NDSU/USDA-ARS

2006 Sclerotinia Initiative Annual Meeting

Bloomington, MN

January 18-20, 2006

National Sclerotinia Initiative

A PROGRAM OF THE

USDA ARS

2006 National Sclerotinia Initiative Annual Meeting

January 18-20, 2006

Holiday Inn Select

Minneapolis (Bloomington), MN

SCLEROTINIA INITIATIVE ABSTRACTS

Sclerotinia Initiative Annual Meeting 2006
Agenda

January 18, 2006

6 - 8 pm Poster Session/Reception (posters will be left up throughout the entire meeting) **(The Grove)**

January 19, 2006

7:15 am Registration/Continental Breakfast **(Cortland Room)**

8:00 am Welcome and Introductions - **Larry Chandler, USDA-ARS, Ft. Collins, CO**

8:05 am ARS National Program Staff Update – **Rich Wilson & Rick Bennett, USDA-ARS, Beltsville, CO**

8:15 am Status of Sclerotinia Initiative in Washington, DC - **Dale Thorenson, U.S. Canola Growers, Washington, D.C.**

8:25 am Meeting Charge - **Larry Chandler**

8:30 am Farmers Have More Choices Than Ever – What Will Affect Their Future Planting Decisions?
– **Mike Krueger, The Money Farm, Fargo, ND**

9:30 am Break **(Cortland Foyer)**

Sclerotinia Research Activities – Session 1 **(Cortland Room)**

Moderator–Howard Schwartz–Colorado State Univ., Ft. Collins, CO

10:00 am Sclerotinia Genome Sequencing Project – **Jeff Rollins - University of Florida, Gainesville, FL**

10:20 am Carrington, ND Research & Extension Center: A resource for Sclerotinia Research – **Blaine Schatz/Bob Henson - North Dakota State University, Carrington, ND**

10:40 am Evaluation of Brassica napus rapeseed accessions for resistance to Sclerotinia under mist-irrigation - **Carl Bradley – North Dakota State University, Fargo, ND**

11:00 am	Genetics and mapping of resistance to Sclerotinia white mold in lentil - **Fred Muehlbauer – USDA-ARS, Pullman, WA**
11:20 am	Introgressing white mold resistance from the secondary gene pool of common bean - **Shree Singh – University of Idaho, Kimberly, ID**
11:40 am	Discussion
noon	Working Lunch **(The Grove)**

Sclerotinia Research Activities – Session 2 **(Cortland Room)**

Moderator – Jerry Miller, USDA-ARS, Fargo, ND

1:15 pm	Guest Speaker Biological control and epidemiology of Sclerotinia - **John Whipps – University of Warwick, Wellesbourne, Warwick, United Kingdom**
2:15 pm	Development of soybean varieties or germplasm resistant to Sclerotinia stem rot - **Dechun Wang – Michigan State University, East Lansing, MI**
2:35 pm	Genetic basis of oxalate sensitivity to Sclerotinia diseases - **Henrik Stotz – Oregon State University, Corvallis, OR**
2:55 pm	Discussion
3:05 pm	Brochure/website update–**Kris Versdahl, Prairie Ag, Red Lake Falls, MN**
3:15 pm	Break & Poster Session **(The Grove)**
4:00 pm	***Industry Panel – What's New in the Commercial Sector*** **(Cortland Rm)**

Moderator – Larry Kleingartner – National Sunflower Association, Bismarck, ND

Panelists:
Pat Duhigg – Seeds 2000 , Breckenridge, MN
Chris Clark – Snack Food Association, Washington, DC
Roger Kaiser – Bayer CropScience, Research Triangle Park, NC
Emmett Lampert – Syngenta Crop Protection, Wimbledon, ND

5:00 pm	Discussion

5:20 pm Adjourn

Dinner on your own

January 20, 2006

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

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

Sclerotinia Research Activities – Session 3 **(Cortland Room)**

Moderator – Scott Halley, North Dakota State University, Langdon, ND

8:00 am Development of Sclerotinia head rot resistant sunflower germplasm - **Tom Gulya – USDA-ARS, Fargo, ND**

8:20 am Characterization of transformants of *Sclerotinia sclerotiorum* – **Weidong Chen – USDA-ARS, Pullman, WA**

8:40 am Resistance improvement of bean through multi-state screening and pathogen characterization - **Jim Steadman – University of Nebraska, Lincoln, NE**

9:00 am Discussion

Strategic Plan Update

Moderator – Bill Kemp – USDA-ARS, Fargo, ND

Germplasm Enhancement:

9:15 am Soybean – **George Graef – University of Nebraska, Lincoln, NE**

9:30 am Sunflower – **Tom Gulya & Gerald Seiler – USDA-ARS, Fargo, ND**

9:45 am Canola – **Carl Bradley & Luis del Rio – North Dakota State University, Fargo, ND**

10:00 am Break **(Cortland Foyer)**

10:30 am	Dry Bean – **Phil Miklas – USDA-ARS, Prosser, WA**
10:45 am	Pea, Lentil & Chickpea–**Fred Muehlbauer – USDA-ARS, Pullman, WA**
11:00 am	Pathogen Biology & Development – **Berlin Nelson – North Dakota State University, Fargo, ND**
11:15 am	Epidemiology & Disease Management – **Luis del Rio - North Dakota State University, Fargo, ND**
11:30 am	Pathogen & Host Genomics – **Steve Clough – USDA-ARS, Urbana, IL; Jeff Rollins, University of Florida, Gainesville, FL; Henrik Stotz – Oregon State University, Corvallis, OR**
noon	Working Lunch **(The Grove)**
1:15 pm	Strategic Plan Discussion and Assignment of Additional Tasks & Wrapup/Initiative Business - **Larry Chandler**
3:00 pm	Adjourn

Sclerotinia Initiative Website:

http://www.whitemoldresearch.com/

http://www.whitemoldresearch.org

http://www.sclerotinia.com

http://www.sclerotinia.org

Sclerotinia Initiative Poster Session

January 18-20, 2006
Minneapolis, MN

Epidemiology & Disease Management

Poster No.	Title	Author
1	A Modified Petzoldt and Dickson Scale for White Mold Rating of Common Bean	H. Terán, M. Lema, H. F. Schwartz, R. Duncan, R. Gilbertson, S. P. Singh
2	Biological Control of *Sclerotinia sclerotiorum*	H. R. Dillard, A. C. Cobb
3	Crop Scequencing and Biological Control to Minimize Sclerotinia Disease Risks, 2005	J. M. Krupinsky, D. L. Tanaka, M. A. Liebig, S. D. Merrill, J. D. Hanson, T. J. Gulya
4	Dispersal of *Sclerotinia sclerotiorum* Ascospores in Canola Fields from Area Source of Inoculum	I. S. Qandah, L. E. del Rio, C. A. Bradley
5	Eco-Tillage and Pesticide Management of White Mold in Dry Bean	H. F. Schwartz, M. A. Brick, K. L. Otto, M. S. McMillan, J. B. Ogg
6	Epidemiology and Resistance to Sclerotinia Head Rot in Wild Sunflower Species	K. Y. Rashid
7	Fungicide Efficacy for Sclerotinia Head Rot Control on Sunflower	S. Halley
8	Fungicides Applied at Four Application Timings to Two Field Pea Cultivars with Differing Flower Durations for White Mold Disease Control	S. Halley, B. G. Schatz, E. Z. Aberle
9	Insertional Mutagenesis of *Sclerotinia sclerotiorum* through Agrobacterium-Mediated Transformation	J. N. Wamatu, D. White, X. Wang, W. Chen
10	Mapping Sclerotinia White Mold Resistance in Lentil	F. Muehlbauer, W. Chen, K. McPhee

Pathogen and Host Genomics & Pathogen Biology and Development

Poster No.	Title	Author
11	Construction of an EST Library for Sclerotinia sclerotiorum and Its Interaction with Pea	M. Kawabe, T. L. Peever, W. Chen, K. McPhee
12	Dry Bean Transformation to Enhance White Mold Resistance	A. R. Armenia, R. F. Allison, J. D. Kelly
13	Gene Expression Profiling of Soybean Challenged with*Sclerotinia sclerotiorum*	B. Calla, T. Vuong, Y. Zhang, S. Hubbard, L. Blahut-Beatty, D. Simmonds, G. Hartman, S. Clough
14	Influence of Soil Texture and Moisture Contents on Carpogenic Germination of *Sclerotinia sclerotiorum* sclerotia	R. Harikrishnan, L. E. del Rio
14	Preliminary QTL Analysis in a Black Bean x Wild Mexican Bean Inbred Backcross Mapping Population for White Mold Resistance	K. A. Terpstra, J. D. Kelly
15	Progress in Mapping QTLs Responsible for Resistance to Sclerotinia Head Rot and Stalk Rot in Two Segregating Sunflower Populations	J. Hu, S. A. Raid, T. J. Gulya, J. F. Miller
16	Transformation of *Sclerotinia sclerotiorum* with the Green Fluorescent Protein Gene (GFP) and Use of GFP to Study Host Resistance	A. De Silva, M. D. Bolton, B. D. Nelson
17	Use of Oxalic Acid to Characterize Soybean Accessions for Partial Resistance to *Sclerotinia sclerotiorum*	A. J. Peltier, C. R. Grau

Germplasm Enhancement and Variety Development

Poster No.	Title	Author
18	Advances in the Development of Sunflower Germplasm with Resistance to Both Sclerotinia Stalk Rot and Head Rot, and Evaluation of Commercial Hybrids for Stalk Rot Resistance	T. J. Gulya, J. F. Miller
19	Development and Testing of Doubled Haploid Canola Lines with Increased Tolerance to Oxalic Acid	D. V. Phillips, C. Bradley, P. Porter
20	Development of Sclerotinia Stem Rot Resistance Germplasm Using Hexaploid Helianthus Species	J. Feng, G. J. Seiler, T. J. Gulya, C. C. Jan
21	Identification of Quantitative Trait Loci Linked to White Mold Resistance in Common Bean	M. A. Brick, J. J. Maxwell, P. F. Byrne, H. F. Schwartz, X. Shan, J. B. Ogg, R. Henson
22	Inheritance of White Mold Resistance in the Interspecific Crosses of Pinto Cultivars Othello and UI 320 and *Phaseolus coccineus* L. Accessions PI 433246 and PI 439534	H. F. Schwartz, K. Otto, H. Terán, M. Lema, S. P. Singh
23	Interspecific Amphiploids of Perennial Helianthus Species x Cultivated Sunflower Possess Valuable Genes for Resistance to Sclerotinia Stem and Head Rot	C. C. Jan, J. Feng, G. J. Seiler, T. J. Gulya
24	Introgressing White Mold Resistance from the Secondary Gene Pool of Common Bean	S. P. Singh, H. F. Schwartz, H. Terán, K. Otto, M. Lema, P. N. Miklas, R. Henson
25	White Mold Resistance in Pea and Lentil Through Breeding and Biotechnology	K. McPhee, W. Chen, B. Schatz, B. Henson, F. Muehlbauer

