], Berlin Nelson[1], Robert Brueggeman[1], and William Underwood[2]

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Funded Plan of Work: Identification of *Sclerotinia sclerotiorum* virulence determinants relevant to infection of multiple host plants by association mapping.

ABSTRACT
Sclerotinia sclerotiorum is one of the most destructive pathogens of sunflower in the United States, causing several distinctive diseases including basal stalk rot initiated by root infection and head rot resulting from infection of the sunflower head. The pathogen has a remarkably broad host range, yet little is currently known about the virulence strategies that allow *S. sclerotiorum* to successfully infect a wide range of plant hosts. The goals of our project are to build on a previous effort toward genotyping a large collection of *S. sclerotiorum* field isolates collected from diverse hosts, to phenotype these isolates for virulence on multiple sunflower genotypes, and to identify and validate candidate genes contributing to the virulence of this fungal pathogen. We have assembled a collection of 252 diverse isolates of *S. sclerotiorum* collected from numerous plant hosts and locations throughout the US. We evaluated this isolate collection for aggressiveness of stem lesion formation on USDA sunflower inbred line HA 207 and we are currently phenotyping the isolates on a second inbred line, HA441. In parallel, we have conducted genotyping-by-sequencing on 227 of the isolates and are currently finalizing variant calling to discover SNP markers to be used in a genome-wide association mapping approach to identify candidate genes associated with aggressiveness on sunflower stem tissue. We are also evaluating candidate *S. sclerotiorum* genes identified as potentially associated with virulence on bean in an initial GWAS effort involving 70 isolates by direct gene replacement in the pathogen as well as host-induced gene silencing in the plant model *Arabidopsis thaliana*. Initial results from host-induced gene silencing suggest that several of these candidate genes are required for full virulence of the pathogen.

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Characterizing resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum*

Kishore Chittem, and Luis E. del Río Mendoza, North Dakota State University, Fargo, ND 58108.

Funded Plan of Work: Characterizing resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum*

ABSTRACT

The objective of this project has been to characterize putative resistance and pathogenicity genes associated with infection of *B. napus* by *S. sclerotiorum.* Several potential *S. sclerotiorum* candidate pathogenicity/ virulence genes and five SNPs linked *B. napus* sclerotinia stem rot resistance QTLs were identified form prior years research funded by the initiative. Gene knock-out mutants were developed for four *S. sclerotiorum* candidate pathogenicity genes SS1G_05491 (cytochrome P450), SS1G_09997 (Glycosyltransferase, *SsBGT1*), SS1G_14184 (Chitin binding domain) and SS1G_04760 (Serine/Threonine kinase) by targeted gene replacement following split marker approach. PCR assays confirmed correct replacement of the genes of interest. The effect of deletion of these genes on growth characteristics on PDA and pathogenicity/ virulence on canola were evaluated. Deletion of cytochrome P450 and *SsBGT1* affected growth characteristics on PDA, resulting in significant reduction in colony diameter and mycelial density, while deletion of the other two genes had no effect on fungal growth. In addition, virulence tests on canola using detached canola leaf assays and stem inoculations indicated that these genes are required for full virulence, but not for pathogenicity. Cleaved Amplified Polymorphic Sequence markers were developed for four SNPs linked to QTL associated with SSR resistance. The utility of these CAPS markers was validated on a set of thirty *B. napus* breeding lines, and two of these markers are currently being adopted by the NDSU breeding program in their germplasm screening for SSR resistance.

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Dissecting RNA silencing pathways in *Sclerotinia sclerotiorum*

Pauline K. Mochama and Shin-Yi Lee Marzano, South Dakota State University, Brookings, SD

Funded Plan of Work: Developing Environmental Friendly Fungicides for Managing White Mold

ABSTRACT

Recent studies have demonstrated that pathogenic fungi can use RNA silencing to silence host immunity genes through the delivery of small RNAs in addition to antiviral defense. This cross-kingdom RNA silencing is bidirectional with plant hosts capable of delivering small RNAs into fungal cells to silence fungal genes. These findings suggest the importance of RNA silencing mechanisms play in white mold growth and virulence. This study aims to disrupt key silencing-related genes in *S. sclerotiorum* in order to dissect the RNA silencing pathways. So far, homologs of the core eukaryotic RNA silencing genes have been disrupted, including single gene mutants of two Dcls, two Agos, three predicted RdRps, a double DCL mutant, and a double AGO mutant. Disruption mutants are being studied for changes in phenotype, virulence, susceptibility to viral infection, and small RNA accumulation compared to the wild-type strain. To examine the effect of viral infection on strains containing null-mutations of Dcl-1, Dcl-2 or both genes, mutants were transfected with full-length RNA transcripts of a hypovirus- SsHV2L and copies of a gemini-related DNA mycovirus- SsHADV1. Results indicate the ΔDcl-1/Dcl-2 mutant shows severe debilitation following virus infection as evidenced by significantly slower growth and altered colony morphology including reduced pigmentation and an absence of sclerotial formation. Dicer mutants infected with SsHADV1 show similar results, with the double Dicer mutant showing much more severe debilitation compared to ΔDcl-1 and ΔDcl-2 single mutants. Additionally, there is a reduction in small RNAs of 21-22nt size in the ΔDcl-1/Dcl-2 mutant compared to wtDK3. Surprisingly, small RNAs in this range were not completely eliminated in the double Dicer mutant which suggests that there are other Dicer-independent pathways of sRNA biogenesis. We are investigating a hypothetical protein representing a RNase L-like protein, as an additional source of small RNAs in *S. sclerotiorum*. This protein is present in yeast and animals but not in plants. Disruption of this gene is underway to make a triple knockout of dicer genes and the RNase L gene using a third selective marker, Bar gene for bialaphos resistance. The small RNA profile of the triple mutant will be informative for designing the RNA fungicides. The findings of these studies will broaden our understanding of RNA silencing pathways in *S. sclerotiorum* and shed light on how these pathways can be exploited in the development of robust technologies to manage the spread and virulence of this fungal plant pathogen.

