2008 National Sclerotinia Initiative Annual Meeting

January 23-25, 2008

Holiday Inn Select

Minneapolis (Bloomington), MN

Sclerotinia Initiative Abstracts

Sclerotinia Initiative 6th Annual Meeting,
January 23-25, 2008
Holiday Inn Select Minneapolis/St. Paul International Airport
Agenda

Wednesday - January 23, 2008

6 - 8 pm	Poster Session/Reception (posters are displayed throughout the entire meeting) (**Cortland**)

Thursday - January 24, 2008

7:30 am	Registration/Continental Breakfast (**Fireside**)
8:15 am	Welcome and Introductions **- Bill Kemp, USDA-Agricultural Research Service, Fargo, ND**
8:25 am	ARS National Program Staff Update – **Rick Bennett, USDA-Agricultural Research Service, Beltsville, MD**
8:45 am	Federal Budget Environment & Implications to Sclerotinia Initiative – **Bev Paul, Gordley Associates, Washington, DC**
9:05 am	Meeting Charge – **Larry Chandler**, **USDA-Agricultural Research Service, Ft. Collins, CO**
9:15 am	***Guest Speaker*** How Far Have We Come? -- Strategic Planning & Reporting Progress – **Rich Wilson (USDA-Agricultural Research Service, National Program Staff –Retired, Raleigh, NC)**
9:45 am	Break (**Fireside Foyer**)
10:30 am	***Featured Speaker*** Developing a Disease Warning System for Sclerotinia Stem Rot in Canola – **Luis del Rio, North Dakota State University, Fargo, ND**
11:00 am	***Guest Speaker*** Farmers Have More Choices Than Ever – What to Plant? – **Mike Krueger, The Money Farm, Fargo, ND**
11:50 am	Discussion
Noon	Working Lunch (**Cortland**)
	Sclerotinia Research Activities – Session 1 (**Fireside**) **Moderator – Larry Chandler (USDA-Agricultural Research Service, Ft. Collins, CO)**

1:30 pm Environmental and Biochemical Factors Affect Expression of Resistance in Soybean to *Sclerotinia sclerotiorum* – **Angelique Peltier, University of Wisconsin, Madison, WI**

1:50 pm Development of Sclerotinia Resistant Germplasm Utilizing Wild *Helianthus* – **C. C. Jan, USDA-Agricultural Research Service, Fargo, ND**

2:10 pm Introgressing White Mold Resistance from the Secondary Gene Pool of Common Bean – **Shree Singh, University of Idaho, Moscow, ID**

2:30 pm Genetics & Mapping of White Mold Resistance in Lentil – **Weidong Chen, USDA-ARS, Pullman, WA**

2:50 pm Discussion

3:00 pm Break & Poster Session (**Cortland**)

Sclerotinia Research Activities – Session 2 (**Fireside**)
Moderator – Martin Chilvers (Washington State University, Pullman, WA)

3:30 pm Identification & Functional Analysis of Candidate (Sclerotinia) Defense-Related Genes in Soybean and Arabidopsis – **Steve Clough, USDA-Agricultural Research Service, Urbana, IL**

3:50 pm Sclerotinia Resistance Enhanced by Accumulation of QTL and Transgenic Approaches **– George Graef, University of Nebraska, Lincoln, NE**

4:10 pm Mapping QTL for White Mold Resistance in an Interspecific Dry Bean Backcross Population – **Mark Brick, Colorado State University, Ft. Collins, CO**

4:30 pm Defense Within the Context of Susceptibility to Sclerotinia **– Henrik Stotz, Oregon State University, Corvallis, OR**

4:50 pm Discussion & Wrap-up

5:00 pm Adjourn

Dinner on your own

Friday - January 25, 2008

7:00 am Steering Committee Breakfast Meeting (**Beacon Conference Room**)

7:15 am Continental Breakfast (**Fireside Foyer**)

Sclerotinia Research Activities – Session 3 (**Fireside**)
Moderator – Kevin McPhee (USDA-Agricultural Research Service, Pullman, WA)

8:00 am Integrated Sclerotinia Field Screening – **DoKyoung Lee, North Dakota State University, Carrington, ND**

8:20 am	White Mold Resistance Identified in Multi-site Tests and Choice of Pathogen Isolates for Resistance Screening Matters – **Jim Steadman, University of Nebraska, Lincoln, NE**
8:40 am	Enhancing Soybean for Resistance to Sclerotinia Stem Rot – **Dechun Wang, Michigan State University, East Lansing, MI**
9:00 am	Cultivar Selection, Plant Spacing and Fungicide Effects upon White Mold Management in Dry Bean – **Howard Schwartz, Colorado State University, Ft. Collins, CO**
9:20 am	Greenhouse Evaluation of Wild Sunflower for Resistance to Sclerotinia Stalk Rot – **Charles Block, USDA-Agricultural Research Service, Plant Introduction Station, Ames, IA**
9:40 am	Break (**Fireside Foyer**)
	Strategic Plan & Annual Report Update (**Fireside**) **Moderator – Bill Kemp (USDA-Agricultural Research Service, Fargo, ND)**
10:00 am	Crop Germplasm Resources & Genetics (Goal 1) – **Phil Miklas, USDA-Agricultural Research Service, Prosser, WA, & George Graef, University of Nebraska, Lincoln, NE**
10:30 am	Pathogen Biology & Development (Goal 2) – **Jim Steadman, University of Nebraska, Lincoln, NE**
11:00 am	Pathogen & Host Genomics (Goal 3) – **Steve Clough – USDA-Agricultural Research Service, Urbana, IL**
11:30 am	Pathogen Epidemiology & Disease Management (Goal 4) – **Luis del Rio - North Dakota State University, Fargo, ND**
Noon	Working Lunch (**Cortland**)
1:15 pm	RFP Process Improvement, Strategic Plan Discussion, Assignment of Additional Tasks & Wrap-up of Initiative Business - **Larry Chandler, Jim Quaratino, Rich Wilson, & Bill Kemp**
2:30 pm	Adjourn (Travel Safely!)

2008 Sclerotinia Initiative Poster Session

Epidemiology & Disease Management		
Poster No.	Title	Author
1	Using fungicides as a management strategy to reduce effects of head rot on sunflower.	S. Halley, B. G. Schatz, E. Aberle, D. K. Lee, K. Misek
2	Effect of partial genetic resistance on efficacy of Topsin fungicide for control of white mold disease in pinto bean.	Phillip Miklas, Lyndon Porter
3	Effect of temperature and period of high relative humidity on white mold resistance of selective germplasm from the *Pisum* core collection.	Lyndon D. Porter, Ginny Coffman
4	Dynamics of *Sclerotinia sclerotiorum* ascospore dispersal in canola fields.	I. S. Qandah, L. E. del Rio
5	Cultivar, plant spacing and fungicide effects upon white mold management in dry bean.	Howard F. Schwartz, Mark A. Brick, Shree P. Singh
Genomics		
Poster No.	Title	Author
6	Identification and functional analysis of candidate Sclerotinia defense genes in soybean.	Bernarda Calla, Daina Simmonds, Steven J. Clough
7	Sequencing of expressed sequence tags of *Sclerotinia sclerotiorum* and *Pisum sativum*.	Martin Chilvers, Tobin Peever, Kevin McPhee, Weidong Chen
8	Further molecular genetic dissection of susceptibility to *Sclerotinia sclerotiorum* in *Arabidopsis*.	X. Guo, A. Doyle, H. Stotz
9	Identification of QTL conditioning partial resistance to white mold in kidney bean line VA19 derived from an interspecific population.	Marilyn Soule, Phil Miklas, Lyndon Porter
Pathogen Biology & Development		
Poster No.	Title	Author
10	White mold resistance identified in multi-site tests and choice of pathogen isolates for resistance screening matters.	Otto-Hanson, L.K., Steadman, J.R.