Advances in the Development of Sunflower Germplasm with Resistance to Both Sclerotinia Stalk Rot and Head Rot, and Evaluation of Commercial Hybrids for Stalk Rot Resistance

T. J. Gulya and J. F. Miller. 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 continue to be the major diseases affecting U.S. production in 2005, with head rot and stalk rot found in 20% and 27% of fields surveyed in seven states, respectively, and affecting 1.6% and 2.3% of the U.S. crop. Regarding germplasm development, two oilseed maintainer lines (HA 451 and 452) and three oilseed restorer lines (RHA 453, 454, 455) with improved tolerance to Sclerotinia head rot and stalk rot were released in the spring of 2005. The lines were derived from Russian and French sources, and thus will expand the diversity in Sclerotinia resistant germplasm. The lines in testcross hybrids had Sclerotinia head rot infection ranging from 8 to 23%, when compared with the check hybrid SF 270 which had 73% infection. Additional oilseed releases of two maintainer and three restorer lines are planned for the spring of 2006. A new breeding program has been initiated to incorporate Sclerotinia head rot and stalk rot resistance into the large-seeded confection sunflowers, with initial germplasm releases projected for the fall of 2006. To provide information for growers and private breeders, 89 experimental and released commercial hybrids were tested for resistance to Sclerotinia stalk rot at five locations in North Dakota and Minnesota using artificial inoculation (as well as being tested for head rot reaction by Dr. Robert Henson, PI of another project). Three locations gave statistical sound stalk rot ratings, with disease incidence of individual hybrids at maturity ranging from 10 to 71%. An experimental hybrid, using USDA lines developed for Sclerotinia resistance (HA 412 x RHA 409), was the sixth best entry with 16% stalk rot, averaged over three locations. Among the top ten entries were one released confection hybrid and two released NuSun hybrids. Retests of the 20 best entries from 2004 trials (at five locations for stalk rot and two locations for head rot) confirmed that a few commercial hybrids were consistently good for both head and stalk rot.

Contact Information: Dr. Tom Gulya, USDA Northern Crop Science Lab, 1307 N. 18th. St., Fargo ND 58105-5677; 701-239-1316; gulyat@fargo.ars.usda.gov

Biological Control of *Sclerotinia sclerotiorum*

Helene R. Dillard and Ann C. Cobb, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY

Funded Plant of Work: Biological Control of *Sclerotinia sclerotiorum*

Abstract:
Research was conducted to evaluate the ability of Contans (commercial product containing *Coniothyrium minitans*) to reduce sclerotial populations of *Sclerotinia sclerotiorum* in fields in New York. The objectives were 1) to quantify decline in populations of sclerotia of *S. sclerotiorum* following a single application of Contans (*C. minitans*), and 2) to determine if applications of Contans results in a reduction in plant disease caused by *S. sclerotiorum*. Using a wet sieving technique to recover sclerotia in soil, we determined baseline levels in soil populations of sclerotia in several naturally infested commercial fields in various cropping systems. We also artificially infested one field at the Geneva Experiment Station with sclerotia of *S. sclerotiorum*. The numbers of sclerotia detected in the unamended soil varied from 0 to 30 per liter of soil. The highest naturally infested field averaged 7.3 sclerotia per liter. This is equivalent to 2.95×10^6 sclerotia per acre to a 4-inch soil depth. Sclerotial populations were reassessed following the application of Contans at 1 to 4 months after application, and in subsequent years in some fields. Populations rapidly declined to below detection levels both with and without application of Contans, but in some fields, the decline was faster following application of Contans. Aggregated distribution of sclerotia in the field made enumeration problematic, but even low levels of sclerotia in the soil represent potentially many infective ascospores. Contans did not decrease sclerotial populations beyond the year of application in these trials, indicating that Contans must be reapplied when susceptible crops are planted. In our trials, disease incidence in subsequent crops following application of Contans was variable. Combining our research results with interactions among product representatives, we generated improved product use recommendations.

Contact Information:

Construction of an EST library for *Sclerotinia sclerotiorum* and its interaction with pea

Masato Kawabe [1], Tobin L Peever [1], Weidong Chen [2], Kevin M[c]Phee [2]. [1] Department of Plant Pathology and [2] USDA-ARS, Washington State University, Pullman, WA 99164

Funded Plan of Work: Sequencing of expressed sequence tags of *Sclerotinia sclerotiorum* and *Pisum sativum*

Abstract:
We have initiated the sequencing of expressed sequence tags from *Sclerotinia sclerotiorum* and its interaction with *Pisum sativum*. The objective of this research is to develop genomic resources for *S. sclerotiorum* and *P. sativum* through the random sequencing of expressed genes. *S. sclerotiorum* isolate WMA1 from pea was used in this study. Before constructing the EST library from culture-grown mycelium, we investigated the genetic relationship between WMA1 and eight *S. sclerotiorum* isolates sampled from legume hosts in various geographic locations using random amplification of polymorphic DNA (RAPD) analysis. The purpose of this preliminary analysis was to ensure that isolate WMA1 was representative of *S. sclerotiorum* genotypes infecting pea. Eleven polymorphic RAPD bands were scored based on the presence (1) or absence (0) of amplicons of similar size. Genetic distances were estimated and clustered to generate a neighbor-joining dendrogram. The dendrogram demonstrated that WMA1 was representative of other *S. sclerotiorum* isolates from pea and could be used for constructing EST libraries.

A cDNA library generated from culture-grown mycelium has already been constructed and transformed into *E. coli*. The interaction cDNA library (genes expressed during infection of pea) is currently being constructed. To date, we have randomly picked more than 2,000 colonies of transformed *E. coli*. Over 1,000 of these randomly-chosen colonies were screened by direct PCR using T7 and SP6 primers designed to the plasmid flanking region. Three hundred thirty four insertions were amplified by PCR and 240 clones have been sequenced using T7 and SP6 primers. Many of the clones have close matches with identified genes from ascomycete fungi including *S. sclerotiorum*. All sequences are currently being analyzed.

Contact Information – Dr. Masato Kawabe, Dept. of Plant Pathology, Washington State Univ., Pullman, WA 99164-6430; 509-335-3742; mkawabe@wsu.edu

Crop Rotation Influences Canola and Wheat Diseases and Production

Paul Porter and Dave LeGare, University of Minnesota, St. Paul & Crookston, MN

Funded Plan of Work: Development of Sclerotinia Management Programs in Canola

Abstract:
A three-year rotation study evaluating the influence of crop rotation and a rye cover crop on sclerotinia incidence and severity in canola was initiated in 2003 (03CRye) and again at a second location near Thief River Falls, MN in 2004 (04CRye).
Canola grown following a rye cover crop compared with no rye cover crop: In the second year for both studies, canola yields were reduced (15.5 and 10.7%) and seed protein content was increased (6.6 and 9.5%) but test weight was not influenced when rye was grown as a cover crop. Canola plant height at harvest was reduced by 6.7% when rye was grown in 03CRye, but was not influenced by the rye in 04CRye. Canola plant biomass was reduced when rye was grown in 03CRye, but not in 04CRye. In 03CRye, time to 30% flowering was delayed by about 1 day, whereas in 04CRye time to beginning flowering was not influenced when rye was grown. Canola seed oil content was not influenced by the rye in 03CRye, whereas in 04CRye the seed oil content was increased by 4.7%. For both studies, sclerotinia disease severity was not influenced when rye was grown, but in 03CRye sclerotinia disease incidence was greater where no rye was grown (but both incidence and severity were quite low).
Canola grown following wheat or canola: In the second year for both studies, canola yield, test weight, plant height, seed protein content, and sclerotinia disease incidence and severity were not influenced by whether the previous year's crop was wheat or canola. In both studies, canola grown after canola delayed time to 30% flowering by about a day compared with canola grown after wheat. Only in 04CRye was canola seed oil content influenced by previous crop, with canola grown after wheat slightly greater.
Wheat grown following a rye cover crop compared with no rye cover crop: In the second year for both studies, wheat yields were reduced (9.2 and 19.1%), wheat test weights were reduced (0.5 and 1.6%), mid-season wheat biomass were reduced (29.0 and 66.4%), and wheat plant heights at harvest were reduced (4.5 and 8.9%) when rye was grown as a cover crop compared with no rye. Heading date was not influenced by rye in 03CRye. Although wheat scab disease severity was not influenced for either study, in 04CRye wheat scab disease incidence was lower when rye was grown. In 04CRye, wheat protein content and 1000 kernel weight were decreased (8.1 and 10.5%, respectively) when rye was grown, but this did not occur in 03CRye.
Wheat grown following wheat or canola: In the second year, wheat yield was reduced by 9.2% when grown wheat on wheat compared with wheat on canola in 03CRye, but in 04CRye there was no statistical difference (in spite of a numerical 8.0% difference). In both studies, protein content was reduced (2.8 and 2.4%) when grown wheat on wheat. Wheat test weights reduced slightly in 04CRye when grown wheat on wheat, but not in 03CRye. For both studies, wheat scab disease incidence and severity were not influenced with wheat on wheat compared with wheat on canola.

Contact Information – Dr. Paul Porter, Dept. of Agronomy and Plant Genetics, Univ. of Minnesota, 1991 Buford Circle, St. Paul MN 55108; 612-625-6719; pporter@umn.edu

Crop Sequencing and Biological Control to Minimize Sclerotinia Disease Risks, 2005

J.M. Krupinsky[1], D.L. Tanaka[1], M.A. Liebig[1], S.D. Merrill[1], J.D. Hanson[1], & T.J. Gulya[2]
USDA-ARS, [1]Northern Great Plains Research Laboratory, Mandan, ND
[2]Northern Crop Science Laboratory, Fargo, ND

Funded Plan of Work: Crop Sequencing and Biological Control to Minimize Sclerotinia and Impact of Sclerotinia Inoculum on Disease.