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Engineering mycovirus to cause loss-of-function of sclerotium production in *S. sclerotiorum*

Jiuhuan Feng and Shin-Yi Lee Marzano, South Dakota State University, Brookings, SD

Funded Plan of Work: N/A

ABSTRACT
Previously we identified a North American mycovirus infecting *Sclerotinia sclerotiorum*, named *Sclerotinia sclerotiorum* hypovirus 2 – Lactuca (SsHV2-L) through metatranscriptomics screening. The full-length viral genome sequence was determined, and the cDNA infectious clone of SsHV2-L was constructed to establish the cause-and-effect relationship between SsHV2L infection and reduced virulence on white mold. The nucleotide sequence of SsHV2-L was 92% identical to another strain, SsHV2-SX247 (NCBI accession: KJ561218) from China, but contained a deletion of 1.2 kb and 524 nt insertion near 5' terminus of the virus genome. We hypothesized that the 1.2 kb region in SsHV2-SX247 genome putatively encodes a viral suppressor of RNA silencing (VSR), which may be the cause of a complete loss of sclerotium production after virus infection. To confirm the putative VSR, we reconstruct SsHV2-SX247 by a combined synthetic biology and directional cloning approach to engineer the SsHV2-L genome. Briefly, the DNA fragment covering the 1.2 kb additional region of SsHV2-SX247 was synthesized commercially as fragment #1, and the cDNA infectious clone of SsHV2-L was double digested by selected enzymes. Also, a second DNA fragment, which was removed from SsHV2L because of double digest, was PCR amplified as fragment #2. By using overlapping primers between the linearized vector and two fragments, a new genome 99% identical to SsHV2-SX247 with putative VSR was reconstructed. The inserts were confirmed by PCR amplification and sequencing. Comparisons of transfection of *S. sclerotiorum* with *in vitro* transcripts of both original and engineered SsHV2-L are underway, and the effects of both viruses on fungal growth and virulence are in progress.

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Enhancing soybean for resistance to Sclerotinia stem rot

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Funded Plan of Work: Enhancing soybean for resistance to Sclerotinia stem rot

ABSTRACT
White mold of soybean, caused by *Sclerotinia sclerotiorum*, is a necrotrophic fungus capable of infecting a wide range of plants. Eight breeding populations consisted of ~1100 $F_{2:3}$ lines have been developed from crosses between new resistance sources and elite cultivars. A population of 112 recombinant inbred lines (RIL) derived from E07048×E06186 were evaluated for white mold resistance again in a naturally infected nursery at Montcalm farm. Two QTLs, located at Chromosome 6 and 10, were repeatedly detected over the three years study. The two QTLs explained 18.1% phenotypic variation. To enlarge training population size for genomic selection, 151 advanced breeding lines were evaluated for white mold resistance in the disease nursery and genotyped with SoySNP6K BeadChip in the past year. To date, genotypic and disease score data of 703 soybean lines has been acquired. The effect of training population size on accuracy began to plateau around 400, but accuracy steadily climbed until the largest possible size (600) was used in this analysis. The present study showed that gBLUP models for genomic prediction in soybean holds good potential to expedite genetic gain of selection for white mold resistance.

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Exploring *Sclerotinia sclerotiorum* effectors during infection of pea

Hyunkyu Sang, Hao-Xun Chang, Jie Wang, Michigan State University, East Lansing, MI, Alejandro Rojas, Duke University, Durham, NC, Kevin E. McPhee, Montana State University, Bozeman, MT, Martin I. Chilvers*, Michigan State University, East Lansing, MI,

Funded Plan of Work: Expression profiling of the pea-Sclerotinia sclerotiorum interaction for genomics assisted breeding

ABSTRACT

Sclerotinia sclerotiorum is a plant pathogenic fungus with over 400 host species including pea (*Pisum sativum* L.). The fungus causes white mold, which is a significant problem in many areas where pea is produced. To improve white mold control in pea, understanding the mechanisms of both pea resistance and *Sclerotinia* pathogenicity is essential. Generally, fungal effectors are used as probes to search for resistance components in plants, but *Sclerotinia* effectors to pea have been poorly studied. RNA-seq analysis of a partially white mold resistant cultivar "PI240515" and susceptible cultivar "Lifter" in response to *S. sclerotiorum* infection (12, 24, and 48 hours post inoculation; hpi) was conducted. 137 putative *S. sclerotiorum* effectors were identified and subjected to gene expression pattern analysis during infection of two pea cultivars, and on agar plugs incubated in Petri dishes containing potato dextrose agar medium. A total of 21 putative effectors were significantly induced in both cultivars compared to the expression on the medium. Eight effectors were highly expressed in the early infection time point (12-24 hpi), and nine effectors showed induced expression at 24-48 hpi and four effectors showed increased expression at 48 hpi only. Interestingly, three putative effectors were significantly less expressed in the resistant cultivar than the susceptible cultivar at 12, 24, and 48 hpi, respectively. The interaction of these three uncharacterized effectors with two pea cultivars are currently being investigated using molecular and biochemical techniques. This study will shed new light on white mold resistance mechanisms in pea and *Sclerotinia* pathogenicity to other economically important crops as well.