Variety Development/Germplasm Enhancement		
Poster No.	Title	Author
11	Evaluation of wild *Helianthus* species for resistance to Sclerotinia stalk rot.	Charles C. Block, Thomas J. Gulya, Laura F. Marek
12	Combining resistance to white mold from common and scarlet runner bean.	Mark A. Brick, Mark A. Newell, Patrick F. Byrne, Howard F. Schwartz, J. Barry Ogg, Barbara Gilmore, James R. Myers
13	Identifying pathogenicity determinants of *Sclerotinia sclerotiorum*.	Weidong Chen, Kevin McPhee, George Vandemark
14	Advances in the development of sunflower germplasm with resistance to both Sclerotinia stalk rot and head rot-2007.	Thomas J. Gulya, Brent S. Hulke, Nikolay F. Balbyshev
15	Incorporating Sclerotinia stem rot resistance from diverse perennial wild species into cultivated sunflower.	Jiuhuan Feng, C. C. Jan, Gerald J. Seiler, Thomas J. Gulya
16	Screening sunflower hybrids for Sclerotinia head rot resistance in the northern Great Plains.	D. K. Lee, Blaine Schatz, Ezra Aberle, Char Hollingsworth, Khalid Rashid, Scott Halley, T. J. Gulya
17	White mold resistance in pea and lentil through breeding and biotechnology.	K. McPhee, W. Chen, B. Schatz, F. Muehlbauer
18	Transfer of Sclerotinia resistance from *Phaseolus coccineus* to *P. vulgaris*: An assessment.	J. R. Myers, J. E. Haggard, B. S. Gilmore, M. Barrett, S. Zimmerman, J. Davis
19	Sequencing of expressed sequence tags of *Sclerotinia sclerotiorum* and *Pisum sativum*.	T. L. Peever, M. I. Chilvers, K. Masato, W. Chen, K. McPhee
20	Environmental and biochemical factors affect expression of resistance in soybean to *Sclerotinia sclerotiorum*.	A. J. Peltier, C. R. Grau
21	Introgressing white mold resistance from the secondary gene pool of common bean.	Shree Singh, Henry Terán, Howard Schwartz, Kris Otto, Margarita Lema
22	Enhancing soybean for resistance to Sclerotinia stem rot.	D. Wang, K. M. Onweller, C. Gu, C. C. Ray
23	QTL for Sclerotinia head rot tolerance in a sunflower population developed from a cross between tolerant lines.	Bing Yue, Scott A. Radi, Brady A. Vick, Xiwen Cai, Shengxue Tang, Steven J. Knapp, Thomas J. Gulya, Jerry F. Miller, Jinguo Hu
24	Progress in mapping QTL for Sclerotinia stalk rot tolerance in a sunflower recombinant inbred population.	Bing Yue, Scott A. Radi, Brady A. Vick, Xiwen Cai, Shengxue Tang, Steven J. Knapp, Thomas Gulya, Jerry MIller, Jinguo Hu

Evaluation of Wild *Helianthus* Species for Resistance to Sclerotinia Stalk Rot

Charles C. Block, USDA-ARS, Ames, IA; Thomas J. Gulya, USDA-ARS, Fargo, ND and Laura F. Marek, North Central Regional Plant Introduction Station, Ames, IA

Funded Plan of Work: Evaluation of Wild *Helianthus* Species for Resistance to Sclerotinia Stalk Rot

ABSTRACT:

Wild *Helianthus* species have been little-explored as a potential source of Sclerotinia stalk rot resistance genes. Variable growth habits and seed dormancy make them difficult to work with in the field. One of our objectives was to develop a reliable greenhouse screening method so that much of the susceptible germplasm could be eliminated and only the most promising materials advanced to field trials. Growth chamber studies of the effects of incubation temperature, pot size, pot shape, growth media, inoculum quantity (*Sclerotinia*-infested millet), and inoculum placement in the pot identified incubation temperature as the most critical factor. The best differentiation of resistance was obtained in the range of 21C to 25C. Susceptibility was often masked at higher temperatures such as 27C to 30C.

Sixty-one accessions were evaluated in replicated greenhouse trials in 2007, representing 11 taxa. Excellent wilt resistance was identified in the perennial hexaploid species, *Helianthus resinosus.* Several accessions of *H. debilis* and *H. argophyllus* also showed promising levels of resistance in the greenhouse. Five *H. resinosus* accessions and one *Helianthus* hybrid (*H. annuus* x *argophyllus*, PI 597912) were placed in field trials at Fargo in 2007. The *H. resinosus* accessions as a group had 92.5% plant survival (no wilt) in the field vs. 94% in the greenhouse. PI 597912 had 50% plant survival in the field vs. 72% in the greenhouse. The most resistant hybrid check had 17% survival in the field vs. 56% in the greenhouse while the susceptible check had 21% survival in the field vs. 12% in the greenhouse.

Current evaluation efforts are focused primarily on the annual diploid *Helianthus* species in the USDA sunflower germplasm collection, with selected perennial accessions included. Much of the wild germplasm is inherently variable, and thus we plan to evaluate a minimum of 40-60 plants from each accession using *Sclerotinia*-infested millet as inoculum. The time in days to first wilt and to permanent wilt are recorded for each plant. After 21 to 24 days, the experiment is halted and the remaining live plants are recorded. Cultivars with known resistant and susceptible field reactions are included in each test group for statistical comparison.

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Combining resistance to white mold from common and scarlet runner bean

M.A. Brick, M.A. Newell, P.F. Byrne, H.F. Schwartz, & J.B. Ogg, Colorado State University, Fort Collins, CO; Barbara Gilmore & James Myers, Oregon State Univ., Corvallis, OR

Funded Plan of Work: Variety Development/Germplasm Enhancement

ABSTRACT:
Resistance to white mold has been found in both common (*P. vulgaris* L.) and scarlet runner bean (*P. coccineus* L.). In 2007, we evaluated the effect of previously reported QTL associated with white mold resistance from common and scarlet runner bean in an interspecific inbred backcross line (IBL) population. The interspecific backcross line (IBL) population was derived from a cross between WM67 and PI 255956. WM67 is a moderately resistant common bean line that possessed resistant QTL found in G122 developed from a cross between G122 and an adapted pinto line. PI255956 is a scarlet runner bean that has high levels of resistance to white mold. Six QTL reported by Maxwell et al. (2007) possessed by WM67 and one reported by Gilmore (2007) possessed by PI 255956 were tested for their effect in the IBL population. Two molecular markers contributed by scarlet runner bean parent PI 255956 accounted for 7.0 ($P<0.05$) and 9.7% ($P<0.05$) of the phenotypic variation in resistance, and two markers contributed by common bean parent WM67 accounted for 10.8 ($P<0.01$) and 12.8% ($P<0.01$) of the phenotypic variation. Four markers could not be evaluated for their associations with WM resistance because they were not polymorphic in the IBL population. The lack of polymorphisms is likely attributed to the low level of recombination between common and scarlet runner bean chromosomes during meiosis, and the selection of gametes with common bean alleles during the development of IBL. We observed severe segregation distortion for all polymorphic molecular markers, and only 16 of the 65 IBL possessed any scarlet runner alleles. Introgression of scarlet runner bean alleles was difficult because most early generation IBL were highly and had crippled phenotype. To show that crossover events did occur between chromosomes from common and scarlet runner bean, we looked for recombination at loci on the same linkage group. Two recombination events were detected. One on linkage group B2b between SSR markers BM152 (1.5%) and BM160 and a second on linkage group B7 between SSR marker BM160 and the *Phs* SCAR marker (15.4%). No other examples of recombination were detected. The results from this study suggest that QTL associated with white mold resistance from common and scarlet runner bean can be combined in an IBL population however due to segregation distortion recombination between common and scarlet runner bean chromosomes is limited. Our results suggest that MAS for resistance QTL from scarlet runner bean into common bean may be a viable method to introgress QTL from scarlet runner bean into common bean to improve white mold resistance in common bean

Contact information – Dr. Mark Brick, Dept. of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523-1170; 970-481-6551; Mark.Brick@colostate.edu.