Abstract:
1) A Crop Sequence Project was conducted to evaluate the impact of previous crops (buckwheat, chickpea, corn, lentil, proso millet, grain sorghum, canola, dry pea, sunflower, and wheat) and crop residue on Sclerotinia diseases. In 2005, 400 sunflower plots were evaluated for disease (29,380 plants rated per evaluation) and harvested. The percentage of Sclerotinia basal stalk rot ranged from 0.6% for the grain sorghum/grain sorghum/spring wheat/sunflower sequence to 17% for the sunflower/sunflower/spring wheat/sunflower sequence. Plots following two years of sunflower were highest for stalk rot compared to two years of the other crops. Crop sequences with spring wheat and grain sorghum in the 1st and/or 2nd year ranked lowest for percentage of stalk rot.
2) The Biological Control Project was evaluated to determine the efficiency of *Coniothyrium minitans* applications under dryland conditions. Treatments after uniform application of sclerotia included: susceptible and resistant crops, and the timing of *C. minitans* (Contans WG®) applications. Sunflower was used as an indicator crop. The percentage of Sclerotinia basal stalk rot for treatments ranged from 0 to 15% for one study. Although statistical differences among treatments were not evident, there was a trend for higher disease levels following crambe. Also, combine yield data showed a lower sunflower yield following crambe compared to the other crops. A total of 228 plots were harvested in 2005 for three studies and samples are being processed. One study will be seeded to canola in 2006 for further evaluation. Another study will be seeded to sunflower in 2006 for further evaluation. Minor differences in soil water measurements were detected among plots. Soil properties were generally not affected by treatments. Soil pH decreased over time in all treatments and crops, with the strongest trend at 0-5 cm. Among crops, soil nitrate increased at all depths under dry pea.
3) A Sclerotinia Inoculum Density Project was evaluated to determine the impact of sclerotia density and tillage on Sclerotinia disease severity under dryland conditions. In 2005, the percentage of basal stalk rot on sunflower under tillage averaged 9% compared to 3% for no-till. Treatments will be evaluated with canola in 2006. This research will contribute to a better understanding of how sclerotia density and tillage influences the incidence of Sclerotinia disease and yield components under dryland field conditions.

Contact Information – Dr. J.M. Krupinsky, USDA-ARS, Northern Great Plains Research Laboratory, Box 459, Mandan, ND 58554-0459; 701-667-3011; krupinsj@mandan.ars.usda.gov

Development of PCR-Based Markers for Resistance to Sclerotinia Stem Rot in Soybean

T. D. Vuong[1], B. Calla[1], B. W. Diers[1], S. J. Clough[1,2], and G. L. Hartman[1,2]
[1]Department of Crop Sciences, University of Illinois, Urbana IL 61801;
[2]USDA-ARS, University of Illinois, Urbana IL 61801

Funded Plan of Work: Development of PCR-based molecular markers for resistance to Sclerotinia stem rot in soybean

Abstract:
Resistance to Sclerotinia stem rot (*S. sclerotiorum*) in soybean has been shown to be quantitatively inherited. Two major QTL associated with disease resistance were mapped to linkage groups (LG) **A2** and **B2**; however, genetic gaps were found in these QTL regions. The development of cDNA microarray technology presents a means of identifying possible genes involved in quantitative resistance traits. The objectives of the present study were to (i) analyze the profiles of differentially expressed genes in stem tissue of soybean seedlings challenged with the fungal pathogen and (ii) develop PCR-based molecular markers associated with differentially expressed genes to determine if they map to identified QTL. Two soybean genotypes, Williams 82 (S) and PI194639 (R), were grown hydroponically in a growth chamber under controlled light intensity and temperature. Total RNA samples from stems collected at 0, 6, 18, and 48 hours post inoculation (hpi) were fluorescently labeled with Cy3 or Cy5 dyes and hybridized onto soybean microarrays containing over 9,000 gene representatives. Data was normalized using the R/maanova package (BioConductor). An ANOVA test was run to determine the significance in the variability of the data. Time point 0 was eliminated to obtain a factorial model balanced for time. Significant differences for 231 genes ($\alpha = 0.01$) were found between treatments (inoculated vs. un-inoculated) across the time points, 18 and 48 h, and varieties. Also, significant differences occurred in 758 genes between treatments across both time points for the resistant variety (PI194639) ($\alpha = 0.05$) and 363 genes for the susceptible variety (Williams 82) ($\alpha = 0.05$). Some of these genes have annotations suggestive of potential involvement in the defense response of soybean against fungal infection and/or colonization. To develop DNA molecular markers, expressed sequence tags (ESTs) of 24 selected genes from the soybean genome database were employed to design primers, consisting 24 forward and 24 reverse oligos. Target region amplification polymorphism (TRAP) technique, in which EST-based oligos were used as fixed primers and combined with arbitrary primers labeled with fluorescent dyes (6-FAM, HEX, and NED) was employed. The markers detected in parental screening were utilized to genotype 155 F4:5 RILs of the Merit x PI194639 population. QTL analysis indicated that although no TRAP markers were mapped to previously identified QTL, several markers were mapped to new genomic regions in MLG **L**, **E** and **B1**. The results showed that the TRAP technique can provide an efficient tool to detect new markers associated with these loci. Additional molecular marker development is in progress.

Contact Information – Glen L. Hartman, USDA-ARS, R. 70 National Soybean Research Center, 1101 W. Peabody Drive, Urbana IL 61801. 217-244-3258; ghartman@uiuc.edu

Dispersal of *Sclerotinia sclerotiorum* ascospores in canola fields from area source of inoculum

Qandah, I., S., L.E. del Rio, C.A. Bradley. Department of Plant Pathology, North Dakota State University, Fargo, ND

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

Abstract:
In 2005, field experiments were established at three locations to study the spatial characteristics of epiphytotics caused by *Sclerotinia sclerotiorum* in canola. A disease gradient that tapered off with distance was observed. Highest disease severity was observed at the edge of the experimental area (0 m from a source of inoculum) and decreased with distance from the source. Regression equations indicated that disease severity was reduced at a rate ranging from 0.5 to 1% per meter; however, disease incidence was reduced at less than 0.5% per meter. The temporal dynamics of spore production was characterized using blue medium dishes (which is a passive sampling method), as well as with two types of volumetric spore traps (active sampling methods) at two locations. Passive spore samplings were conducted at Cando and Langdon on the 1, 7, 9, 15, and 21 of July. Spore peaks where observed at both locations only on the first three dates when air temperature inside the canopy was on average <70° F and relative humidity was higher than 80%. However, much larger numbers of spores were detected using volumetric spore traps.

Contact Information - Dr. Luis del Rio, Dept. of Plant Pathology, North Dakota State Univ., 306 Walster Hall, Box 5012, Fargo, ND 58105; 701-231-7073; luis.delrio-mendoza@ndsu.edu

Dry Bean Transformation to Enhance White Mold Resistance

Armenia AR[1], Allison RF[2], and Kelly JD[1].
[1]Crop and Soil Sciences, and [2]Plant Biology, Michigan State University, East Lansing, MI

Funded Plan of Work: Improving white mold resistance by transforming dry bean with the Germin oxalate oxidase gene

Abstract:
The potential of dry beans engineered to express the wheat germin gene which encodes an oxalate oxidase may provide an opportunity to control the oxalic acid generated upon infection by *Sclerotinia sclerotiorum*. Matterhorn and Olathe seedlings were electrotransformed with the pBKS*bar/gf-2.8* constructed to contain the wheat germin, *gf-2.8* and *bar* genes while employing various pretreatments including hormones that were injected into the apical meristems prior to transformation. To date, over 1000 Matterhorn and Olathe plants have been transformed and herbicide screening of the T_1 progeny was completed. PCR analysis of the herbicide resistant plants revealed integration of *gf-2.8* in a few Matterhorn and Olathe plants. Oxalic acid and fungal bioassays to evaluate resistance to white mold in T_2 plants are currently underway.

Contact Information – Dr. James D. Kelly, Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824, 517-355-0271 x181: kellyj@msu.edu

Eco-Tillage, Biopesticide and Resistance Management of White Mold in Dry Bean

Howard F. Schwartz and Mark A. Brick, Colorado State University, Fort Collins, CO

2004-2005 Funded Plan of Work:
Eco-Tillage, Biopesticide and Resistance Management of White Mold in Dry Bean

Abstract:
During 2004 and 2005, we investigated the roles of plant growth habit (pinto Type III vine- 'Montrose' vs Type II upright- 'Vision'), plant spacing (1 vs 2 lines per 75 cm wide bed), and timely application of chemicals (none, thiophanate methyl = Topsin, and boscalid = Endura) within an Integrated Pest Management context. Unfortunately, the regional drought effects of 2004 and 2005 did not support appreciable disease development in spite of repeated inoculations with the pathogen. Agronomic data were analyzed as a factorial, and plant spacing was the only significant main effect for yield (kg/hectare). There was a significant interaction between cultivar and spacing, as one would expect when comparing vine (Montrose) and upright (Vision) growth habits in different plant spacings. Both cultivars yielded more with the 2-line spacing that provided more uniform distribution and utilization of resources (light, moisture, nutrients). Yield of the vine cultivar was increased by 10% and 0%, while the yield of the upright cultivar was increased by 50% and 9% in 2004 and 2005, respectively. Additional testing of released cultivars and future releases is warranted under varying environmental conditions and disease pressure in the dry bean producing regions with a history of white mold.

During 2004 and 2005, we evaluated the efficacy of thiophanate methyl and boscalid applied to foliage of susceptible cultivars 'Montrose' and 'Vision' before inoculation of leaf disks with the white mold pathogen. The rate of leaf colonization by the fungus was recorded over time (2 to 5 days post-inoculation and incubation at 23°C). This series of laboratory experiments demonstrated that thiophanate methyl provided very good control (80 to 84%) of white mold, even after 5 days of incubation. The newer fungicide boscalid provided even greater control (97 to 99%). A second set of experiments evaluated the response (efficacy) of these fungicides applied in varying gallonage to foliage of susceptible cultivar 'Montrose' before inoculation of leaf disks with the white mold pathogen. The fungicides were applied in 46 to 2337 liters of water per hectare (5 to 250 gallons of water per acre), and thiophanate methyl provided very good control (84 to 96%) of white mold, even after 5 days of incubation. Boscalid provided less control (39 to 93%) with different gallonages, but still offers a lot of potential for enhanced fungicide management for future IPM programs on dry bean and other crops that are affected by *Sclerotinia sclerotiorum*. Both fungicides were more efficacious when applied in 234 or more liters of water per hectare (25 or more gallons or water per acre). These rates are typically associated with ground rig or low volume chemigation equipment; while rates less than 234 liters per hectare are typically associated with aerial equipment. A final series of experiments demonstrated that thiophanate methyl and boscalid, applied in 46 to 2337 liters of water per hectare (5 to 250 gallons of water per acre), provided very good control of white mold, even after a simulated rain event (6.35 mm or 0.25 inches in a 15 minute period). Thiophanate methyl provided 93 to 95% control and boscalid provided 87 to 95% with different gallonages and the post-treatment rain event. Both fungicides provided 90 to 98% control when there was no simulated rain event post-treatment. Future experiments should investigate the effectiveness of adjuvants combined with fungicides against different isolates of Sclerotinia on susceptible cultivars of dry bean and other white mold hosts.