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Exploring the genetics of lesion and nodal resistance in pea (*Pisum sativum* L.) to *Sclerotinia sclerotiorum* using genome-wide association studies and RNA-seq

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ABSTRACT

The disease white mold caused by the fungus *Sclerotinia sclerotiorum* is a significant threat to pea production and improved resistance to this disease is needed. Nodal resistance in plants is a phenomenon where a fungal infection is prevented from passing through a node and the infection is limited to an internode region. Nodal resistance has been observed in some pathosystems such as the pea (*Pisum sativum* L.)-*S. sclerotiorum* pathosystem. Other than nodal resistance, different pea lines display different levels of stem lesion size restriction, referred to as lesion resistance. It is unclear whether the genetics of lesion resistance and nodal resistance are identical or different. This study applied genome-wide association studies (GWAS) and RNA-Seq to understand the genetic makeup of these two types of resistance. The time series RNA-Seq experiment consisted of two pea lines (the susceptible 'Lifter' and the partially resistant PI 240515), two treatments (mock samples and *S. sclerotiorum* inoculated samples), and three time points (12, 24, and 48 hours post-inoculation). Integrated results from GWAS and RNA-Seq analyses identified different redox-related transcripts for lesion and nodal resistances. A transcript encoding a glutathione S-transferase was the only shared resistance source for both phenotypes. There were more leucine rich-repeat containing transcripts found for lesion resistance, while different candidate resistance transcripts such as a VQ motif-containing protein and a myo-inositol oxygenase were found for nodal resistance. This study demonstrated the robustness of combining GWAS and RNA-Seq for identifying white mold resistance in pea, and results suggest different genetics underlying lesion and nodal resistance.

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Fine mapping of loci for resistance to Sclerotinia stem rot in the wild perennial *Glycine latifolia*

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ABSTRACT

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is a damaging disease of soybean (*Glycine max*). Partial resistance to the disease is available in the soybean primary gene pool, but higher levels of resistance to Sclerotinia stem rot can be found among soybeans wild perennial relatives. Because it has not been possible to recover fertile recombinant hybrid plants between soybean and its perennial relatives, it has not been possible to utilize those genes for resistance to Sclerotinia stem rot or other pathogens and pests in soybean improvement. Advances in high-throughput genome sequencing and gene mapping along with gene editing technologies provide mechanisms to bypass barriers to standard genetic hybridization. *Glycine latifolia*, one of soybean's 26 perennial relatives, is recognized for its resistance to multiple abiotic stresses and plant diseases, including Sclerotinia stem rot. To map loci for resistance to Sclerotinia stem rot in *G. latifolia*, populations of 324 F6 recombinant inbred lines (RILs) and over 3000 individual F2 seed were produced from crosses between *G. latifolia* plant introduction (PI) 559298 (resistant) and PI 559300 (susceptible). Assays were initiated to evaluate the RIL population for sensitivity to Sclerotinia stem rot and oxalic acid, a pathogenicity determinant for *S. sclerotiorum*, and by genotyping by sequencing.

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Fungicide sensitivity of 120 *Sclerotinia sclerotiorum* isolates determined with resistance factor analyses

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Funded Plan of Work: Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas

ABSTRACT
Sclerotinia sclerotiorum is a necrotrophic fungal pathogen of more than 400 plant species. One method of disease control is fungicide applications. Although *S. sclerotiorum* is predicted to have low risk of resistance development based on expected population genetic variability, intensive use of fungicides can select for fungicide resistance. Our recent work showed that a discriminatory dose method (DD) was a more reliable method over use of a spiral gradient. In the present work, we sought to characterize fungicide sensitivity using a preliminary set of 120 *S. sclerotiorum* isolates from soybean (n=96) and dry bean (n=24) that came from diverse sources, including Brazil (n=100), Argentina (n=5), and the U.S.A. (n=15). To characterize fungicide sensitivity, a discriminatory dose (DD) was obtained from a baseline subset of 23 isolates to determine sensitivity (EC_{50}) to 8 fungicides. The average and standard deviation (SD) of EC_{50} of each fungicide was used to create a single DD for assessments. Subsequently, the EC_{50} was determined for isolates in the 90^{th} percentile of growth. Resistance factor (RF_1) is typically calculated as a ratio of the observed EC_{50} compared to a baseline EC_{50}. Since the distribution of EC_{50} is different for each fungicide mode of action, two complementary calculations (RF_2 and RF_3) were developed to account for range and variance of each fungicide. Denominator of RF_2 used the range of the baseline EC_{50}, and denominator of RF_3 used 2 x SD from the baseline EC_{50}. Reduced sensitivity for boscalid was identified and RF_1 was higher (5.39, 3.98, and 3.69 respectively). Reduced sensitivity to both dicarboximide fungicides was also found, however, RF_3 yielded higher results (1.98, 5.87, and 14.1 for iprodione, and 2.65, 10.18, and 13.40 for procymidone). Overall, there was a low frequency of reduced sensitivity isolates, although further sensitivity assays are currently underway to evaluate more U.S.A. isolates.