Mapping QTL for White Mold Resistance from Common and Scarlet Runner Bean Using an Interspecific Backcross Population

M.A. Brick, M.A. Newell, P.F. Byrne, H.F. Schwartz, & J.B. Ogg, Colorado State University, Fort Collins, CO, & Barbara Gilmore & James Myers, Oregon State Univ., Corvallis, OR

Funded Plan of Work: Variety Development/Germplasm Enhancement

ABSTRACT:
Genetic resistance to white mold has been reported in both common (*Phaseolus vulgaris* L.) and scarlet runner (*P. coccineus* L.) beans. From funding received from the USDA Sclerotinia Initiative during the past four years, we were able to (1) validate the utility of previously reported quantitative trait loci (QTL) for white mold resistance derived from G122, and (2) identify additional QTL for resistance in common bean. In 2007, we (1) validated the effect of previously reported QTL associated with white mold resistance in an independent common bean recombinant inbred line (RIL) population, and (2) evaluated the effect of previously reported QTL associated with white mold resistance in both common and scarlet runner bean in an interspecific inbred backcross line (IBL) population. In common bean RIL population G 122/Montrose, two molecular markers previously reported linked to resistant QTL were associated with resistance and accounted for 13.7 (P<0.05) and 33.7% (P<0.01) of the phenotypic variation. One additional QTL on linkage group (LG) B7 was also marginally associated with resistance and accounted for 10.7% (P<0.06) of the phenotypic variation in resistance. Results indicate that transmission of resistance QTL from G 122 can be accomplished using marker assisted selection. In the IBL population WM67/PI 255956, four molecular markers were associated with resistance. Two of the molecular markers were contributed by scarlet runner bean PI 255956 and two from common bean WM67. Molecular markers contributed by scarlet runner bean parent PI 255956 accounted for 7.0 (P<0.05) and 9.7% (P<0.05) of the phenotypic variation in resistance, and markers contributed by common bean parent WM67 accounted for 10.8 (P<0.01) and 12.8% (P<0.01) of the phenotypic variation. The results from this study suggest that QTL associated with white mold resistance from common and scarlet runner bean can be combined in an IBL population. Due to poor chromosome recombination we observed segregation distortion for all polymorphic molecular markers. Furthermore, only 16 of the 65 IBL possessed any of the scarlet runner alleles, and many of the early generation lines were highly sterile and had abnormal phenotype. Because we were unable to obtain a large mapping population that contained proportional scarlet runner alleles, we were unable to map the IBL population. Additional backcrossing to a common bean parent may enable mapping of QTL in future populations. Our results suggest that MAS for resistance QTL from scarlet runner bean into common bean may be a viable method to introgress QTL from scarlet runner bean into common bean to improve white mold resistance in common bean

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Identification and functional analysis of candidate Sclerotinia defense genes in soybean

Bernarda Calla, University of Illinois, Urbana, IL; Daina Simmonds, AgCanada, Ottawa, Ontario; Steven J. Clough, USDA-ARS and University of Illinois, Urbana, IL

Funded Plan of Work: Identification and functional analysis of candidate defense-related genes to *Sclerotinia sclerotiorum* in soybean and Arabidopsis

ABSTRACT:
We have conducted a series of microarray studies that enabled us to identify genes that are significantly differentially expressed in soybean plants in response to *Sclerotinia sclerotiorum.* We are expanding these studies to include effects of oxalic acid, a major virulence factor *S. sclerotiorum*. To assist with the identification of key defense genes, we assigned genes into functional categories based on the annotation of their closest sequence match in public databases and we clustered the genes across multiple experiments. Candidate defense genes will be further characterized by quantitative real-time RT-PCR to verify that the correct gene was identified in the microarray experiments. Promising genes will be functionally characterized by obtaining knockouts of these genes in soybean and/or *Arabidopsis thaliana*. For Arabidopsis we will obtain the T-DNA insertion mutants of the genes that are of interest and that have high sequence identity with a soybean gene. To obtain knockouts in soybean we will use a viral induced gene silencing system and/or generate stable transgenics utilizing RNAi constructs. Additionally, overexpression of candidate defense genes will be studied in Arabidopsis and/or soybean. Promising candidate defense genes will be mapped to see if they map to known QTLs related to defense to *S. sclerotiorum*.

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Role of leaf wetness on *Sclerotinia sclerotiorum* disease development

Luis del Rio, Dept. of Plant Pathology, 306 Walster Hall,
North Dakota State University, Fargo ND

Funded Plan of Work: Developing a disease-warning system for Sclerotinia stem rot of canola

Abstract:

Ascospores are the primary inoculum for most diseases caused by *Sclerotinia sclerotiorum*. Typically, ascospores that land on floral parts germinate and colonize the tissue and use it as a source of energy to penetrate into branches and stems. Successful colonization of tissues by ascospores, under favorable temperature conditions, depends on the availability and duration of moisture on the leaves. In order to model the relationship between moisture and disease development controlled conditions experiments were conducted using a completely randomized design with four replications; and each experiment was replicated three times. Re-hydrated dry bean flowers were dry-inoculated with *S. sclerotiorum* ascospores and placed on pinto bean 'Maverick' seedling leaves. Inoculated seedlings were incubated alternating periods of wet and dry leaf conditions of varied duration. The number of foliar lesions produced was measured over time. Colonization of flowers by ascospores of *S. sclerotiorum* took place even when wet periods as short as 8 hours were alternated with periods of up to 24 hours of dry conditions. A logistic regression model indicated that in general a minimum of 65 hours of cumulative moisture in a period of six days was necessary to have 50% chance of infection. Such probability increased to 80% when cumulative moisture was 70 hours.

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Introgressing Sclerotinia Stalk Rot Resistance from Diverse Wild Perennial Species into Cultivated Sunflower

J. Feng[1], Z. Liu[1], G. J. Seiler[2], T. J. Gulya[2], C. C. Jan[2]

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Funded Plan of Work: Development of Sclerotinia Resistant Germplasm Utilizing Wild *Helianthus* Species

ABSTRACT:
Cultivated sunflower lacks a sufficient level of resistance to Sclerotinia stalk rot, but high levels of resistance have been reported in perennial *Helianthus* species. The objectives were to 1) reconfirm the resistance in perennial *Helianthus* species and in previously developed interspecific amphiploids, and 2) transfer the resistance genes into a cultivated background. We have completed a 3-year project funded by the Sclerotinia Initiative and are proposing a new 4-year project based on the progress of the past 3 years. In the first two years of the previous project, field and greenhouse evaluations indicated excellent stalk rot resistance in six amphiploids. Resistant amphiploids were crossed with HA 410 in 2006, and BC_2F_1 plants with chromosome numbers ranging from 2n=34 to 41 were established in the greenhouse for further backcrosses or seed increase for field evaluation. In addition, interspecific F_1 progeny were produced between stalk rot resistant hexaploid *H. californicus* and *H. schweinitzii* and HA 410. The F_1 plants were resistant to stalk rot and had good backcross seed set. Continued backcrossing with HA 410 resulted in BC_4F_1 plants with improved pollen and seed fertility and with 2n chromosome numbers between 34 and 40. These progenies can be further backcrossed or seed increased for field evaluation. In the third year (2007), stalk rot resistant diploid perennial *H. maximiliani, H. giganteus,* and *H. grosseserratus* were crossed with HA 410 and F_1 progeny obtained via embryo rescue. A sufficient number of backcrossed seeds were obtained to continue the project. In summary, wild perennial *Helianthus* species and interspecific amphiploids involving wild perennial *Helianthus* species were found to be highly resistant to Sclerotinia stalk rot. Embryo rescue proved to be essential for the establishment of the F_1 interspecific hybrids. F_1 hybrids had low pollen and seed set fertility but sufficient backcrossed seed were obtained for most crosses. Backcrossed progeny with 2n=34 were obtained from all selected resistant sources and selfed to produce seed for replicated field tests. The continuation of this project will produce a large number of segregating families for selecting resistance to the most devastating disease of sunflower. In addition, understanding the genetics of the resistance and the follow-up QTL molecular mapping will provide useful tools for marker-assisted selection for Sclerotinia resistance breeding.

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Sclerotinia resistance enhanced by accumulation of QTL and transgenic approaches

George L. Graef, Thomas E. Clemente, James R. Steadman, Tamra Jackson
University of Nebraska, Lincoln, NE

Funded Plan of Work: Sclerotinia resistance enhanced by accumulation of QTL and transgenic approaches

ABSTRACT

This research is being conducted to increase the level of resistance to *Sclerotinia sclerotiorum* in soybean cultivars and to develop and evaluate improved disease control and resistance options for producers. The first goal is to increase the level of resistance to *S. sclerotiorum* in soybean. Objective 1 is to combine quantitative trait loci (QTL) that were previously mapped and identified with the resistance phenotype into single breeding lines. We identified 40 F5:6 lines with the smallest lesion size that were evaluated during 2006 for reaction to *S. sclerotiorum* in 12 replications of a lattice design using the detached leaf test (DLT). Nineteen of the lines had a lesion size equal to or smaller than the best parent in the cross, and better than the resistant check NKS19-90. The 19 selected F5:7 lines were evaluated again during 2007 using the DLT, as well as in multi-location tests to evaluate yield and agronomic characteristics. Objective 2 is to determine if a novel antifungal synthetic peptide expressed in soybean will confer resistance to *S. sclerotiorum.* We developed transformed plants with a codon-optimized gene-expression cassette for the antifungal peptide that contains the barley alpha-amylase signal sequence to export the peptide to the apoplast. We conducted the DLT on T2 populations from seven independent transformation events during summer 2007. Results indicated no significant difference between the plants with the lytic peptide and those without the inserted gene expression cassette. We will follow with development and evaluation of homozygous T2-derived lines during 2008. The second goal is to improve the use of calcium cyanamide as a control option for *S. sclerotiorum*. Our previous results indicate that the *cah* gene has no negative effects on yield in the transgenic lines vs. the non-transgenic control. Furthermore, Perlka application reduced germination of sclerotia and increased yield. It is unlikely, however, that the results for sclerotinia reaction alone will be sufficient to justify regulatory approval expenses for a transgenic event. Perlka has been shown to affect other pathogens as well as nematodes, and it has herbicidal activity. These effects together could make an attractive disease management package for producers. Objective 1 is to evaluate effects of Perlka™ (granular Ca-cyanamide) on soybean cyst nematode, *Heterodera glycines* (Ichinohe). Results over four environments during 2006 and 2007 show some possible reduction in SCN egg counts for the 100 kg/ha Perlka treatment at planting.