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Efficient insertional mutagenesis of *Sclerotinia sclerotiorum* using Agrobacterium-mediated transformation

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Funded Plan of Work: Identifying virulence factors of *Sclerotinia sclerotiorum* through insertional mutagenesis

Abstract:
Sclerotinia sclerotiorum, the causal agent of stem rot or white mold, is one of the most serious pathogen of many economically important crops. Despite extensive investigations on the pathogen and the disease, the genetic factors that control the pathogenesis of *S. sclerotiorum* are incompletely understood, except for the recent studies on the *pac*1 gene and the *pg*1 gene. *S. sclerotiorum* was transformed using protoplast-based protocols for targeted mutagenesis by Rollins in 2003. A more convenient transformation system without protoplast preparation is sought in order to study other genetic factors involved in pathogenesis. An insertional mutagenesis protocol using *Agrobacterium tumefacience*-mediated transformation is developed to transform *S. sclerotiorum*. Both mycelial fragments and ascospores were successfully used in transformation with hygromycin phosphotransferase (*hph*) gene as a selection marker. Two promoters were compared for their efficiency in expressing the *hph* gene in *S. sclerotiorum*, and the *Aspergillus nidulans trp*C promoter was better than the Cauliflower mosaic virus 35S promoter CaMV35s. Because of the uneven growth rates of the fungus and the bacterium, a low temperature condition of co-cultivation enhanced transformation efficiency. More than130 transformants have been generated and screened. Transformants showed normal growth on PDA containing hygromycin B which inhibits growth of wild type strains. The inserted T-DNA was detected in the transformants using PCR and Southern hybridization. In pathogenicity assays, 16 transformants showed reduced virulence or lost pathogenicity to lentil. We also developed a detached stem assay to test virulence of transformants. The results of detached stem assay were generally correlated with whole plant assay conducted in the greenhouse. The insertional mutagenesis shall be a useful tool in studying the genetic factors of virulence of the important pathogen *S. sclerotiorum*.

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Epidemiology and resistance to Sclerotinia head rot in wild sunflower species

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Funded Plan of Work: Resistance and control of Sclerotinia head rot in sunflower.

Abstract:
Field trials were conducted in 2004 and 2005 at the Agriculture and Agri-Food Canada Research Station, Morden Manitoba to assess the efficacy of nine fungicides in controlling sclerotinia head rot in sunflower. A susceptible oilseed hybrid was used in 3-row plots with a randomized complete block design and four replicates. Rows were 3 m long and 0.75 m apart. Each fungicide was applied in three treatments: a single application at early flowering (early), a single application at late flowering (late), and two applications at early and late flowering. All plots in the test were artificially inoculated with ascospore suspension and ground sclerotinia-infected millet seed at 24 hrs after each fungicide application, and a misting system was operated to provide dew and high humidity to enhance the infection process and the disease development. Head rot was assessed weekly on each plant using the scale of 0 to 5 where 0 is health and no infection, and 5 is very severe infections and collapsed heads. Disease index was calculated, yield was measured and the percentage of sclerotia in harvested seed was estimated for each plot. All fungicide applications reduced the head rot disease index, but only a single application of the JAU6476, Lance, and Topsin significantly reduced the disease index from 6.1 to 3.8-4.2. Two applications of Ronilan, Benlate, and Fluazinam reduced the disease index to 4.3-4.4. Rovral and Quadris reduced the disease index to 4.7. Most early fungicide applications resulted in reduction in yield losses from sclerotinia head rot. A single application of JAU6476, Topsin, Benlate, and Ronilan significantly improved yield by 31-40%. Two applications of Benlate, and Ronilan resulted in 46-50% yield improvement. Most fungicide applications resulted in reduced sclerotia in the harvested seed samples, however, significant reductions were obtained with JAU6476, Benlate, Lance, Ronilan, and Topsin.

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Epidemiology and resistance to Sclerotinia head rot in wild sunflower species

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&
Gerald J. Seiler, USDA, ARS, NCSL, Fargo, ND

Funded Plan of Work: Epidemiology and control of Sclerotinia head rot in sunflower and wild sunflower species

Abstract:
Field trials were conducted in 2002-2005 to understand the epidemiology of the Sclerotinia infections to wild sunflower heads and stems, to establish methodology for assessing wild sunflower germplasm, and to identify sources of resistance. In 2002-2003, 96 accessions of perennial wild sunflower species *Helianthus maximiliani* and *H. nuttallii* were tested in a 4-replicated randomized complete block design with various inoculum types, inoculation at three growth stages, and using three head coverings. Ground sclerotinia-infected millet seed and ascospores inoculated at the mid flowering stage and covered with brown paper bags proved to be the most appropriate method to create disease epidemics to differentiate between susceptible and resistant genotypes. In 2004-2005, 400 accessions of the perennial wild sunflower species were evaluated using the standard artificial inoculation procedure established in previous years with a combination of ascospores and ground sclerotinia-infected millet seed. Different groups of plants (5-10) from each accession were inoculated at the early flowering and late flowering stages, and were covered with light-brown paper bags.

The typical symptoms of sclerotinia infection on wild sunflower plants were stem bleaching, shredding, and the formation of tiny cylindrical sclerotia inside the stems, while the heads were shriveled, dry with little or no seed setting. Some changes were observed in the reaction of several accessions from year to year, however, most wild accessions identified with resistance to head rot in 2002-2003 remained resistant in 2004 and 2005. Several accessions remained healthy in the four years of testing under the various artificial inoculation methods. Such accessions are believed to have genetic resistance to Sclerotinia head rot and mid-stem infection. Present research is focusing on studying the genetics of this resistance and the transfer of the resistance genes to sunflower breeding lines for future hybrid development. Such accessions will be deposited at the Plant Gene Resources of Canada, AAFC and at the USDA Sunflower Research Unit.

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Evaluation of Canola Cultivars for Resistance to Sclerotinia

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Funded Plan of Work: Evaluation of Canola Cultivars for Resistance to Sclerotinia

Abstract:
The objective of this project is to identify canola (*Brassica* napus L.) cultivars which are less susceptible to Sclerotinia. In 2005, field trials were conducted at the North Dakota State University Carrington Research Extension Center and an on-farm site near Red Lake Falls, Minnesota. Twenty-six canola cultivars, representing current production varieties and private breeding lines, were evaluated in a randomized complete block design with four replicates. Plot size was approximately seven 7-inch rows x 25 feet. At flowering, plots were inoculated with ascospores (foliar spray) and misted until physiological maturity to provide a favorable environment for disease development. Disease incidence and severity were evaluated, as well as plant height and lodging at maturity and grain yield, test weight, and oil concentration at harvest. Data were analyzed by standard statistical procedures and means were compared by F-protected LSD. Weather during flowering was exceptionally hot and dry and the inoculations with ascospores were probably ineffective. Minimal disease pressure was achieved at Red Lake Falls (only two plots had disease incidence greater than 6%). At Carrington, heavy misting late in the season stimulated germination of residual sclerotia from the 2004 sunflower head rot trial, which resulted in significant disease pressure (incidences ranging from 4.5 to 68.5%). However, the disease attacked relatively late, favoring the entries which were close to physiological maturity (a 14-day range was recorded in the date of physiological maturity). Highly significant negative correlations were observed between disease incidence and days to flowering and physiological maturity, plant height, yield, and seed size. Disease was highly and positively correlated with lodging. One application of Endura fungicide reduced Sclerotinia incidence in both varieties which were treated, but yield was not increased due to the lateness of disease pressure.

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Fungicide Efficacy for Sclerotinia Head Rot Control on Sunflower

S. Halley, Langdon Research Extension Center North Dakota State University

Funded Plan of Work: Sunflower Head Rot Screening Nursery and Fungicide Evaluation

Abstract:
Sunflowers (*Helianthus annuus* L.) are important component of rotations in the plains region of the United States. Sunflowers fit very well in rotations with small grains and cool season crops and are one of the few crops that can root deep, use excess soil water, and recycle nitrogen deep from the root zone. Unfortunately sunflowers are commonly grown in rotations with crops with differing degrees of susceptibility to white mold disease. White mold, caused by the pathogen *Sclerotinia sclerotiorum,* can affect sunflowers in several ways. Head rot is the most economically devastating of these infection modes. Severe yield losses from the disease have growers searching for control measures. Breeding for resistance is in its infancy and alternative measures will likely be needed short term to keep the industry viable through years when environmental conditions conducive to white mold epidemics occur. *Sclerotinia sclerotiorum* over winters as sclerotia formed in the stem and seed head. These sclerotia contaminate the harvested sunflower and are nearly impossible to remove with seed cleaners. Confection type sunflowers suffer the greatest economic loss. This project is part of an initial effort to evaluate potential fungicides as a means to reduce these losses. Compounds for testing were requested based on their potential for controlling white mold in crops. In addition a baseline set of treatments was included to compare results with other locations. Disease was induced by spraying ascospores to the sunflower head at flowering and providing a wet environment by misting a confection type cultivar throughout flowering. Disease levels were very high in the trial although fields in the surrounding region had little infection. No differences were measured in disease levels, yield, and 200 seed weight.

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Fungicides Applied at Four Application Timings to Two Field Pea Cultivars with Differing Flower Durations for White Mold Disease Control

Scott Halley, North Dakota State University-Langdon Research Extension Center Langdon, ND and Blaine G. Schatz and Ezra Z. Aberle, North Dakota State University-Carrington Research Extension Center Carrington, ND

Funded Plan of Work: Fungicides and Application Timings for Sclerotinia Disease Control on Field Pea

Abstract:
Field pea acreages in North Dakota and Montana have increased since 2000 making the region the largest producer of field pea in the United States. Field pea fits well in rotations with small grain and other broadleaf crops All of these broadleaf crops, sunflower, lentil, chickpea, soybean, canola, and many weed species are also susceptible to white mold disease caused by the pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary. The length of time (many years) that the principal reproductive organism of Sclerotinia (sclerotia) remains viable in the soil and the lack of resistance available in currently planted cultivars make research to find alternative control measures necessary. Studies were conducted at the Carrington and Langdon Research Extension Centers in 2004 and 2005 to evaluate the disease susceptibility of two cultivars, Integra and Eclipse with determinate and indeterminant flowering periods respectively, and the efficacy of boscalid (Endura), prothioconazole (Bayer experimental JAU 6476), and thiophanate methyl (Topsin M) fungicide applied at 10%, 40%, 100%, or 10 + 100% flowering growth stages. Disease incidence was reduced on the stem by 28% and branches by fungicide applications. Quality factors, 1.) Protein was reduced with JAU 6476 fungicide when applied at Langdon in 2004, 2.) Seed weight was increased in a high disease environment at Carrington in 2005 with a JAU 6476 application, and 3.) Test weight was greater when Endura fungicide was applied but less when TopsinM was applied to Eclipse. Test weights were affected by environments, fungicides, and cultivars. Field pea yield was increased at Langdon by 17.2 bu/acre with the determinant cultivar Integra in 2004 and 8.5 and 12.3 bushels/acre at Carrington in 2005 with an application of JAU 6476 compared to Endura and Topsin M.