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Genetically Defined Oxalate-Minus Mutants of *Sclerotinia sclerotiorum* are Pathogenic on Many Host Plants

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Funded Plan of Work: Characterizing pathogenicity effectors of Sclerotinia sclerotiorum preferentially expressed under acidic conditions and during plant infection

ABSTRACT

The fungus *Sclerotinia sclerotiorum* causes the devastating whit mold disease on more than 400 plant species including many economically important crops such as common bean, canola, sunflower, soybean and cool season legumes, and produces copious amounts of oxalic acid, which, for over a quarter century, has been claimed as the pathogenicity determinant. The claim was based on UV-induced mutants that concomitantly lost oxalic acid production, sclerotial formation and pathogenicity. The evidence so far supporting such a claim has not fulfilled the molecular Koch's postulates because the UV mutants are genetically undefined and harbor defects other than oxalate production, such as defect in normal growth and developmental defect in sclerotial production. In addition to the inability to complete its life cycle, the defects in growth rate and inability to develop sclerotia in the UV-induced mutants could explain, at least in part, the lack of pathogenicity. Using two independent mutagenesis techniques, we previously generated mutants of *S. sclerotiorum* that completely lost oxalic acid production. The oxalate-minus mutants accumulated fumaric acid, produced functional sclerotia and have reduced ability to acidify the environment relative to the wild type strain. In this study, the oxalate-minus mutants were tested for pathogenicity on plants representing five plant families (Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae and Solanaceae). The oxalate-minus mutants retained pathogenicity and produced lesions similar in size as those by the wild type strain on many of the tested plants. Previously we showed that acidifying the host tissue enhanced lesion expansion of the oxalate-minus mutants, whereas supplementing with oxalate did not. These results suggest that it is acidic pH, not oxalic acid *per se*, that establishes the optimum conditions for growth, reproduction, pathogenicity and virulence expression of *S. sclerotiorum*. Exonerating oxalic acid as the primary pathogenicity determinant will stimulate research into identifying additional candidate genes as pathogenicity factors towards better understanding and managing Sclerotinia diseases.

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Genome wide association study (GWAS) for white mold resistance in common bean

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Funded Plan of Work: White mold resistance-QTL: identification, interactions, and fine mapping in common bean

ABSTRACT

Snap bean shows a higher level of susceptibility to white mold compared to dry bean. This group is also a potential source of resistance QTL not found in dry bean. Our overall goal is to identify, evaluate and use resistance QTL from this germplasm pool. Genome wide association study (GWAS) was conducted to detect markers significantly associated with white mold resistance in two panels of snap bean cultivars: BeanCAP Snap Bean Diversity Panel (SBDP) (n= 137) and the Snap Bean Association Panel (SnAP) (n=376). The objectives of the present study were: 1) to verify previously reported QTLs detected in other populations and studies, 2) to detect novel QTLs associated with white mold resistance and 3) to identify new sources of resistance to this disease in common bean, with particular emphasis on snap bean. The SBDP was evaluated for white mold resistance in the field for two years. The SnAP was screened for white mold resistance in greenhouse using the seedling straw test. The SnAP was genotyped using genotyping-by-sequencing (GBS) for which 40,023 SNPs were generated. GWAS was performed using the R package FarmCPU. A total of 146 SNPs were detected on all 11 chromosomes. Twenty SNPs were detected for the straw test while 126 significant SNPs were detected in one or both years in the field. SNPs grouped into 39 chromosomal regions. Thirteen regions contained SNPs from one year in the field, 10 regions had SNPs from both years, 10 regions contained SNPs from one year and the straw test, 4 regions had SNPs from both years in the field and straw test, and two regions were identified by SNPs associated with the straw test only. The regions overlapped with 13 previously identified QTLs. Eight regions corresponded to meta-QTL previously identified by us. Twenty-five were newly identified in this study. NY6020-5 and Unidor were the most outstanding snap bean cultivars in the field tests for both years while Homestyle and Top Crop were the most resistant snap beam cultivars in straw test. This study provides validation of some previously identified QTL and provides novel sources of resistance to be exploited in common bean.

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Identification and characterization of pathogenicity effectors of *Sclerotinia sclerotiorum*

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Funded Plan of Work: Characterizing pathogenicity effectors of Sclerotinia sclerotiorum preferentially expressed under acidic conditions and during plant infection

ABSTRATCT

Sclerotinia sclerotiorum causes the whit mold disease on many economically important crops such as common bean, canola, sunflower, soybean and cool season legumes. Despite numerous investigations, the pathogenic mechanisms of *S. sclerotiorum* are not fully understood. Our previous investigations using genetically defined oxalate-minus mutants showed that it is acidic pH, not oxalate per se, that sets the optimal condition of virulence of *S. sclerotiorum*. This research was designed to identify and characterize pathogenicity effectors that are preferentially expressed at acidic pH and during plant infection in both the wild type strain WMA1 and also in the oxalate-minus mutant strain KO7 of *S. sclerotiorum*. For in vitro transcriptome study, total mRNAs were isolated from wild type WMA1 and oxalate-minus mutant KO7 cultured on PDA buffered at pH 4.2, 5.5 and 6.2. Total mRNAs were isolated from infected plants for in vivo transcriptome study, with total mRNA from non-infected plants as controls. Strand-specific cDNA libraries were constructed from the mRNAs and the mRNA libraries are bar-coded with appropriate adaptors, and pooled together, and paired-end DNA sequencing was carried out in the HiSeq 2000 platform. After trimming adaptor sequences, all the remaining clean reads were mapped to the *S. sclerotiorum* reference genome. Reads per kb per million reads (RPKM) were used to compare relative expressions among samples. The transcriptome data were analyzed to identify genes that are up-regulated at pH 4.2 over high pHs 5.5 and 6.2 for both the wild type and the mutant strains, and to identify genes that are up-regulated in the wild type than in the mutant in infected green bean leaves and genes that are up-regulated in mutant strain in infected faba bean leaves than in infected green bean leaves. Finally the genes that are commonly up-regulated in all the above conditions were identified. Using this approach, we have identified 56 genes that are preferentially expressed in all the conditions, and 17 of those 56 up-regulated genes contained the a signal peptide, indicating they are potentially secreted proteins and are potential pathogenicity effectors. Specific knock-out of one of the identified genes resulted mutants that have significantly reduced virulence, and the gene product was found to specifically bind to a host cell wall protein based on BiFC and Yeast two-hybrid analyses. The detailed analysis of the interaction between the pathogen effector protein and the host cell wall protein will be presented at the NSI annual meeting.