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Advances in the Development of Sunflower Germplasm with Resistance to Both Sclerotinia Stalk Rot and Head Rot – 2007.

T. J. Gulya, B. S. Hulke, N. F. Balbyshev. Sunflower Research Unit, USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58105-5677.

Funded Plan of Work: Development of Sunflower Germplasm with Resistance to Sclerotinia Stalk Rot and Head Rot

ABSTRACT:

Sclerotinia diseases remain the most significant of all diseases on both oilseed and confection sunflower production in the U.S. In 2007 Sclerotinia stalk rot and head rot affected 30% and 26%, respectively, of fields surveyed in North Dakota, South Dakota, Minnesota, Kansas, Colorado and Texas. Stalk rot and head rot affected 1.3% and 2.7% of the 1.97 million acre U.S. crop, with the greatest incidence and severity in North Dakota, where 52% of the U.S. crop is produced. 2007 was a transition year for the USDA Sunflower Unit with the retirement of our long-time geneticist in January and the hiring of a new geneticist in September. While no major breeding efforts were done during the summer of 2007, there were eight oilseed lines and eight confection genetic stocks released in the fall of 2006. In an effort to identify new sources of stalk rot resistance, a portion of the USDA cultivated sunflower germplasm collection from the Plant Introduction Station (Ames, IA) was tested in one location with artificial inoculation. One-hundred-fifty accessions out of approximately 800 without Sclerotinia data were tested in 2007. Entries in the trial ranged from 0% infection (6 entries) to 80%, with a trial mean of 26% infection. The most resistant entries originated from Russia, Spain, and Yugoslavia and will be retested in multiple inoculated stalk rot trials and head rot trials in 2008 for verification. Concerted efforts will be made in 2008 to evaluate the remaining Plant Introductions for stalk rot, and in subsequent years for head rot, in an effort to identify germplasm with resistance to both diseases. Ninety-five commercial hybrids were evaluated for stalk rot resistance at four locations, all with artificial inoculation, and these same 95 entries were tested by NDSU researchers at Carrington, ND for head rot resistance. Three locations generated usable stalk rot data, and this has been forwarded to NDSU for publication in their annual sunflower bulletin A-652 (http://www.ag.ndsu.edu/pubs/plantsci/rowcrops/a652w.htm). Stalk rot severity, averaged over three locations, ranged from a low of 6% to a high of 63% infection at maturity. Head rot data for commercial hybrids, supplied by the NDSU researchers, helped to identify hybrids possessing good levels of resistance to both head rot and stalk rot.

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Further Molecular Genetic Dissection of Susceptibility to *Sclerotinia sclerotiorum* in *Arabidopsis*

Xiaomei Guo, Ariel Doyle, and Henrik Stotz, Oregon State University, Corvallis, OR

Funded Plan of Work: Genetic Mechanisms of Resistance to *Sclerotinia sclerotiorum*

ABSTRACT:

Our published data [Guo & Stotz (2007) *Mol. Plant-Microbe Interact.* 20: 1384-1395] show that *S. sclerotiorum* activates jasmonate, salicylic acid, and ethylene signaling. Mutations in the jasmonate receptor *coi1* and central regulators of salicylic acid and ethylene signaling, *npr1* and *ein2*, respectively, increase susceptibility to *S. sclerotiorum*. Thus, activation of these three defense pathways is implicated in defense to *S. sclerotiorum*.

In addition to defense signaling, phytoalexin biosynthesis appears to be required for defense because the *pad3* mutant is hypersusceptible to *S. sclerotiorum*. *PAD3* encodes the cytochrome P450 enzyme CYP71B15, which catalyzes the last step in camalexin biosynthesis.

The contribution of abscisic acid (ABA) signaling to defense against *S. sclerotiorum* has been further characterized. The ABA-insensitive *abi1* and *abi2* mutants are hypersensitive to oxalic acid and hypersusceptible to *S. sclerotiorum*. Wilting symptoms and water loss are enhanced in *abi1* mutant, suggesting that this mutant cannot counteract the detrimental effects of oxalic acid in the absence of ABA signaling. Guard cells respond to oxalic acid with an increase in production of reactive oxygen species (ROS). However, in the presence of ABA, the known ABA-induced increase in ROS production is blocked by oxalic acid. This observation is consistent with the published interference with elicitor-induced ROS production. It is unknown which cellular compartments respond differently to ABA or oxalic acid. Alternatively, spatial or temporal differences in ROS production may exist within guard cells. We are in the process of testing oxalate-triggered ROS production in the *abi1* mutant because this protein phosphatase 2C blocks ROS production in response to ABA. We hypothesize that ROS production is increased in *abi1* mutants after treatment with oxalic acid.

One of the mutants identified in a yeast screen for oxalate sensitivity was *vma5Δ* [Cheng et al. (2007) *Appl. Environ. Microbiol.* 73: 5919-5927]. The *Arabidopsis* ortholog *det3* was shown to be supersensitive to oxalic acid. In addition, the *det3* mutant is hypersusceptible to *S. sclerotiorum*. *DET3* encodes the C-subunit of the vacuolar H^+-ATPase. We have also found evidence for activation of the plasma membrane H^+-ATPase by oxalic acid based on vanadate inhibition of oxalate-induced opening of stomatal pores in leaves of *Vicia faba*. Thus, pH homeostasis is apparently required to dampen oxalate stress.

In conclusion, dicot plants are able to mount defense responses while, at the same time, being susceptible to virulence factors like oxalic acid.

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Using Fungicides as a Management Strategy to Reduce Effects of Head Rot on Sunflower

S. Halley(1), B. G. Schatz(2), E. Aberle(2), D.K. Lee(2), and K. Misek(1)

ABSTRACT: Fungicides have been used effectively to reduce the effects of white mold disease caused by the pathogen *Sclerotinia sclerotiorum* L. on soybeans, dry beans, canola and several other crops. White mold disease affects over 400 species of crops including sunflower and has been studied extensively on crops grown in some regions. Sunflower has not been one of those crops. Sunflower is infected by mycelial germination from the sclerotia through the root system, mid stalk infection from ascospores infecting through a leaf, and by carpenogenic germination of the sclerotia. During carpenogenic germination one or more apothecia develops from sclerotia near the soil surface and expels ascospores into the boundary atmospheric layer. When conditions are right these spores deposit on the flowering sunflower head and infect causing what is commonly known as head rot. Using fungicide is of interest to producers as a management strategy because levels of resistance to head rot in cultivar evaluations have not been adequate. The sclerotia can live up to eight years in the soil limiting benefit of rotation to non susceptible grass crops as a management strategy and because more than one environmental condition needs to be met for an epidemic to occur. The need for effective strategies has been quite unpredictable. Epidemics occur when the sclerotia are present close to the soil surface (1.5 inches or less) and the soil water is near field capacity for 12 days to germinate sclerotia. After expulsion of the ascospores, the sunflower must be at flowering growth stage and have free water and high relative humidity levels to allow the germination of ascospores to infect the heads. Ascospores are very small and travel long distances when the atmosphere is turbulent, a common condition on the prairies.

Fungicides evaluations were initiated in 2005 with funding support from the Sclerotinia Initiative. Statistical differences among treatments have not previously been reported although numerical yield increases have exceeded 600 lbs/acre. Measuring differences among fungicides treatments has many challenges. The crop is affected by many pests including insects, birds, deer, bacteria and fungi. In 2007 studies were conducted at the Carrington and Langdon Research Extension Centers focusing on screening fungicides for efficacy. An additional study was conducted at Langdon for Winfield Solutions LLC evaluating adjuvants for enhancement of Endura (boscalid) fungicide efficacy. Three proprietary and one Topsin M (thiophanate methyl) fungicide treatments reduced white mold incidence at Langdon compared to an untreated plot. No statistical differences were measured at Carrington but the Topsin M treatment numerically increased yield by 550 lbs. Adjuvants were not effective in reducing white mold incidence compared to Endura fungicide alone but may react differently with a different fungicide chemistry.