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Gene expression profiling of soybean challenged with *Sclerotinia sclerotiorum*

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Funded Plan of Work: Soybean genome response to Sclerotinia and oxalate, its major virulence factor

Abstract:
Sclerotinia sclerotiorum infects soybean leading to the destructive disease called white mold or Sclerotinia stem rot. Oxalate is a major virulence factor of *S. sclerotiorum*. We are utilizing a combination of the partially resistant PI and the susceptible Williams 82 as well as a resistant transgenic soybean plant that constitutively produces the oxalate-degrading enzyme, oxalate oxidase (OxO), and its susceptible parent AC Colibri to study how soybean plants respond to *S. sclerotiorum*. Cut stems of PI and Williams 82 were inoculated with agar plugs containing actively growing fungal cultures and stems collected at various time points to determine gene expression in response to *S. sclerotiorum*. In a separate experiment, leaflets of an OxO transgenic plant (line 80(30)-1) and its parent AC Colibri were inoculated using infected flowers. For each experiment, samples were obtained at multiple time points and frozen in liquid nitrogen within 30 seconds of collection. RNA was isolated from the samples, reverse transcribed using amino allyl incorporation for indirect labeling. Cy3 and Cy5 were assigned as labels according to loop designs for the experiments. Samples were hybridized on soybean cDNA microarrays (ca. 38,000 genes total) and images were obtained for each of the hybridized slides. A response measurement in log2 ratio intensity was obtained. Preliminary analyses on biological replicates were conducted for both experiments and will be presented. Significance of these results and identification of differentially expressed genes will be assessed using various statistical tools such as R/MAANOVA and SAS after conducting additional biological replications for the experiment.

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Genetics and mapping of resistance to Sclerotinia white mold in lentil

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Funded Plan of Work: Genetics and mapping of resistance to Sclerotinia white mold in lentil

Abstract:

Lentil (*Lens culinaris*) is an essential rotational crop in the Pacific Northwest and northern Plains states where it is grown in predominantly cereal based cropping systems. Sclerotinia white mold of lentil, caused by *Sclerotinia sclerotiorum*, limits productivity and affects crop quality. Preliminary screening of lentil germplasm has identified cultivars and lines with resistance/tolerance to the disease. Those lines are currently being used in the crossing program to develop lentil cultivars with improved resistance to the disease. Also, information on the genetics of resistance/tolerance in lentil would assist breeding efforts and accelerate the development of resistant varieties.

The goal of the project is to determine the mode of inheritance and genetic linkage map positions of the genes for white mold resistance in lentil and to use that information to breed white mold resistant cultivars. To determine the genetics of resistance to white mold in lentil we are establishing genetically defined populations of F_6 derived recombinant inbred lines (RILs) from crosses of resistant x susceptible parents and plan to evaluate those RILs in replicated trials under greenhouse and field conditions. RILs can be used in replicated trials at more than one location and in repeated experiments over years to insure a good quality data set for genetic mapping of the resistance genes.

Currently, we are using an F_2 population from the Pennell x Pardina cross to develop a preliminary genetic map of lentil using a combination of SSRs, RFLPs, AFLPs and RAPDs. That population is currently in the F_4 and we will continue development of that population to F_6 derived RILs. The map under development is in a rudimentary stage but we plan to further populate the map with markers using "Joinmap" to combine information from several lentil maps that have been published. Several other crosses are being similarly advanced to RILs for further evaluations. Resistant selections from these and other crosses made in the breeding program will be potential candidates for release as resistant cultivars. Current status of the project will be discussed.

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Identification of Quantitative Trait Loci Linked to White Mold Resistance in Common Bean

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Funded Plan of Work: Variety Development/Germplam Enhancement

Abstract:
The long-term goal of this project is to pyramid genes that confer white mold resistance found in the Andean common bean line G 122 with resistance genes from *P. coccineus*. The specific objectives for this years' research were to: 1) Identify QTL linked to resistance in a CSU RIL population derived from G 122 X Pinto CO 72548, 2) Complete mapping of the RIL population using molecular markers, and 3) Initiate pyramiding of resistance genes from G 122 with those found in *P. coccineus* accession PI 225956. Two RIL had higher resistance than G 122 based on the straw test. CSU RIL lines 31 and 67 had DSI 3.2 and 3.4, respectively compared to resistant parent G 122 with DSI 4.5. Both lines also showed lower levels of disease infection in the field however, the difference was not significant. One hundred twenty four molecular markers were used to map the CSU RIL population based on AFLP, SSR, RAPD and SCAR markers. A significant relationship ($P < 0.01$) was found between the B7 QTL (Miklas et al., 2001) and white mold reaction in both the greenhouse straw test and field. Based on single factor analysis, the B7 QTL explained 8.8% and 9.4% of the phenotypic variation in the straw test and field severity, respectively. In total, four markers were found to be significantly associated with white mold resistance in this RIL Population using single factor analysis. Based on composite interval mapping (CIM) strong evidence (LOD >2.9) indicated that three QTL influenced physiological resistance to white mold. The QTL were linked with marker loci a5p4195, ataca300, and *Phs* on linkage groups B2, B6a, and B7, respectively. The ataca300 region of B6a had the largest effect and accounted for 19.3% of the phenotypic variation for white mold reaction in the straw test. The a5p4195 region of the B2 linkage group accounted for 17.6%, and the *Phs* region of the B7 linkage group accounted for 16.3% of the phenotypic variation for the white mold reaction. To date we have developed 140 early generation inbred backcross lines (IBL) from a cross between the most resistant CSU RIL 67 and *P.coccineus* PI 255956. Currently the lines are BC_1F_2 and are being advanced via single seed descent. By fall 2006, we will have 140 $BC_1F_{5:4}$ IBL to achieve our long term goal of combining resistance genes from common bean with resistance from scarlet runner bean, and to validate the effect of QTL from both G 122 and PI 255956 using molecular markers.

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Improved white mold resistance in common bean from multi-site tests and progress in pathogen characterization

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

Abstract:
No complete resistance to *Sclerotinia sclerotiorum*, the cause of white mold, has been reported in common bean. To find the most consistent source of resistance, putative sources of partial resistance were tested at multiple sites located in most of the major bean production areas in the U.S. Pathogen isolates also were collected from collaborators from various locations within U.S. bean production areas to determine mycelial compatibility groupings (MCGs), aggressiveness (virulence) and molecular genotype. Over all tests and locations in the past two years, Cornell 501 and 602 were identified as having WM resistance and 501 was released for the snap bean industry. In 2005, the number of lines tested in greenhouse screenings tripled (a result of SI funding) and sources of resistance in black and red kidney classes were identified in field tests. The nine *S. sclerotiorum* isolates routinely used for greenhouse/lab screening for WM resistance in common bean from ID, ND, WI, WA, NE, CO, MI, OR, and NY, plus a reference isolate (1980) used as the type isolate for genome sequencing of *S. sclerotiorum*, were tested in a matrix for MCGs. Only two of the mycelial interactions, NY-WI and NE-OR, were compatible (clones); all other isolates were unique. Consistency of results and pathogen virulence discrimination in WM-resistance screening conducted in 2003 and 2004 indicated that the straw test was a more reliable screening method than the detached leaf test for common beans. Further testing of an alternative, non-destructive stem screening method which limits cell wall degrading enzymes and avoids wounding will continue. Variation in isolate virulence and MCGs may help explain why greenhouse/lab screening results often do not agree across different test sites. Microsatellites, locus-specific , multi-allelic genetic markers, have been used successfully in revealing variation within *S. sclerotiorum* populations. Sirjunsingh and Kohn developed a set of 25 primers to identify microsatellites in *S. sclerotiorum.* A subset of eight of these primers produced variant profiles in the nine screening isolates used in the MCG and virulence tests. Our next step will be microsatellite profiling of the 150 field isolates collected from our collaborators in the major bean production areas in the U.S.

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Influence of soil texture and moisture contents on carpogenic germination of Sclerotinia sclerotiorum sclerotia

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Abstract:
Experiments were conducted under laboratory conditions to evaluate the effects of varying sand and moisture contents on carpogenic germination of Sclerotinia sclerotiorum sclerotia. A clay loam and a sandy soil were physically mixed in various proportions to give a total of five soil textures differing in both clay and sand contents. Fifty grams of soil from each texture group were moistened to 25, 50 or 100% of their respective field capacity (FC) and placed in individual Petri dishes (10 x 2 cm). Each treatment was replicated four times and the whole experiment repeated once. Twenty laboratory-produced sclerotia were soaked in water overnight and then embedded in each dish. Dishes were sealed with Parafilm and incubated at 15 ± 1 C under 12 h light/dark cycles for the duration of the study. Starting on the third week of incubation and for the next 12 weeks, data on carpogenic germination (GERM), and stipe (ST) and apothecial formation (AP) were recorded every seven days. At the termination of the experiment, observations on %GERM, total ST (#), and total AP (#) were subjected to ANOVA analysis using PROC GLM in SAS. Results indicated significant ($P < 0.0001$) effects of sand content and moisture content on all variables evaluated. A significant negative linear relationship between sand content and %GERM, total ST (#), and total AP (#) ($R^2 = 0.30$ to 0.40) was detected when soils were at 25% FC. However, when soils were at 50% or 100% FC the association between these variables and sand content was positive; the association was particularly high for soils at 100% FC ($R^2 = > 0.81$). These results suggest that the nature of the association between soil moisture and carpogenic germination may depend on the texture of the soil.

Contact Information:

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:
Dry bean losses from white mold (WM) vary from 30% to 90%. Only low levels of resistance exist in dry bean. But, high levels of resistance exist in *Phaseolus* species in the secondary gene pool. Our goal is to introgress high levels of WM resistance from the secondary gene pool. Of 132 interspecific breeding lines (IBL) from populations of 'ICA Pijao' with the three species in secondary gene pool (*P. coccineus, P. costaricensis,* and *P. polyanthus* = *P. dumosus*) evaluated in the FY 2004, 51 IBL resistant (disease scores ≥3) or intermediate (disease scores >3 and ≤6) were selected for evaluation in FY2005. Also, 119 single plant selections were made from the 51 IBL, and their seed increased in the greenhouse. The specific objectives in FY 2005 were to (i) screen 51 IBL in the greenhouse in Colorado (using the straw-test) and Idaho (using the cut-branch method), and in the field in Idaho, (ii) screen 119 single-plant selections from the 51 IBL in the greenhouse and field in Idaho, and (iii) develop a new group of approximately 700 interspecific inbred and inbred-backcross breeding lines from six populations of white mold susceptible pinto Othello and UI 320 by resistant *P. coccineus* accessions PI 433246 and PI 439534. Seventeen of the 51 IBL and 10 resistant and two susceptible checks were included in a replicated field trial in Idaho, North Dakota, and Washington; and two IBL for the field test and four for the greenhouse test in the National Bean White Mold Nursery (NBWMN) in 2005. The 119 single plant selections from the 51 IBL were grown in a replicated field trial in Idaho in 2005. Three mycelial inoculations were made during flowering, and solid-set sprinkler system was used to maintain high humidity in Idaho. In spite of that, inadequate WM infection occurred which did not permit field evaluations. The greenhouse evaluation of all 170 interspecific genotypes is in progress in Colorado and Idaho. Although a few looked promising, all 17 IBL and 10 resistant checks were variable for white mold reaction. A new large-seeded dry bean breeding line A 195 with high white mold resistance was identified. Also, the "straw-test" was modified to facilitate a better differentiation among resistant, intermediate, and susceptible genotypes. Development of a new set of IBL from crosses among pinto bean and *P. coccineus* is continuing in the greenhouse in Idaho. Resistant IBL will be screened in greenhouse and field in FY2007, seed of the most promising IBL will be increased for germplasm release and registration, and two manuscripts will be prepared for publication in refereed journals. Four IBL will be tested in the NBWMN, used in a white mold control study, and information shared with bean growers, researchers, and other clientele.