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Identification of major QTL conditioning partial resistance to white mold in dry bean

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Funded Plan of Work: White mold resistance-QTL: identification, interactions, and fine mapping in common bean

ABSTRACT
Cultivars with partial resistance to white mold are an important component of an integrated disease control strategy (genotype, cultural practices, and fungicides). Our overall goal is to identify, characterize, and utilize resistance QTL to facilitate breeding cultivars with improved partial resistance to white mold. More than 35 QTL from 14 bi-parental populations developed, phenotyped, and genotyped by this collaborative project have been discovered to date. Nine meta-QTL were identified as major targets for breeding efforts and further genomic analyses. Two meta-QTL WM7.2 and WM8.3 have been fine-mapped, WM2.2 is in the process (McClean's Lab), and genetic populations for fine mapping meta-QTLWM5.4 and WM7.5 are in development. The following crosses were recently conducted [PT12-37/USPT-WM-12; PT11-13/USPT-WM12; PT9-5-6/USPT-WM-12; CO14790-3/USPT-WM-12; PT11-13/PRP-153; PT10-12-1/PRP-153; PT12-37/VCP-13; PT9-17/VCP-13] for development of recombinant inbred line mapping populations to examine combinations of QTL in a susceptible pinto bean background and to deploy new QTL toward breeding pinto bean with improved white mold resistance. While other crosses, selection and generation advancement, of breeding lines and genetic populations is ongoing we are identifying and characterizing QTL from genome wide association studies (GWAS). There are four large germplasm panels [SnAP – 376 snap bean accessions (Myers' lab); ADP – 258 determinate large seeded Andean accessions; MDP – 298 Middle American accessions; and DDP – 192 Durango accessions] that have been phenotyped for reaction to white mold and genotyped with SNPs (McClean's lab). The GWAS for the ADP identified five QTL WM4.3, WM6.1, and WM11.1, and new QTL on Pv08 (~WM8.4) and Pv10 (~WM10.1). Seven QTL were identified in the MDP: WM1.2, WM2.2, WM2.3, WM5.4, and WM8.4, and new QTL ~WM1.4 and ~WM11.2. These results will be: i) combined with the GWAS findings for SnAP and DDP, and ii) integrated with the comprehensive linkage and physical maps which possess all the WM QTL discovered to date. New QTL targets for breeding and fine mapping are expected.

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Improving reliability of sunflower phenotyping for resistance to basal stalk rot caused by *S. sclerotiorum*

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Funded Plan of Work: Improving stalk rot phenotyping by evaluation of environment, pathogen, and host factors for S. sclerotiorum infection in sunflower disease nurseries

ABSTRACT
Sclerotinia sclerotiorum causes three distinct diseases on sunflower, basal stalk rot (BSR), mid-stalk rot, and head rot. BSR and head rot are typically among the most prevalent diseases of sunflower and can cause significant losses in years where environmental conditions are favorable. BSR is unusual among diseases caused by *S. sclerotiorum* in that it is a root rot initiated by invasion of sunflower roots by mycelium derived from myceliogenic germination of sclerotia in the soil. Consequently, evaluation of sunflower germplasm and experimental materials requires inoculation of the root zone with *S. sclerotiorum* mycelium. The ARS Sunflower & Plant Biology Research Unit has previously developed an inoculation method for field-scale evaluation of sunflower materials for BSR resistance using custom-build equipment to deposit *Sclerotinia*-infested millet seed into a furrow next to sunflower plants. This technique is capable of producing sufficient and even disease pressure in some years. However, the reliability of this method is relatively poor and frequent failures to produce disease pressure adequate to facilitate genetic mapping efforts are encountered. Thus, the goal of our project is to evaluate potential improvements to this methodology and to develop alternative procedures to supplement this method. During the 2017 field season, we evaluated several alterations and amendments to the field inoculation procedure, including the use of mixed *S. sclerotiorum* isolates selected for their aggressiveness on stem tissues in greenhouse testing as well as the use of straw mulch to modulate soil temperature and moisture. Our results indicated that straw mulch made a modest, but significant impact on disease development, but other alterations were not effective. In addition, we have initiated an effort to develop and validate a greenhouse-based method to supplement field phenotyping for BSR.