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Screening Sunflower Hybrids for Sclerotinia Head Rot Resistance in the Northern Great Plains

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Funded Plan of Work: Sunflower Head Rot Screening Nursery

ABSTRACT:
Sclerotinia head rot (*Sclerotinia sclerotiorum*) is a devastating disease of sunflower (*Helianthus annuus* L.) and no resistant commercial hybrids are available. A long-term germplasm screening nursery was established in 2000 at the North Dakota State University Carrington Research Extension Center. Sclerotinia head rot was not a major limiting factor for sunflower yield in 2007. However, extended precipitation and foggy conditions caused some level of sclerotinia pressure during the late season (2007 Crop Survey). In 2007, the initial screening of eighty entries was conducted at Carrington, ND, Crookston, MN, and Morden, Canada and twenty of the best entries for both head rot and stalk rot in the 2006 initial screening were evaluated at Carrington, ND, Langdon, ND, Morden, Canada, and Oakes, ND. Entries consist of production hybrids and experimental lines submitted by private breeding programs. Individual heads are inoculated with ascospores and plots were misted to provide favorable conditions for disease development. After several weeks of misting, inoculated heads are evaluated for incidence and severity of head rot. Tests were verified using a susceptible check and a resistant check. Incidence of the resistant check was 2.5% and 92.5% and incidence of the susceptible check was 30% and 100% for Carrington and Oakes, respectively. Langdon and Morden had limited disease pressure for both the repeat test and the initial screening. The range of incidence levels for the repeat evaluation was 0 - 39% and 72 - 100% for Carrington and Oakes, respectively. There was no correlation between locations due to the very high incidence in Oakes. However, the repeat test showed that a repeated evaluation is necessary to confirm previous observations. For the initial screening, good disease pressure was achieved at Carrington (0 - 45.9%) and Crookston (21 – 98%) and highly significant correlation existed between entry rankings at the two sites ($r = 0.66$, $P < 0.001$). Further testing is needed to confirm these observations at various locations in 2008.

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White mold resistance in pea and lentil through breeding and biotechnology

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Funded Plan of Work: Improved resistance to *S. sclerotiorum* in pea and lentil through breeding and biotechnology

ABSTRACT:
Two approaches to develop resistance to *Sclerotinia sclerotiorum* in pea and lentil have been initiated. The first approach involved screening 37 pea genotypes and 306 plant introductions under field conditions at Carrington, ND. One hundred fifty plant introductions were evaluated for reaction to Sclerotinia white mold in single replicate evaluations in 2006 and 156 were evaluated in 2007. Research plots for advanced lines and cultivars consisted of seven rows spaced 18 cm apart and 7.6 m long and were arranged in a randomized complete block design with 4 replicates. At early bloom all plots were inoculated with ascospores. Immediately after inoculation a misting system was used to maintain high humidity and favor disease development. The misting system was run for 2-4 minutes every half hour, 24 hours/day, for 4 weeks. Disease was scored periodically and growth and development data were recorded. Disease scores at Carrington in 2006 were not as severe as 2005 due to dry and unfavorable conditions. In 2005, statistically significant differences were observed in all parameters measured at Carrington except days to physiological maturity where disease progression did not allow accurate assessment of physiological maturity (and powdery mildew) in all plots. Several entries showed relatively high levels of susceptibility on the first evaluation date and on the final evaluation date, 'Arvika' (forage pea) and 'CDC Sonata' showed the lowest disease occurrence in 2005. In 2006, 'Admiral' (0.5) and 'Carneval' (1.0) had the lowest disease ratings and Arvika had a score of 2.8 compared to 3.8 in 2005. A highly significant negative correlation was observed between yield, days to beginning and end bloom and disease rating. Disease reaction scores for 150 accessions evaluated in 2006 ranged from 0.0 to 5.0 on a scale of 0 to 10 where, 0 = no disease and 10 = all plants showing symptoms. Genotypes with the greatest level of resistance will be used to develop genetic mapping populations for inheritance studies. Evalation of the PI accessions should be repeated to verify disease reaction from single replicate nurseries. The second approach involved introducing the oxalate oxidase gene from barley (*Hordeum vulgare* L.) into pea and lentil through *Agrobacterium tumefaciens*-mediated transformation. The oxalate oxidase gene was successfully cloned from barley cDNA and incorporated into a twin binary vector. Transformation experiments using two pea cultivars, 'Mukta' and 'Joel', and one lentil cultivar, 'Pardina', have been completed; however, now transformants have been recovered. Additional experiments are planned using a modified T-DNA in an attempt to improve transformation efficiency. The twin binary vector will allow the selectable marker gene, *nptII*, to be separated from the oxalate oxidase gene through natural Mendelian segregation. This will be beneficial if deregulation of the transformants is pursued.

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Effect of partial genetic resistance on efficacy of Topsin fungicide for control of white mold disease in pinto bean

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Funded Plan of Work: Contribution of partial genetic resistance to white mold disease management in pinto bean

ABSTRACT:
Pinto bean is the most important dry bean market class grown in the U.S., but is one of the most susceptible to white mold disease. Developing pinto bean with partial resistance is a major goal of plant breeders, but the effect of partial resistance on efficacy of fungicide application for disease management is unknown. Our goal was to document the effect partially resistant or less susceptible lines and cultivars have on overall white mold disease control in pinto bean. The pinto breeding line USPT-WM-1 with partial resistance, less susceptible pinto cultivars Maverick and Winchester, upright pinto cultivar Aztec with potential disease avoidance, and Montrose pinto as a susceptible check, were selected for use in this study. The select pintos were grown in replicated trials in multiple environments under moderate to severe disease pressure. A commercial fungicide Topsin M was applied at recommended rates and bloom stages. Four spray treatments were used, 0, 1, 2, and 3, applications. Across years and genotypes Topsin M reduced disease severity by 32, 37, and 53%, for 1, 2, and 3 applications, respectively, but only had a positive yield response for 1 (14%) and 2 applications (23%), suggesting that a third application is unnecessary and two applications is economically superior to one. Two Topsin M applications improved yield the most (26%) for the four most susceptible pintos and benefited yield (4%) of the partially resistant cultivar USPT-WM-1 the least because this line has stable yield potential under moderate to severe disease pressure regardless of fungicide application. It was observed that reduced lodging, increased plant height, and late maturity were moderately correlated (~45%) with reduced disease severity, indicating importance of disease avoidance traits in combating white mold disease. Aztec with disease avoidance traits alone was highly susceptible in the absence of a fungicide application. USPT-WM-1 has disease avoidance traits too but the avoidance is augmented by physiological resistance which is lacking in Aztec. In summary results suggest that pinto cultivars with combination of partial resistance and disease avoidance traits will yield effectively under moderate white mold disease pressure without Topsin M fungicide.

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Transfer of *Sclerotinia* resistance from *Phaseolus coccineus* to *P. vulgaris*: An assessment

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Funded plan of work: Mapping Sclerotinia Resistance in common bean: Characterizing *P. coccineus* resistance QTL

ABSTRACT:
White mold (*Sclerotinia sclerotiorum*) is a serious disease of common bean (*Phaseolus vulgaris.* One of the best sources of resistance to white mold is *P. coccineus* (runner bean). In our program, we first screened the *P. coccineus* USDA-NPGS plant introduction collection to identify accessions with highest levels of resistance. We crossed one of these (PI255956) to a susceptible *P. coccineus* heirloom variety ('Wolven Pole') to create an F_2 mapping population. A total of 215 RAPD, SSR, and AFLP markers were placed in thirteen linkage groups and spanned a total distance of 797 cM. We characterized the population phenotypically for white mold resistance using the straw test, and mapped molecular markers and QTL associated with resistance. Subsequently, we crossed and backcrossed PI255956 to the highly susceptible Oregon 91G common bean cultivar to create a $BC_2F_{4:6}$ advanced QTL backcross inbred population that was used for phenotypic characterization and mapping. Seventy seven SSR and 59 AFLP markers were scoreable in this population. The linkage map constructed consisted of 11 linkage groups that correspond to 9 of the 11 core map linkage groups based on known SSR marker locations, and a single LG with no anchoring loci. The backcross-inbred population was tested with the straw test repeated three times, an oxalate tolerance test, and in a single field environment. With the P. coccineus intraspecific population, we discovered two QTL for a five week straw test that explained a total of 90% of the phenotypic variation. These QTL were located on an unlinked fragment associated with an AFLP and two RAPD markers. In the backcross inbred population, 29 potential loci were identified through single factor analysis but only one on B09 explained 6% of phenotypic variation when subjected to composite interval mapping analysis. Several linkage groups (b01, b04, and b05) were not represented in this population and a high level of segregation distortion was observed, with more heterozygotes than expected. Eight backcross-inbred interspecific lines (811/43-4, 826/48-3, 828/48-5, 853/6-9, 861/13-14, 891/15-2, 903/20-2, and 904/20-3) have shown white mold resistance similar to G122, NY6020, and Ex Rico over two field seasons.