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Mapping Sclerotinia white mold resistance in lentil

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Funded Plan of Work: Genetics and mapping of resistance to Sclerotinia white mold in lentil

Abstract:
White mold caused by *Sclerotinia sclerotiorum* is an important disease of lentils where conditions are conducive. Management of white mold in lentil is mainly through the planting of resistant cultivars. Tolerance to white mold has been identified in lentil germplasm and is being used to develop improved cultivars. Our goal in this study was to develop an improved genetic linkage map of lentil and locate the regions of the genome that are important for resistance/tolerance to the disease. Nine lentil crosses are being advanced by single seed descent to produce F_6 derived recombinant inbred lines (RILs) for use in phenotyping and for mapping the important genes. In order to accelerate the development of the genetic linkage map, we are currently using the F_2 population from the cross of Pennell (tolerant) with Pardina (highly susceptible). Molecular markers for map development using this population will include SSRs developed for lentil at ICARDA, RFLPs previously developed for lentil in our lab, and EST derived SSRs from *Medicago truncatula.* Marker density will be increased through the use of dominant markers such as AFLPs and RAPDs. The phenotypic data on the F_3 families of the Pennell x Pardina cross will be used in conjunction with the genetic linkage map to determine quantitative trait loci for white mold resistance in lentil. The current status of the project is described.

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Molecular Mechanisms of Oxalate Action in White Mold Infection

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Funded Plan of Work: Genetic basis of oxalate sensitivity in relationship to *Sclerotinia* diseases

Abstract:

Oxalic acid is an important virulence factor of *Sclerotinia sclerotiorum* that promotes pectin degradation via cell wall acidification and calcium chelation, suppresses the oxidative burst, and kills plant cells. Susceptibility of *Phaseolus vulgaris* and *P. coccineus* to *S. sclerotiorum* has been correlated with oxalate sensitivity. However, induction of oxalate oxidase activity was found to be correlated with high oxalate concentrations and susceptibility to *S. sclerotiorum* in *P. coccineus*, suggesting that this enzyme responds to oxalate stress but is not causally linked to resistance. Oxalate-induced wilting in response to white mold infection is at least partially dependent on stomatal opening. The response of guard cells to oxalic acid is vanadate-dependent, suggesting activation of the plasma membrane H^+-ATPase. While these findings provide important insights into the physiology and biochemistry of oxalate toxicity, the genetic basis for sensing and responding to oxalate is essentially unknown.

Genetic model organisms, including *Arabidopsis thaliana* and the yeast *Saccharomyces cerevisiae* can be used to gain insights into the molecular mechanisms of oxalate action in white mold infections. Differences in oxalate sensitivity and susceptibility to oxalate-deficient *S. sclerotiorum* are quantifiable in *Arabidopsis*, and efforts are under way to effectively screen for genetic differences in susceptibility to *S. sclerotiorum*. A combination of *Arabidopsis* ecotypes, EMS and candidate mutants will be used to identify genes that influence this host-pathogen interaction. In the meantime, a genetic screen using deletion mutants of *S. cerevisiae* has revealed 105 genes involved in oxalate tolerance. A subset of 23 mutants was found to be highly sensitive to oxalate ($\leq$ 2 mM) but relatively insensitive to malonate and formate. Seven of the genes deleted in these mutants were yeast-specific, thus limiting the number of candidates for further analysis to 16. Among these genes is *RIB4* encoding lumazine synthase, an enzyme involved in riboflavin biosynthesis. *COS1*, the plant ortholog of *RIB4*, has been reported to be involved in defense-related jasmonate signaling. Based on this finding, the hypothesis that jasmonate signaling enhances induced resistance to *S. sclerotiorum* will be tested.

These studies are expected to enhance mapping of quantitative trait loci for partial white mold resistance and to provide new opportunities for genetic engineering. In addition, jasmonate spraying may be considered for increasing field resistance to *S. sclerotiorum*.

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Potential Marker-Assisted Selection for Resistance to White Mold in Pinto and Great Northern Bean

Phillip Miklas, USDA-ARS, Vegetable and Forage Crop Research Unit, Prosser, WA

Funded Plan of Work: Towards Marker-Assisted Breeding for White Mold Resistance in Common Bean

Abstract:
The identification of quantitative trait loci (QTL) with major-effect on resistance provides an opportunity to use marker-assisted breeding to expedite development of cultivars with enhanced levels of white mold resistance. Two such QTL with major effect on expression of resistance in both greenhouse and field environments derive from different sources, G 122, a large-seeded landrace from India, and NY6020-4, a snap bean breeding line from Cornell University. The QTL from G 122 and NY6020-4 reside on different linkage groups, B7 and B8, respectively. Tightly linked DNA markers were used to introgress the QTL into susceptible pinto bean cultivars 'Winchester' (Pop I and II, -B7 QTL) and 'Maverick' (Pop III and IV, -B8 QTL), and great northern cultivar 'Matterhorn' (Pop V, -B8 QTL), using up to three marker-assisted backcrosses. Five resulting populations consisting of 50 (Pop I), 38 (Pop II), 52 (Pop III), 33 (Pop IV), and 41 (Pop V) $BC_2F_{4:6}$ or $BC_3F_{4:6}$ backcross inbred lines were tested in greenhouse straw tests and field disease nurseries for reaction to white mold. The B7 QTL had major effect in the greenhouse straw test explaining 42% and 64% of the variation in reaction, respectively, for Pop I and II. The B7 QTL exhibited less effect in the field, 17% and 12% for Pop I and II, respectively, in part, because of poor disease pressure. Similarly, the B8 QTL had major influence in the straw test explaining 47, 35, and 26% of the variation in disease reaction for Pop III, IV, and V, respectively. The B8 QTL was also expressed in the field as measured for Pop IV (17%) and Pop V (27%). Field data was missing for Pop III. No linkage drag due to presence of the markers was observed for agronomic traits such as harvest maturity, seed weight, and yield, but overall maturity was later and yield was depressed in the backcross inbred lines compared to the recurrent parents. Although the QTL explained a major portion of the phenotypic variation for disease reaction in the backcross populations they had only minor effect on the level of resistance conferred. In summary, this study validates the effect of the B7 QTL (G122) and B8 QTL (NY6020-4) for white mold resistance in different populations and genetic backgrounds and confirms the utility of the linked markers for marker-assisted backcrossing, selection, and integration of the QTL into different market classes. Further research and germplasm development will be needed to recover pinto bean and great northern bean possessing both B7 and B8 QTL for white mold resistance with acceptable maturity, seed quality, and yield.

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Preliminary QTL Analysis in a Black Bean x Wild Mexican Bean Inbred Backcross Mapping Population for White Mold Resistance

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Funded Plan of Work: Identification of Defense Response Genes for White Mold Resistance in Dry Bean

Abstract:
White Mold (*Sclerotinia sclerotiorum*) is a serious yield-limiting disease of common bean (*Phaseolus vulgaris*). A $BC_2F_{3:4}$ mapping population was developed using the Mexican black bean cultivar 'Tacana' as the recurrent parent and the Mexican wild accession PI 318695 as parents. Utilizing greenhouse straw test data, white mold field ratings, SSR and SRAP marker data, a QTL analysis was conducted to identify QTL associated with white mold resistance in this mapping population. The results of this QTL analysis will be discussed.

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Progress in Development and Testing of Doubled Haploid Canola Lines with Increased Tolerance to Oxalic Acid

D.V. Phillips, University of Georgia; Carl Bradley, North Dakota State University; and Paul Porter, University of Minnesota

Funded Plan of Work: A Novel Approach to Develop Elite, Sclerotinia Resistant Canola Cultivars

Development of DH lines and Screening by the leaf wilt test.

The production of DH lines has greatly exceeded expectations. The target was to produce about 75 DH lines that had been screened for oxalic acid tolerance. Dr. Kott has delivered seed of 377 lines (335 spring and 42 winter). With the large number of lines she was able to do an additional high level oxalic acid leaf wilt test that identified 101 lines with exceptional tolerance. The only remaining activity in this stage is harvest of the seed increase of 42 lines; these should be available for field testing in North Dakota in 2006.

Greenhouse inoculation on petioles and elongated stems during flowering.

All of the 173 spring lines that were delivered in 2004 have been inoculated on the petioles in the greenhouse. At least 3 replications of each line have been inoculated and addition replications are in process. The original plan was to inoculate all plants that survived the petiole inoculation on the elongated stem. Since most of the plants were killed by the petiole inoculation, the survivors are being grown out for seed increase and the progeny will be tested by stem inoculation. The 162 spring types and 42 winters delivered in late November, 2005 will be inoculated this winter.

Slightly less than 25% of the lines had a mean survival of 14 days or more after petiole inoculation. Less than 2% of individual plants survived for 21 days after inoculation. However, most that survived that long, lived on to produce seed.

Field testing for Sclerotinia resistance, agronomic traits and yield.

A total of 166 lines (156 DH lines and 10 original lines) were field tested in North Dakota in hill plots under mist irrigation. These plots were inoculated with infested millet seed. A total of 90 lines (80 DH lines and 10 original lines) were tested in Minnesota in row plots under mist irrigation. These plots were inoculated with ascospores. A total of 160 lines (150 DH lines and 10 original lines) were planted in a field nursery in north Georgia in October, 2005. These lines will receive natural inoculum (ascospores) and will be irrigated at flowering time if necessary. Preliminary data from this nursery will be available in early April and final results in May, 2006. The additional 162 spring lines and 42 winter lines arrived one month too late to be included in the Georgia 2005 test.

The hot dry weather at the Minnesota site resulted in extremely low disease incidence despite inoculation and misting. No useful disease data was obtained in 2005.