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Inheritance of Sclerotinia basal stalk rot resistance derived from sunflower wild species

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Funded Plan of Work: QTL mapping of Sclerotinia basal stalk rot resistance derived from sunflower wild species

ABSTRACT
In 2017, we completed the first year Sclerotinia basal stalk rot (BSR) screening trials of three advanced backcross (AB) mapping populations derived from crosses of three wild annual sunflower species, *H. petiolaris* (AB-pop1), *H. praecox* (AB-pop2), and *H. argophyllus* (AB-pop3) with cultivated sunflower lines. All field screening trials were conducted using a randomized complete block design with three replications. Commercial hybrids Croplan 305 and Cargill 272 were used as resistant and susceptible checks, respectively. AB-pop1 and AB-pop2 were evaluated at Carrington, ND and Staples, MN along with the recurrent parents, HA 89 and HA 485. Analysis of variance of 174 BC_2F_4 families of AB-pop1 revealed a significant genetic variation for BSR disease incidence (DI) at both locations. The mean BSR DI at Carrington and Staples was 51.9 and 15.1%, and ranged from 4.8 to 88.6 and 0 to 43.1%, respectively, suggesting a lower BSR incidence at Staples. Genotype x environment (G×E) interaction effects of the trait was also highly significant, indicating differential response of BC_2F_4 families at the two locations. In order to develop a linkage map, genotyping of AB-pop1 using the GBS approach is currently underway. AB-pop2 consisting of 174 BC_2F_4 families grown at Staples was late maturing and could not be scored for BSR DI due to poor disease development. Analysis of variance revealed a significant genetic variation for BSR DI at Carrington. The mean BSR score was 12.3% for this population with a low of 0% to as high as 51.5%. The distribution of BSR DI data was highly skewed towards lower values with 56% of the families scored less than 10% DI. Because of limited space at Staples, AB-pop3 consisting of 134 BC_1F_4 families was evaluated only at Carrington along with the recurrent parent, HA 89. The genetic variation for BSR DI was also highly significant with a mean DI of 20.3%, ranging from 0 to 72.3%. The distribution of BSR DI was also highly skewed towards lower values with 39% of the families having less than 10% DI. In 2017, we planted 55 *H. petiolaris* derived BC_2F_3 families, 44 *H. praecox* derived BC_2F_3 families, and 48 *H. argophyllus* derived BC_1F_3 families at Glyndon, MN, and bagged ~1,500 heads for seed increase to conduct multi-environment BSR field evaluations in North Dakota and Minnesota in the summer of 2018. Crossing different germplasm lines conditioning partial resistance to BSR is underway to increase BSR resistance by pyramiding QTL.

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Leveraging *Arabidopsis* genomic resources to identify genes governing quantitative resistance to *Sclerotinia*

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ABSTRACT

Sclerotinia exhibits an exceptionally broad host range and is apparently capable of causing disease on most dicot plants. In contrast to resistance against biotrophic and hemi-biotrophic plant pathogens, no major dominant genes conditioning qualitative resistance to *S. sclerotiorum* have been discovered in any species of host plant. Nonetheless, phenotypic variability for partial resistance to *S. sclerotiorum* is observed for affected plant species. Partial resistance is quantitative, conditioned by many genetic loci each making a relatively small contribution to the overall level of resistance. Consequently, fine-mapping of resistance loci is challenging and the identities and functions of genes governing resistance to *Sclerotinia* have mostly remained elusive. The goal of this project is to utilize the genomic and functional genetic resources available for the model dicot *Arabidopsis thaliana* to identify and functionally validate genes conferring *Sclerotinia* resistance. We have acquired a core collection of 360 diverse *Arabidopsis* accessions and, over the past year, we have evaluated 300 accessions for resistance to the sequenced *S. sclerotiorum* reference isolate 1980. As anticipated for the quantitative nature of resistance typical for this pathogen, we observed a continuous distribution of resistance/susceptibility, with some accessions exhibiting considerable partial resistance. We have used our resistance phenotyping data along with millions of SNP markers available for these accessions from whole-genome resequencing efforts to conduct genome-wide association mapping of *Sclerotinia* resistance. This effort has yielded a number of loci associated with resistance, ranging from ~10 to over 100 depending on GWAS parameters, stringency of significance cutoff, and timing of disease evaluations (4 days or 7 days post inoculation). At most loci, clear and obvious candidate genes are identified with defined annotations and often functional information. Our initial results suggest that resistance to this pathogen is governed by genes involved in defensive secondary metabolite biosynthesis, pathogen detection, regulation of jasmonic acid and ethylene metabolism and signaling, and cell wall modification including lignin production. We plan to functionally validate a subset of candidate genes using available insertional mutants and we are currently phenotyping the accessions with a second, more aggressive *Sclerotinia* isolate to determine if some defense loci/mechanisms are specific to individual isolates of the pathogen. This dataset will be useful to identify plausible candidate genes within mapped intervals for affected crop species and to potentially develop new strategies for more efficient resistance breeding efforts.

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New sources of white mold resistance derived from wide crosses in common bean and evaluated in the greenhouse and field using multi-site screening nurseries

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Funded Plan of Work: Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas