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White Mold Resistance Identified in Multi-site Tests and Choice of Pathogen Isolates for Resistance Screening Matters

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Funded Plan of Work: A search for improved resistance in common bean through multi-site screening and pathogen characterization.

Abstract:
One goal was to identify resistance to white mold in adapted common bean lines. The hypothesis was that screening difficulties can be reduced by using multiple location screening sites and understanding the role of pathogen variation in the screening system. Every location now uses the same protocol for rating the greenhouse screening straw test and field screening tests. In six field and seven greenhouse tests reported so far in 2007, Cornell 604, 605 and 606 as well as A195 and VA19 were identified as having partial WM resistance. In 2007-08 more lines (22) have been added for greenhouse screening (a reflection of SI-supported projects). The multi-site field tests combined with greenhouse results helped to identify disease escape or avoidance in B05055 similar to Bunsi. B05055 was ranked among the susceptible lines in the greenhouse but was intermediate in the field. A second goal was to determine variability of the pathogen. Mycelial compatibility groupings (MCGs) were used to test the isolates for clonality. When the ten greenhouse isolates and the 146 field screening isolates were tested by the MCG assay, 64 MCGs were identified; 36 of those MCGs (over half) were composed of a single isolate; and 6 of the 64 MCGs were formed by screening isolates from more than one location, e.g. an MCG was formed by greenhouse screening isolates, Minnesota field isolates, and Washington field isolates. When the isolate aggressiveness, using the straw test, was compared both within and between MCGs the isolates came from, the MCGs were significantly different, but the isolates within MCGs did not differ in aggressiveness. This data supports the hypothesis that the differences in aggressiveness can be associated with the MCG that the screening isolates form or are members of. The differences in aggressiveness caused by genetic variation in the isolates were important to consider when screening for resistance. A common isolate(s) is recommended for use across greenhouse screening sites. Pathogen variability exists in both greenhouse and field screening isolates. Use of multi-sites can provide more convincing evidence for putative resistance in bean lines.

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Sequencing of expressed sequence tags of *Sclerotinia sclerotiorum* and *Pisum sativum*

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The aim of the project was to develop expressed sequence tags (ESTs) for both the pathogen, *Sclerotinia sclerotiorum* and the host, *Pisum sativum,* so that genome-wide gene expression studies could be conducted. It is important to develop genomic resources for *S. sclerotiorum* that are relevant to the interaction between *S. sclerotiorum* and *P. sativum*. Currently, nothing is known about the genetic mechanisms that control the basic biology and pathology of *S. sclerotiorum* interacting with pea. The development of ESTs will enable the identification and characterization of resistance and pathogenicity genes from host and pathogen. Additionally, ESTs from *S. sclerotiorum* will be useful to refine the annotation of the *S. sclerotiorum* genome sequence.

As detailed in our previous progress report, we have developed three EST libraries using *S. sclerotiorum* isolate WMA1. However, our EST libraries included many clones which had high sequence similarity to bacteria (more than 70% clones from the fungus grown on PDB and more than 50% clones when grown on YPG). This result raised the possibility of contamination of the culture medium by bacteria or symbiotic bacteria associated with strain WMA1. Subsequent experiments make the former possibility highly unlikely and favor the hypothesis of symbiotic bacteria associated with the fungal hyphae. Further research is required to definitively determine the presence of these bacteria inside the fungal hyphae and to elucidate their interaction with this fungus and possible role in pathogenesis. Collaborators in Japan are currently using fluorescent in situ hybridization (FISH) to confirm the presence of the bacteria in strain WMA1.

We have adopted a novel approach to generating EST data for the interaction between *Sclerotinia* and pea. Briefly, the pea cv. 'Lifter', identified as having partial resistance to *S. sclerotiorum,* has been inoculated with an isolate of *S. sclerotiorum* known to be free of bacteria. Total RNA was isolated from advancing lesions to capture expressed transcripts from both pathogen and host, which was confirmed with quantitative RT-PCR. The quality of the RNA sample was qualified on an Agilent Bioanalyzer and is currently being converted into a 'normalized' cDNA pool. The normalization process reduces the abundance of highly expressed transcripts so that rare transcripts are better represented by the cDNA pool. It is this normalized cDNA pool which we will have sequenced using massively parallel sequencing on a 454 pyrosequencing platform, specifically the GS FLX from Roche. A single full plate run on the GS FLX instrument can yield up to 400,000 sequence reads of approximately 250 bp in length. The shorter sequence length delivered by the GS FLX is overcome by the sheer number of reads that are possible when compared to traditional Sanger sequencing, substantially decreasing the cost per base pair of sequence data. We predict that sequencing the normalized cDNA pool will yield a substantial number of genes involved in pathogenicity and resistance responses. The plant and fungal transcripts will be sorted by mapping the reads to the *S. sclerotiorum* genome and where possible ESTs will be assembled into larger contiguous reads.

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Environmental and biochemical factors affect expression of resistance in soybean to *Sclerotinia sclerotiorum*

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Funded Plan of Work**:** Unraveling the genetics of resistance in soybean to *Sclerotinia sclerotiorum* using multiple evaluation criteria

ABSTRACT

Sclerotinia stem rot (SSR) continues to be a major disease of soybean in the upper Midwest. The occurrence and severity of SSR in the field is highly dependant upon prevailing environmental conditions, which is problematic when evaluating soybean germplasm for resistance. Numerous methods are available to evaluate soybean germplasm for SSR resistance in greenhouses and other forms of controlled environments. Frequently, results from controlled environments do not correlate to results from field trials. In previous studies we determined that oxalic acid was not a reliable method to differentiate soybean accessions with differing reactions to *S. sclerotiorum*. We also determined that a controlled light environment of 337 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) is most conducive to the study of the soybean x *S. sclerotiorum* interaction. This environment resulted in the detection of differences in reaction to *S. sclerotiorum* among soybean accessions, and was used to reliably predict their field performance. For the host, we developed a breeding line, W04-1002, that has consistently expressed a high level of partial and environmentally insensitive resistance to *S. sclerotiorum*. W04-1002 and five other soybean lines were used to study the role of preformed lignin concentration on the reaction of soybean to *S. sclerotiorum*. The identification of an environmentally stable plant trait that is associated with resistance to *S. sclerotiorum* could be used to indirectly screen for resistance to the pathogen. No studies to date have been conducted to specifically address the relationship between lignin concentration in soybean stems and SSR. We hypothesize that plants with low stem lignin are more susceptible and exhibit greater disease severity than plants with high lignin concentrations. Results are presented from a series of field studies in which both SSR and stem lignin concentration were evaluated for six soybean accessions that vary in response to *S. sclerotiorum.* Stems were sampled at reproductive soybean growth stages that correspond to particular events in the growth and development of the soybean plant and the SSR disease cycle. The lignin concentration of stem component samples was quantified using the acetyl bromide procedure. Soybean accessions expressed different disease phenotypes in both 2004 and 2006 field trials. Lignin concentrations differed among accessions for each stem section sampled at the R3 or beginning pod, growth stage (*P* values ranged from 0.0993 to < 0.0001). Positive ranked relationships were observed between accession SSR disease severity and lignin concentration for each plant part assayed. Accession lignin concentrations of the internode between the fourth and fifth trifoliate leaves correlated best to disease severity data from each year (*P* = 0.02 to 0.005). However, we determined that lignin content of internode and node tissues is inversely related to disease severity caused by *S. sclerotiorum*. W04-1002 was found to have a lower lignin content compared to less resistant, and susceptible soybean accessions. W04-1002 is considered a novel source of resistance to *S. sclerotiorum*. Experiments are planned to determine the relationship between stem lignin concentration and SSR resistance QTLs identified in previous studies.