Despite the hot dry conditions, moderate disease incidence was obtained at the North Dakota test site at Fargo, ND. The mean disease rating for the lines ranged from 0 to 5.0 on a 0 to 5 scale. However, only 4% of the DH lines had severe damage and 43% had no lesions or only superficial lesions. The CV for this test was high and no correlation with the results of the greenhouse inoculations or the leaf wilt test will be attempted until additional replications are available.

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Progress in mapping QTLs responsible for resistance to Sclerotinia head rot and stalk rot in two segregating sunflower populations

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

Abstract:
Two segregating F_2 populations were developed by selfing a USDA experimental hybrid (HA 441 x RHA 439) and a proprietary commercial hybrid (Proseed 9405). These two populations were grown in three research plots during the 2005 growing season. The first plot (Mapleton, ND) was inoculated with Sclerotinia-infected millet seeds for stalk rot development under natural conditions. Only eight and ten plants out of the 192 plants developed stalk rot in Proseed 9405 and HA 441 x RHA 439 populations, respectively. The second plot (Fargo, ND) was inoculated with an ascospores solution (~5000 spores/ml) for studying head rot, which was developed under artificial misting irrigation. For disease reading, we used the zero (resistant) to five (susceptible) scoring system. The population could be divided into roughly two groups, and with a few plants scored between 1 and 4. We tested 192 plants for each population. There were 105 and 70 plants with disease scores of zero in the (HA 441/RHA 439) and Proseed 9405 F_2 populations, respectively. This could be the result of the fact that the USDA hybrid possesses more resistance genes than Proseed 9405, because the former produced more resistant progeny. Between each pair of parents, about 600 high quality polymorphic TRAP markers have been scored, which will be sufficient to construct linkage maps for QTL mapping. The third plot (Fargo, ND) was for generating F_3 seeds, and selfed seeds were harvested from 300 individuals for the replicated F_2-F_3 row field tests to locate QTLs conferring head rot tolerance in these two populations during the 2006 growing season. It will be possible to pyramid the tolerant QTLs into an elite USDA sunflower line if the two populations have different QTLs responsible for Sclerotinia tolerance.

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Progress in Transferring Sclerotinia Resistance from *Phaseolus coccineus* to *P. vulgaris* via the Advanced Backcross QTL method.

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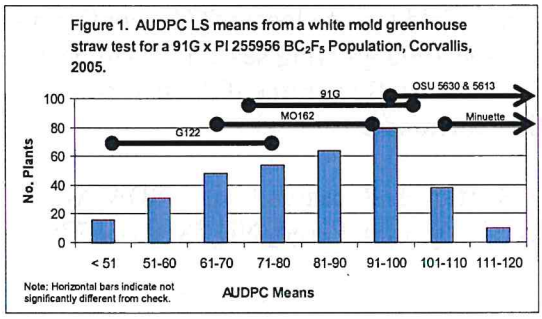
Funded plan of work: Mapping and Transfer of *Sclerotinia* Resistance from Scarlet Runner to Common Bean

Abstract:
This work builds on our initial screening for physiological resistance to white mold of all available *P. coccineus* accessions from the USDA NPGS plant introduction collection. The most resistant materials identified were used to develop a genetic map within the *P. coccineus* genome, investigate the physiological basis of resistance, and to transfer resistance into common bean (*P. vulgaris*). From our intraspecific mapping studies, we found that at least six randomly distributed QTL accounting for a total of 63% of genetic variation for resistance were present. We established that oxalate tolerance was strongly associated with resistance to white mold, but other resistance factors may also be involved. Several *P. coccineus* accessions with high levels of resistance were crossed and backcrossed to various common bean cultivars to establish populations for the transfer of resistance into *P. vulgaris*. Certain parental combinations were more productive than others, and as a result, we focused mainly on three populations: 91G x PI 255956 (BC_2F_5); 91G x PI 433251B, G122 x PI 433251B, and MO162 x PI 433251B (all in the BC_2F_2). Minimum population size in each backcross was 50 individuals in order to have a high probability of transferring complete resistance. The 91G x PI 255956 population has also been tested twice with the straw test, and once in the field. While in earlier tests resistance was bimodally distributed, in our field test and more recent straw test, resistance shows a normal distribution (Fig. 1) The latest straw test was read three times so that area under the disease progress curve (AUDPC) could be calculated. In the first straw test and the field trial, no families were better than G 122, but two were significantly more resistant than MO 162. In the third test (Fig 1.) one family was significantly better than G 122 and 18 were significantly more resistant than MO 162. The data shows that the 91G x PI 255956 population carries a high level of resistance, but because of the quantitative nature of resistance, it may be necessary to intercross individuals to achieve higher levels. Mapping studies are under way to examine the genetic architecture of resistance in the interspecific populations.



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QTL for white mold resistance in I9365-31 dry bean derived from *P. vulgaris* x *P. coccineus*

Phillip Miklas, USDA-ARS, Vegetable and Forage Crop Research Unit, Prosser, WA

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. I9365-31 is a black bean line that possesses resistance to white mold putatively derived from scarlet-runner bean. The objective of this research was to characterize resistance of I9365-31 to white mold in a mapping population tested across multiple field (two) and greenhouse environments (five). A recombinant inbred population consisting of 109 $F_{5:8}$ lines was developed from the cross Raven/I9365-31. 'Raven' is a commercial black bean susceptible to white mold. Separate R and S bulks for greenhouse and field reactions to white mold were used in bulked-segregant analyses to identify markers associated with resistance in the field and greenhouse. There were four independent quantitative trait loci (QTL) identified that conditioned resistance to white mold in the field. All four QTL were expressed (explaining from 9% to 24% of the phenotypic variation for disease score) across multiple environments (two years). The QTL were associated with disease avoidance traits, canopy porosity (7% to 24%) and canopy height (7% to 11%). Two major independent QTL conditioning resistance in the greenhouse were stably expressed across five separate straw tests. Phenotypic variation explained by the QTL ranged from 22% to 37%. Both QTL had minor expression in the field (5% to 9%). The QTL for physiological resistance to white mold as detected by the straw test had less expression in the field than previous QTL identified for other sources (G122, NY6020-4). Less expression of these QTL in the field is likely due to the increased importance of disease avoidance traits in conferring field resistance in this population. Composite interval mapping and integration of the QTL on the core map will be the next step. To determine utility for indirect selection, the major QTL detected by the straw test will be validated in different genetic backgrounds using marker-assisted backcrossing.

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Response of *Brassica napus* Germ Plasm Accessions to Sclerotinia Stem Rot

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Funded Plan of Work: Evaluation of *Brassica napus* accessions for resistance to *Sclerotinia* under mist-irrigation

Abstract:
Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, is a major disease of canola (*Brassica napus*) grown in North Dakota. Although differences in susceptibility exist, no commercial canola cultivars are marketed as having resistance to SSR. The National Plant Germplasm System (NPGS) currently maintains over 500 accessions of *B. napus*. The objective of this project is to evaluate these accessions and identify those with good levels of resistance to SSR. A series of greenhouse trials were conducted to evaluate all accessions for potential resistance to SSR using a petiole inoculation technique. Accessions identified from the greenhouse trials with potential resistance to SSR were further evaluated in field trials conducted in 2004 and 2005. In the field trials, accessions were inoculated with *S. sclerotiorum* – infested millet seed and mist-irrigated to increase disease pressure. Due to poor germination of some of the accessions, not all of the potentially resistant accessions were evaluated under all conditions (greenhouse trials, 2004 field trial, and 2005 field trial). Accessions identified as having some resistance in the greenhouse trials did not always express this resistance in the field and vice-versa; however, a limited number of accessions appeared to possess partial resistance to SSR in both greenhouse and field trials. These accessions could potentially be used in a canola breeding program to develop cultivars with improved levels of resistance to SSR.

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Sclerotinia resistance enhanced by accumulation of QTL and transgenic approaches

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

Abstract:
This project has two goals involving research on germplasm enhancement and variety development, including biotechnology. The first goal is to increase the level of resistance to *Sclerotinia 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. Three different populations were developed that combine resistance QTL from different sources. After screening over 4,000 plants through the F2, F3, and F4 generations we obtained F4-derived soybean lines that are homozygous for the desired marker alleles for the 8 QTL on 7 different linkage groups. Evaluation of 48 F4:5 lines representing 35 F4 families identified 10 F4 families that had significantly smaller average lesion size compared with the most resistant parent in the cross. The individual F5 lines with the smallest lesion size will be evaluated during 2006 for reaction to *S. sclerotiorum* using the detached leaf assay, and in multiple-location tests for yield and agronomic traits. Objective 2 is to determine if a novel antifungal synthetic peptide expressed in soybean will confer resistance to *S. sclerotiorum.* The T4 plants lines homozygous for the transgene, based on glufosinate resistance of the plants, were evaluated in 12 replications using the detached leaf assay. The leaf test results from the 2005 experiment indicate no significant differences in lesion size between the controls and the transgenic lines containing the D4E1 peptide. We are introducing a revised codon-optimized gene expression cassette into soybean that contains the barley alpha-amylase signal sequence to export the peptide to the apoplast. Evaluation will also occur during 2006, and if results indicate promise, crosses to high-yield soybean cultivars will be initiated. The second goal is to improve the use of calcium cyanamide as a control option for *S. sclerotiorum*. Two controlled-environment experiments conducted during 2004 confirmed the inhibitory effect of Perlka (Ca-cynamide) on germination of sclerotia. We evaluated 10 transgenic *cah*-gene lines, along with the non-transformed parental cultivar Thorne in 5 environments, with Perlka application of 0 kg ha^{-1}, 100 kg ha^{-1}, and 400 kg ha^{-1}. During 2004 the tests were grown at two disease locations and one non-disease location, and Perlka was applied at the R1 (beginning flower) growth stage. During 2005, the experiment was grown at two disease locations and a Perlka application at the V3-V4 stage was added as a treatment. Our results indicate no difference between the parental line and the transgenic lines for yield and disease resistance (DSI). The 400 kg ha^{-1} Perlka treatment resulted in higher yield.