ABSTRACT
The goal of our work over the past two years is to identify putative sources of resistance to white mold in adapted backgrounds at multiple sites located in most of the major bean-production areas of the northern states. This is the second year of our project funding. Our approach combines evaluations in greenhouses using a straw test method that is consistent in identification of sources of resistance in adapted and non-adapted bean germplasm and a multi-site field evaluation at white mold nurseries in several locations throughout the major dry bean production regions of the United States. A benefit of the straw test is that it requires only 24 bean seeds per site (~200 seeds) for evaluations to confirm resistance. A benefit of our multi-site field testing is that it mitigates variability in weather from site-to-site that can hinder effective evaluations. In 2017, breeders sent 12 lines for evaluations, which were pinto, navy, black, and small red bean seed classes. Greenhouse trials were conducted using these 12 lines, plus the controls (G122, Beryl, and Bunsi). In preliminary analysis of greenhouse data from four sites, we identified two pinto lines (NE5-16-98 and NE5-16-101) that had straw test ratings with better performance than the control (G122) and significantly reduced disease ratings compared to all other lines, except ND122386. Field trial preliminary data from six sites did not show a significant difference in lines (ANOVA; $p = 0.051$), however, four lines (NE5-16-98, NDZ14083, N14229, and NE5-16-101) had lower disease ratings than Bunsi, although none were lower than G122. Collectively, these preliminary results suggest the two pinto lines with disease resistance in the greenhouse show promise for increased disease resistance in the field. Over the past two years, we have evaluated a total of 20 bean lines in the greenhouse and/or multi-site fields, which is important for identification of new sources of resistance to white mold.

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Optimizing fungicide application methods for improved Sclerotinia disease management in sunflowers, soybeans, and dry edible beans

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ABSTRACT

Management of Sclerotinia stem rot in soybeans and dry beans and Sclerotinia head rot in sunflowers is constrained by the difficulty of achieving satisfactory fungicide deposition to the interior of the canopy in soybeans and dry beans and to the front of sunflower heads, the sites where pathogen infection generally occurs. Field studies were conducted in Carrington and Oakes, ND in 2017 to identify application methods that optimize fungicide deposition to the interior of dry bean and soybean canopies and to the front of sunflower heads with the goal of improving disease control and agronomic outcomes. The impacts of nozzle spray pattern, droplet size, application pressure, boom height, and spray volume were evaluated, and the 'Undercover 360' drop nozzle (360 Yield Center; Morton, IL) was tested. All applications were made with a tractor-mounted boom equipped with a pulse-width modulation system to permit a constant driving speed across all treatments, and applications were pressurized with carbon dioxide. The application of fungicides to soybeans and dry beans through standard boom-mounted nozzles was generally optimized with spray nozzles and application pressures that produced primarily medium to coarse spray droplets (300-350 micron diameter). The application of fungicides through drop nozzles was highly effective in soybeans and modestly improved outcomes in bush-type dry edible beans. In vine-type dry edible beans, drop nozzles could only be utilized at bloom initiation (prior to canopy closure) and did not confer improved disease control relative to standard application methods. In sunflowers, fungicides were effective against Sclerotinia head rot when applied through drop nozzles but not with standard fungicide applications over the top of the canopy. The results suggest that improvements in Sclerotinia disease control may be possible in soybeans and dry beans by modifying application methods. In soybeans and dry beans, fungicides are often applied with boom-mounted nozzles using application pressures that produce spray droplets primarily in the fine to medium spectrum (100 to 250 microns). In sunflowers, fungicides were most effective against head rot when sunflowers were inoculated with ascospores of *Sclerotinia sclerotiorum* 1 to 2 days after fungicides were applied and were not effective when sunflowers were inoculated with *S. sclerotiorum* 7 days after fungicides were applied, suggesting that fungicides might only be a useful tool for managing head rot in sunflowers when applications are made shortly before periods of rain, cool temperatures, and high humidity conducive for pathogen infection.

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Population structure and phenotypic variation of *Sclerotinia sclerotiorum* from dry bean in the USA

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Funded Plan of Work: Improved white mold resistance in dry and snap beans through multi-site screening and pathogen characterization throughout major production areas

ABSTRACT:
The ascomycete pathogen and causal agent of white mold on dry bean, *Sclerotinia sclerotiorum*, is a necrotrophic pathogen on over 400 known host plants. Currently, there are no known cultivars of dry bean with complete resistance to white mold. For over 20 years, bean breeders have used white mold screening nurseries (WMSN) with natural populations of *S. sclerotiorum* to screen new cultivars for resistance. It is thus important to know if the genetic diversity in populations of *S. sclerotiorum* within these nurseries a) reflect the genetic diversity of the populations in the surrounding region and b) are stable over time. Furthermore, previous studies have investigated the correlation between mycelial compatibility groups (MCG) and multilocus haplotypes (MLH), but none have formally tested these patterns. We genotyped 366 isolates of *S. sclerotiorum* from producer fields and WMSN surveyed over 10 years in 2003–2012 representing 11 states in the United States of America, Australia, France, and Mexico at 11 microsatellite loci resulting in 165 MLHs. Populations were loosely structured over space and time based on analysis of molecular variance and discriminant analysis of principal components, but not by cultivar, aggressiveness, or field source. Our study suggests that breeders should continue to test dry bean lines in several WMSN across the USA to account for both the phenotypic and genotypic variation that exists across regions. All aspects of this project from raw data, R analysis pipeline, and figure generation are publicly available and reproducible online. Thus, this represents a gold standard for open science research and a case study for reproducible research in the *Sclerotinia* community that is attainable by anyone in our field of work.