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Effect of temperature and period of high relative humidity on white mold resistance of selective germplasm from the *Pisum* core collection.

Lyndon D. Porter and Ginny Coffman, USDA-ARS, Prosser, WA

Funded Plan of Work: Effect of temperature and period of 100% relative humidity on white mold resistance of selective germplasm from the *Pisum* core collection

ABSTRACT:

White mold, caused by *Sclerotinia sclerotiorum*, is a common foliar pathogen of peas that can cause serious disease in both irrigated and dryland peas in the Pacific Northwest and pea production areas in the Midwest of the United States. Fungicides effective in managing white mold in peas are not economical, therefore the development of resistant cultivars is essential. The present research assessed the resistance of eleven pea lines to *S. sclerotiorum*. Ten of these lines had previously been identified as having potential resistance to *S. sclerotiorum*. The lines were inoculated with an isolate of *S. sclerotiorum* and incubated at six temperatures (60, 65, 70, 75, 85 ºF) and four incubation periods (12, 24, 48, 72 hours) and maintained in an incubator at 90-100% RH. Resistance to *S. sclerotiorum* was identified in these lines based on lesion expansion, incubation period, and nodal or internodal stem resistance at different temperatures. Lesion expansion at incubation periods of 24, 48 and 72 hours was greatest at temperatures of 75-85, 70 and 70 ºF for 11, 8 and 10 of the eleven pea lines tested, respectively, indicating that the rate of lesion expansion is greatest at high temperatures (75-85 ºF) during the first 24 hours, and then favored by 70 ºF at incubation periods of 48 to 72 hours. All of the pea lines tested except lines 1204-3, PI166084 and the susceptible control, Bolero, were highly resistant (mean nodal resistance score less than 1, 1 = lesion did not expand beyond the initial inoculation point) to *S. sclerotiorum* at all the temperatures tested when incubated for a 12-hour period. At the 24-hour incubation period, only three lines (PI103709, PI164972, and PI169603) were highly resistant at any of the temperatures tested, and these three lines were only resistant at 85 ºF. Line PI169603 was the only line that was highly resistant at an incubation period of 24 hours and a temperature of 75 ºF. There were no pea lines that were highly resistant to *S. sclerotiorum* at any of the temperatures tested when the incubation periods were greater than 24 hours. The pea lines PI169603 and PI240515 appear to have the best resistance to *S. sclerotiorum* across all the temperatures and incubation periods based on lesion expansion and nodal resistance values.

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Dynamics of *Sclerotinia sclerotiorum* ascospore dispersal in canola fields

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Funded Plan of Work: Epidemiological studies on Sclerotinia stem rot of canola

Abstract:
The dynamics of *Sclerotinia sclerotiorum*'s ascospore dispersal patterns were studied in the summers of 2005-2007 at two commercial canola fields in North Dakota using several 7-days volumetric spore samplers. Daily ascospore concentrations were surveyed between June 24 and the end of July (canola flowering period) each season. Disease incidence was estimated at the end of the growing season in the area surrounding each spore sampler. Regression analysis was used to relate spore concentrations to disease incidence. Significant differences in the number of ascospores trapped and in the amount of disease produced were detected between seasons. In 2005 the average number of ascospores trapped ranged from 23 to 139 spores per cubic meter of air per day; while in 2006 it ranged from 0 to 10 spores. A significant association (R^2= 0.99) was detected between spore concentrations and disease incidence in 2005. No measurable amounts of disease were recorded in 2006. Highest spore concentrations in the air were detected between 10:00 A.M. and 1:00 P.M. in 2005; however, in 2006 highest peaks were observed much earlier (between 3 A.M. and 7 A.M.) In both years average air temperature <21°C and relative humidity >80% were associated with peaks of spore production. Data from 2007 is currently being analyzed.

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Cultivar, Plant Spacing and Fungicide Effects upon White Mold Management in Dry Bean

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Funded Plan of Work: Cultivar, Plant Spacing and Fungicide Effects upon White Mold Management in Dry Bean

ABSTRACT:
During 2006 – 2007, we investigated the roles of cultural practices (plant spacing), and timely application of multi-action pesticides in reducing damage from white mold to dry bean cultivars with varying degrees of resistance (plant architecture – disease avoidance, interspecific resistance). The Colorado experiment was adversely affected by herbicide drift in 2007 from an adjacent pasture during the vegetative stage of growth, and only a light amount of white mold developed after repeated inoculation. Agronomic responses revealed that there was a noticeable increase in plot yield when plant population was increased 50% from 1 line to 2 lines. The % increase in yield when comparing 1 to 2 lines was 74% and 55% in 2006 but only 14% and 0% in 2007 for Montrose and Vision, respectively. With an average yield of 20 cwt/A (2240 kg/ha) and average grower price of $0.20/pound ($0.44/kg), the increased plant population (2 lines) could provide an addition return of 11 cwt valued at $220/A (1232 kg valued at $542/ha) for a type II cultivar like Vision in the absence of white mold or if the cultivar was resistant to the pathogen.

In Idaho during 2007, fungicide protection reduced white mold infection by 11 – 15% with a 12 - 40% yield increase for the susceptible Type III Montrose; while infection of the susceptible Type II Vision was reduced 24 - 39% with a 20 - 32% increase in yield for low and high plant density. Partially resistant CO23704 and CO54150 lines exhibited more white mold infection at the higher density with a 6 – 14% increase in yield when protected with a fungicide. The resistant WM54 and WM55 exhibited low infection with either density and/or fungicide program; and WM55 was significantly more productive than WM54. Increased plant density did not provide an increase in yield, regardless of the fungicide program, for any entry tested in 2007. Assuming this modest disease control and associated yield gain with an upright, susceptible cultivar like Vision, 1 fungicide application, and 2 lines per bed, a grower could net an additional $190/A or $512/ha at a cost of $30/A or $74/ha for the fungicide application in the presence of white mold.

These objectives support the SI areas of Germplasm Enhancement and Variety Development (25%) and Epidemiology & Disease Management (75%).

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Introgressing White Mold Resistance from the Secondary Gene Pool of Common Bean

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Funded Plan of Work: Introgressing White Mold Resistance from the Secondary Gene Pool of Common Bean

ABSTRACT:
Low levels of white mold (WM, caused by *Sclerotinia sclerotiorum* Lib. de Bary) resistance occur in the common bean (*Phaseolus vulgaris* L.) whereas *Phaseolus* species of the secondary gene pool (*P. coccineus*, *P. polyanthus*, *P. costaricensis)* possess higher levels of resistance. The objectives in the FY2007 were to (1) pure-line five interspecific breeding lines (IBL) derived from crosses of 'ICA Pijao' with *P. coccineus* (G 35172), *P. costaricensis* (S 33720), and *P. polyanthus* (G 35877), (2) compare the WM reaction of the IBL with known sources of WM resistance, and (3) initiate screening of a new group of 482 IBL derived from crosses of pinto 'Othello' and 'UI 320' with highly WM resistant *P. coccineus* accessions PI 433246 and PI 439534. The five IBL that had survived repeated greenhouse and field screenings from 2002 to 2006 and were still variable for WM score were screened under WM pressure in the greenhouse at Fort Collins (CO) and Kimberly (ID). Plants with the lowest WM score were harvested separately. Seed from each selected plants at Fort Collins and Kimberly were screened in the greenhouse at Kimberly using two replicates. By inoculating each plant up to three times, each time using three mycelial plugs from a 48 hr old culture of *S. sclerotiorum* a more stringent and severe WM pressure was created. Consequently, one of three IBL from *P.* coccineus G 35172 (VCW 51) and the only one that had survived from *P. polyanthus* G 35877 (VPW 20) until 2006 turned out to be susceptible (WM scores >6). However, two IBL (VCW 54 and VCW 55) derived from congruity-backcrossing with *P. coccineus* G 35172 (Pijao/G 35172//Pijao/3/G 35172) and one (VRW 32) derived through recurrent-backcrossing with *P. costaricensis* S 33720 (Pijao//Pijao/S 33720) had high levels of and were true-breeding for WM resistance and other traits. These three IBL and ICA Pijao, ICA Bunsi, G 122, and I 9365-25 were again evaluated in the greenhouse and in the field in Idaho in 2007. A randomized complete block design with three replicates was used. Plants in the greenhouse were inoculated three times using the cut-stem method and three mycelial plugs each time. Three mycelial inoculations also were made in the field during flowering using a power-driven backpack solo sprayer. White mold reaction was recorded on a single-plant basis 28 days post-inoculation and verified at maturity. VCW 54 and VCW 55 had very high levels of WM resistance and VRW 32 had slightly lower level of resistance. Nonetheless, the three IBL developed through this Sclerotinia Initiative funded project seem to possess much higher levels of WM resistance than previously available. Also, the three IBL possess unique combinations of flower and seed colors and have an upright growth habit. Seed of these IBL is being multiplied in an off-season nursery in Puerto Rico for more extensive greenhouse and field-testing through the national Bean White Mold Nursery in 2008 onwards. Use of these three IBL in breeding and genetics studies, for WM resistance cultivar development, and determining optimum agronomic practices for an integrated management to combat WM problems in the USA should be maximized. Screening of a new group of 482 IBL derived from crosses of pinto Othello and UI 320 with highly WM resistant *P. coccineus* PI 433246 and PI 439534 is currently in progress in the greenhouse.

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Identification of QTL conditioning partial resistance to white mold in kidney bean line VA19 derived from an interspecific population

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Funded Plan of Work: Genetic characterization of scarlet-runner bean derived resistance to white mold in common bean

ABSTRACT:
Scarlet-runner bean (*Phaseolus coccineus* L.), a representative species of the secondary gene pool of common bean, is a potential source of white mold resistance for improving dry bean and snap bean. VA19 is a light-red kidney bean line that possesses resistance to white mold putatively derived from scarlet-runner bean. The objective of this research was to characterize resistance of VA19 to white mold in multiple field and greenhouse environments. A recombinant inbred population Benton/VA19 consisting of 79 F_5-derived RILs was used for QTL analysis. 'Benton' is a susceptible snap bean. Separate R and S bulks based upon combined field and greenhouse reactions to white mold were used in bulked-segregant analysis to identify sequence-related amplified polymorphic (SRAP) markers associated with resistance. There were three QTL identified. Two QTL conditioned physiological resistance in the greenhouse. These QTL mapped to linkage groups B2 and B4. The QTL on B2 was detected by both the straw test (R^2=38%) and a non-wounding technique (27%). This QTL was also expressed in the field (11%). The QTL on B4 was detected primarily by the non-wounding greenhouse screening method and had a minor effect (6%). The third QTL on linkage group B8 conditioned partial field resistance to white mold (11%) and was not associated with disease avoidance traits. Preliminary examination suggests that the B2 and B8 QTL derived from VA19 are independent of QTL derived from other resistance sources which map to the same linkage groups. Cloning and sequencing of QTL-linked SRAP markers is underway for potential identification of candidate genes underlying partial resistance and for development of user friendly SCAR markers for MAS of the QTL.

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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:
In a previous project funded by the National Sclerotinia Initiative, 1,147 lines derived from crosses in which either or both parents were partially resistant to Sclerotinia stem rot were evaluated for yield and other agronomic traits. Eighteen advanced lines with potential resistance to Sclerotinia were selected for further evaluation. In 2007, these 18 lines were tested at four locations in 6-row plots with two replications at each location for yield and other agronomic traits. Five of the 18 lines were among the top 10 highest yielding lines and were higher yielding than the check IA2021 and IA2052. The 18 lines were also evaluated for Sclerotinia stem rot resistance in our disease nursery where we test commercial soybean varieties annually for Sclerotinia stem rot resistance. Of the top 5 highest yielding lines, two had a DSI (disease severity index, ranging from 0% to 100% with 0% as the most resistant) less than 1.7% in the test where the average DSI of all 61 lines were 9.9% and the maximum DSI was 45%.

In 2007, 392 lines from seven populations segregating for resistance from five new PIs were evaluated in single 3-foot row plots with two replications for agronomic traits such as yield and lodging. The 392 lines are currently being evaluated for Sclerotinia stem rot resistance in the greenhouse using the spray-mycelium method we developed. Leaf samples were collected from these 392 lines for DNA extractions. The 392 lines will be used to validate 33 reported QTLs for resistance to Sclerotinia stem rot.

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Identifying Pathogenicity determinants of *Sclerotinia sclerotiorum*

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Funded Plan of Work: Identify Virulence Factors of *Scleortinia sclerotiorum* through Insertional Mutagenesis

ABSTRACT

Sclerotinia sclerotiorum causes white mold and other diseases on more than 400 plant species, and many of the host plants are economically important. Diseases caused in economically important plants by *S. sclerotiorum* occur worldwide, inflicting considerable damage. The diseases are difficult to manage either culturally or chemically. Host resistance to this fungus has been inadequate. One obstacle that hampers effective management of the disease is that we lack an adequate understanding of the mechanistic interactions between the pathogen and host plant. Because of its wide host range, it is speculated that the pathogen must possess rather general pathogenic mechanisms such as production and secretion of oxalic acid and hydrolytic enzymes. However, very little is known about the pathogenic genetic determinants of this devastating pathogen. This research is aimed at increasing our understanding of pathogenic determinants of *S. sclerotiorum*. Four putative genes in pathogenesis have been identified through this research.

We have identified four putative genes of pathogenicity factors through sequencing disrupted genes in selected non-pathogenic transformants. The four putative genes are a haloacid dehalogenase-like hydrolase gene in transformant Y22, a nucleoside phosphatase genes in transformant M139.2, a gene coding for esterase of the alpha-beta hydrolase in transformant M-2, and a GTPase gene in the transformant M-69. These putative genes need to be confirmed for their roles in pathogenicity through complementation tests and targeted mutagenesis. The main impact is on increasing our understanding of pathogen biology and development, specifically pathogenicity genes.

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Progress in mapping QTL for Sclerotina stalk rot tolerance in a sunflower recombinant inbred population

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Funded Plan of Work: Map the quantitative trait loci responsible for Sclerotinia tolerance in two USDA sunflower lines

ABSTRACT:

The stalk rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a serious disease of sunflower (*Helianthus annuus* L.), and mapping quantitative trait loci (QTL) for resistance to Sclerotinia should facilitate the development of marker-assisted selection strategies for enhancing resistance. In this study QTL mapping for tolerance to Sclerotinia stalk rot was conducted in a recombinant inbred line (RIL) population developed from a cross between RHA280 and RHA801. The RILs were artificially inoculated using mycelium-bearing millet seeds in both greenhouse and field conditions. In 2006, disease severity, on a scale from 0 (highly resistant) to 9 (susceptible), ranged from 1.7 to 8.3 among RILs in the two greenhouse tests. Disease incidence was used to evaluate the stalk rot tolerance in field tests, and it ranged from 0 to 27.5% and 0 to 52.5% in the two field tests (Fargo and Grandin, ND), respectively. In 2007, we repeated the same tests. Unfortunately, the field tests did not produce useful data for an extremely low disease incidence at both locations due to unfavorable weather condition after inoculation. However, the greenhouse test was successful and the disease severity scores ranged from 1.2 to 8.7 among the RILs, very close to that of the previous year. In general, phenotypic correlations for RIL disease tolerance traits between tests were positive and significant. Three QTL for disease severity were identified in greenhouse tests and two QTL for disease incidence were identified in field tests. Individual QTL explained 9.1% to 15.4% of the phenotypic variation, and different QTL were detected in different tests, indicating strong environmental effects. More tests are needed to confirm the location of these QTL.

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QTL for Sclerotinia Head Rot Tolerance in a Sunflower Population Developed from a Cross between Tolerant Lines

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Funded Plan of Work: Map the quantitative trait loci responsible for Sclerotinia tolerance in two USDA sunflower lines

ABSTRACT:
One hundred and twenty-three $F_{2:3}$ and $F_{2:4}$ families derived from a cross between HA 441 and RHA 439, both showing partial tolerance to Sclerotinia head rot, were used for the current study. A genetic map with 180 TRAP, 32 SSR, 11 INDEL, and 2 morphological markers was constructed. The map has 19 linkage groups and spans a genetic distance of 1797.6 cM. Disease incidence (DI) and disease severity (DS) were evaluated in three field tests in a randomized complete block design with two replicates. Strong positive correlations were detected among the traits investigated in different tests. Nine DI and seven DS quantitative trait loci (QTL) were identified with LOD scores ranging from 2.4 to 11.8. QTL were found in ten linkage groups. One DI QTL was detected in all three experiments and four (two DI and two DS) QTL were identified in two of the three experiments. Identification of the tolerance QTL will facilitate marker-assisted selection of the disease tolerance in germplasm development and breeding. Although a positive correlation existed between the two disease indexes, most of the respective QTL were located in different chromosomal regions, suggesting a different genetic basis for the two indexes.

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