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Rescue of Sclerotinia sclerotiorum Hypovirulence-Associated DNA Virus 1 (SsHADV-1) for White Mold Control

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Funded Plan of Work: N/A

ABSTRACT

Sclerotinia Sclerotiorum is a cosmopolitan pathogen that causes necrotic effects on more than 400 plant species, many of which have not been adequately controlled by conventional technologies. Gemycircularviruses are stable, ubiquitous and infect fungi mainly. Among them, sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) shows debilitating effect on white mold. The ability of the virus to be transmitted extracellularly allowed the performance of field-trials in Asia that reduced the severity of white mold on rapeseed. In USA, the presence of this virus reported till now was only sequences detected from metagenomic sequencing. Without a phenotype or a live culture, we cannot develop the virocontrol strategy. The goal of this study is to test the delivery of gemycircularvirus-based mycoviral fungicides directly or from viral genomes integrated into plant chromosomes to create or enhance defence mechanisms in a plant to resist *S. sclerotiorum*. In this study, we designed and constructed a SsHADV-1 clone that consists of a dimer of the corresponding viral genome starting from synthetic fragments. Further, a soybean leaf-associated gemycircularvirus (SlaGemV1) was discovered from metatranscriptomic screening and the dimer has been cloned from the leaf DNA extract. So far, the dimer clone of SsHADV-1 is shown to be sufficient to start self-replicating inside the fungal host. The dimer clone of SlaGemV1 is being introduced into *S. sclerotiorum* to determine the infectivity. The presence of replicating SsHADV-1 has been confirmed using rolling circle amplification (RCA) and sanger sequencing in fungal strains, DK3 and 1980. SsHADV-1 infected DK-3 and 1980 shows phenotypic differences compared to wild type. The growth rate of fungus and plant infectivity are severely decreased with SsHADV-1 infection as compare to wild type fungus. Transgenic plants are being developed that express episomal copies of SsHADV-1 or SlaGemV1. The viral dimer cassettes are being engineered into *Arabidopsis* as a proof of concept to show that by integrating into plant genomes these naturally occurring viral elements detected from fungal isolates or indirectly from plant tissues, it is a promising approach to enhance resistance and contribute to the integrated management of white mold in affected crops.

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Understanding and Improving Basal Resistance to *Sclerotinia sclerotiorum*

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Funded Plan of Work: Enhancing basal resistance to *Sclerotinia sclerotiorum* in Brassica

ABSTRACT

The goal of the project is to use the newly identified *Arabidopsis thaliana* gene hypersusceptible to *S. sclerotiorum* (*HSS1*) for engineering high levels of disease resistance in canola. Through map-based cloning, we have determined that *HSS1* encodes the Mediator complex subunit MED16, indicating that MED16 is a key component regulating basal resistance to *S. sclerotiorum*. We found that the *Brassica napus* MED16 is biologically functional, since the *BnHSS1* homolog complemented the *A. thaliana hss1* mutant. Unfortunately, overexpression of either the *Arabidopsis HSS1* gene or the *BnHSS1* homolog did not provide an increase in resistance to *S. sclerotiorum*. To better understand these results, we analyzed the fate of the HSS1/MED16 protein during infection and discovered that *S. sclerotiorum* infection induces a modification followed by complete degradation of HSS1/MED16. We have screened other novel forms of HSS1/MED16 from fungi and non-host plants for their ability to confer resistance to *S. sclerotiorum*. We found that the rice *HSS1* (*OsHSS1*) homolog complemented the *A. thaliana hss1* mutant and the *S. sclerotiorum HSS1* homolog did not. Over-expression of *OsHSS1* in the *A. thaliana hss1* mutant background increased resistance to *S. sclerotiorum* above the wild-type level. The Mediator complex is emerging as a master regulator of plant immunity against pathogens, especially necrotrophic fungal pathogens, which underlines our discovery of the critical role of *HSS1* in basal resistance against *S. sclerotiorum*. We are now focusing our efforts on engineering the MED16 signaling cascade to determine if a further increase in resistance is possible.

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Using genomic selection to optimize prediction of *Sclerotinia* and agronomic phenotypes for more efficient breeding

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Funded Plan of Work: Using GS to optimize prediction of Sclerotinia and agronomic phenotypes for more efficient breeding.

ABSTRACT
Sunflower breeding has made huge gains in disease resistance and quality traits that are simply inherited, but lacks efficiency to adequately deal with Sclerotinia resistance, because of the complex genetic architecture. Many genes of small effect must work in concert to facilitate partial resistance. Lines exist with high levels of resistance in the field, as demonstrated by recent germplasm evaluations. They do not, however, bring the most favorable yield and agronomic characteristics to sunflower hybrids. The primary goal of this work is to better balance the intensity and efficiency of selection for Sclerotinia resistance and other agronomic traits, to make more breeding progress per generation on all traits proportional to their actual value to the producer. Genomic Selection (GS) is a new statistical technology we would like to investigate for this purpose. Up to this point in the project, we have used GBS or skim sequencing to genotype all of the lines we have evaluated for yield, agronomic traits, Phomopsis stalk canker, and Sclerotinia head and stalk rots since 2008. We have found that trio imputation with 5 to 10x whole genome shotgun data from parental lines can result in dense data sets for breeding populations, and that for single populations, can result in around 2000-3000 markers that show almost all of the recombination history in a biparental population with additional redundancy to account for genotyping error, which is described in a new paper in Frontiers in Plant Science. For the remainder of the project, we are extending imputation out to more complex population designs such as grandparent situations (three or four way crosses), and running final models for genomic selection to understand best practices for the technology. We are currently analyzing sequence data from our association mapping population to develop a second-generation model that has the needed higher density than our original Illumina chip data and will assemble the updated model this spring for publication. We have also grown a portion of our association population in the field, sampled the rhizosphere at the late vegetative/early reproductive stage, and are characterizing the microbial population that would interact with the Sclerotinia pathogen in a basal root infection scenario. Our report will provide the most up-to-date knowledge from these investigations.

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