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Sclerotinia stem rot and head rot resistant germplasm development utilizing wild perennial *Helianthus* species

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Funded Plan of Work: Development of Sclerotinia resistant germplasm utilizing wild *Helianthus* species

Abstract:
Moderately *Sclerotinia* stem rot tolerant line HA 410 was used to cross with resistant wild perennial hexaploid *Helianthus* species *H. californicus* and *H. schweinitzii* in 2005. All four crosses *H. californicus* 2376 × HA 410, *H. schweinitzii* 2404 × HA 410, *H. schweinitzii* 2405 × HA 410, and *H. schweinitzii* 2415 × HA 410 produced many good quality embryos, and the success of the embryo rescue was high, with the seedling survival rate of 55.65%, 35.71%, 40.71%, and 25.58%, respectively. Greenhouse and field evaluations indicated excellent stem rot resistance for interspecific F_1 progeny and HA 410. Pollen stainability of crosses *Californicus* 2376 × HA 410, *Schweinitzii* 2404 × HA 410, *Schweinitzii* 2405× HA 410, and *Schweinitzii* 2415× HA 410 were 37.77%, 45.12%, 54.23% and 31.59%, respectively, suggesting good F_1 fertility. Most F_1 progenies had acceptable backcross seed set, but with much lower sib-pollinated seed set. Field evaluation of stem and head rot resistance using artificial inoculation was also conducted using interspecific amphiploids of wild perennials *H. grossesserratus, H. hirsutus, H. maximiliani, H. nuttallii, H. strumosus* crossed with P21, intercrossed amphiploids involving *H. divaracatus* and *H. grossesserratus*, and BC_1F_1 of amphiploid involving *H. strumosus*. While HA 441 was mostly susceptible, all the amphiploids had better resistance than HA 410 for stem rot. Due to the later flowering of most amphiploids, head rot resistance was observed only on an amphiploid of *H. nuttallii* x P21, which segregated 11 resistant and 16 susceptible plants as compared with the all 17 plants susceptible for the tolerant check HA 441. Therefore, we believe it is possible to transfer new resistance genes from the two perennial species and the amphiploids to develop new germplasm lines superior to HA 410 and HA 441 for stem rot and head rot resistance, respectively.

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Sunflower Head Rot Screening Nursery and Fungicide Evaluation

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Khalid Rashid, Agriculture and Agri-Food Canada, Morden Manitoba
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Funded Plan of Work: Sunflower Head Rot Screening Nursery and Fungicide Evaluation

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. Entries consist of production hybrids and experimental lines submitted by private breeding programs. Individual heads are inoculated with ascospores and plots are misted to provide favorable conditions for disease development. After several weeks of misting, inoculated heads are evaluated for head rot symptoms. Progress toward resistant commercial hybrids is difficult to assess, since entries vary from year to year and increasingly more entries are experimental lines. However, there are signs of progress. Every year, several entries, including some confection types, are rated more resistant than the resistant check. Promising germplasm does exist in both oilseed and confection types. However, more emphasis is needed on commercial hybrids and more site-years of data are needed to verify previous results and to release the information to growers. To correct this situation, the project was significantly expanded in 2005. The initial screening nursery was conducted in Morden, Manitoba, as well as in Carrington. Good disease pressure was achieved in Carrington (18 - 100% incidence), but not in Morden (0 – 13.5% incidence). Approximately 20 of the best entries in the 2004 initial screening were evaluated in 2005 at Carrington, Morden, the NDSU Langdon R/E Center, and South Dakota State University - Brookings. As in the initial screening, Morden had poor disease pressure. The Brookings plots were lost to flooding. Good disease pressured was achieved at both Carrington (31 – 89% incidence) and Langdon (2 – 58 % incidence) and a highly significant correlation existed between entry rankings at the two sites (0.76, P = 0.0006). In 2006, a further expansion of the project is anticipated to include the NDSU research site at Oakes and an additional location. In a sister project (Tom Gulya, P.I.), the same set of 20 best entries was evaluated for Sclerotinia stalk rot. In addition, fungicide treatments and application strategies for head rot control were evaluated at Carrington, Langdon, Morden, and Brookings in 2005. In Carrington, the products and rates tested did not significantly reduce head rot, but Endura showed some promise. Application procedures are likely in need of refinement, since the fungicides tested have shown efficacy on Sclerotinia in other crops. In Langdon, fungicide effectiveness varied greatly with the adjuvants tested, which will form the basis of future work.

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The Drop-mycelium Greenhouse Evaluation Method in Prediction of Field Resistance to *Sclerotinia sclerotiorum* in Soybean

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Funded Plan of Work: Development of soybean varieties or germplasm resistant to Sclerotinia stem rot

Abstract:
The drop-mycelium method is a recently developed low-cost and high-efficiency greenhouse method to evaluate soybean for resistance to *Sclerotinia Sclerotiorum* (Lib.) de Bary. No information is available on the consistency between the results obtained with this method and the results obtained in field evaluations. The objective of this research was to assess the consistency of the results between the drop-mycelium method and field evaluations. Thirty five soybean genotypes with different levels of resistance to *S. Sclerotiorum* were used. The greenhouse evaluation with the drop-mycelium method was carried out at Michigan State University. Field evaluations were conducted in Wisconsin, Ohio, and Iowa. Disease Severity Index (DSI) was used to measure the resistance in the fields and plant mortality was used to assess the resistance in the greenhouse. Three replications were employed across all the experiments. In the greenhouse, 10 plants per genotype in each replication were inoculated at the V3 growth stage. The correlation coefficient between the plant mortality in the greenhouse and the DSI obtained from the field in Wisconsin was the highest and the most significant ($R = 0.68$, $P < 0.0001$), implying that they are highly correlated. The correlation coefficients between the greenhouse plant mortality and the field DSIs from Ohio and Iowa were the same ($R = 0.40$, $P < 0.05$). Among the field tests, the range of correlation coefficients was from 0.76 ($P < 0.0001$) between Wisconsin and Iowa to 0.39 ($P < 0.05$) between Ohio and Iowa. The results from this study showed that the drop-mycelium method is a viable greenhouse evaluation method to predict the field resistance to *S. Sclerotiorum* in soybean.

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Transformation of *Sclerotinia sclerotiorum* with the green fluorescent protein gene (GFP) and use of GFP to study host resistance.

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Funded Plan of Work: Innovative Methods to Identify Resistance to *Sclerotinia sclerotiorum.*

Abstract:
Sclerotinia sclerotiorum is a plant pathogenic fungus that causes important diseases on a wide variety of broadleaf crops grown in the USA. Although there has been considerable work on identifying resistance in susceptible crops, incorporation of resistance into commercial cultivars remains a difficult objective largely due to the lack of rapid and effective methods for greenhouse, field, or laboratory screening. Fungal biomass levels in infected tissues should be a strong indicator of host resistance because restricting pathogen growth is an important aspect of the resistance response. We hypothesized that the expression of the green fluorescent protein (GFP) in a GFP-tagged fungus should correlate with total fungal biomass, and thus be a tool to study host resistance. Two isolates of *S. sclerotiorum* (ND30 and ND21) were transformed using standard protoplast/polyethylene glycol-mediated transformation and two GFP constructs (gGFP and pCT74) with constitutive promoters. Twenty putative hygomycin resistant transformants were obtained in 7 to 12 days and 7 were selected based on GFP expression for further study. Expression of fluorescence was stable in selected isolates after serial transfers on potato dextrose agar over 21 days. The presence of the GFP gene was confirmed by amplification of a 606 bp region of genomic DNA from selected transformants representing a GFP gene sequence. These transformants were pathogenic on soybean, bean, canola, and sunflower; however in culture they grew slower than the wild type. Infected tissues were examined on a Leitz Wetzlar epifluorescence microscope, or a Nikon E600 CARV Confocal cell imaging system both equipped with filters for GFP excitation and emission. Hyphae of transformants fluoresced in and on host tissue and could be distinguished from the plant cells. Using soybean as the test plant, excised leaves were inoculated with the wild-type and GFP-tagged *S. sclerotiorum.* Total protein was extracted from leaf samples using standard techniques. Protein concentration was standardized for all samples and the fluorescence levels were measured on a BioTek Synergy HT multi-detection microplate reader. Fluorescence levels were up to seven times greater in the tissue infected with the GFP isolate than in tissues infected with the non-GFP (wild type) isolate or non infected tissues. These results demonstrate that the GFP transformed pathogen was readily detected in tissue with this methodology. Studies are in progress to determine if differences in host resistance of crops can be measured using this technology.

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Use of oxalic acid to characterize soybean accessions for partial resistance to *Sclerotinia sclerotiorum*

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Abstract:
Different methods of assessment may be needed to assign the function of soybean QTL putatively linked to physiological resistance to *Sclerotinia sclerotiorum*. Efforts have been made to characterize the genetics of partial resistance through the identification of QTL. Several QTL are linked to phenotypes associated with disease escape mechanisms. Other QTL are linked to phenotypes most likely associated with physiological resistance, but explain only 9.6% of the variability observed among interaction phenotypes. It is not known whether specific combinations of QTL in the same soybean genotype would confer higher degrees, and more environmentally stable forms of resistance. We hypothesize that various QTL are associated with different mechanisms of physiologically-based resistance. Phenotypic data derived from multiple methods of disease assessments would provide guidance as to which QTL could be combined to achieve a more complete and environmentally stable form of resistance. Using multiple assessments to evaluate plants with genetically characterized resistance will help link phenotype to genotype, leading to a greater understanding of resistance. Oxalic acid is a putative virulence factor associated with pathogenesis by *Sclerotinia sclerotiorum*. Researchers have used oxalic acid with varying success to challenge plants in efforts to screen accessions of various host species for resistance to *S. sclerotiorum*. The use of oxalic acid rather than the pathogen has been perceived to reduce variability associated with the plant / *S. sclerotiorum* interaction. In a preliminary experiment we used oxalic acid (20 mM) to challenge trifoliate leaves removed from flowering soybean plants of varying reaction to *S. sclerotiorum*. We assessed tolerance to oxalic acid by measuring the percent mid-rib discoloration of each leaflet. Three resistant accessions, W04.1000, W04.1001, and W04.1002, exhibited a high level of tolerance and differed from NK S19-90, the resistant check, which performed in a similar manner to W01.1305 and BSR-101, the susceptible checks. The results of this preliminary experiment suggest that resistance to *S. sclerotiorum* and tolerance to oxalic acid may be conferred by unique genes or gene combinations. Various aspects of these results were repeated in subsequent experiments. Experiments are currently underway to optimize methodology, incubation environment and assessment variables. We plan to utilize three methods of assessment: field data, inoculation in controlled light environments, and the oxalic acid assay, to differentiate genotypic and phenotypic differences among soybean accessions reported to express partial resistance to *S. sclerotiorum*.

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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 were undertaken in 2005. The first approach involved screening 37 pea genotypes under field conditions at Carrington, ND and 24 and 12 genotypes of pea and lentil, respectively, in a grower's field near Spangle, WA. Research plots at Carrington consisted of seven rows spaced 18cm apart and 7.6 m long and were arranged in a randomized complete block design with 4 replicates. During the flowering period, all plots were inoculated with a solution of ascospores on 6 July (3.32 million ascospores/plot) and again on 11 July (2.40 million ascospores/plot). Beginning immediately after the first inoculation, a misting system was employed to maintain a humid environment to 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, development, and grain yield and quality data were recorded. Natural infection from inoculum present in the soil was relied on for infection at the Spangle location. Unfortunately, environmental conditions at the Spangle location were not conducive to disease development in the relatively open canopy of peas while some disease was observed among the lentil genotypes. Statistically significant differences were observed in all parameters measured at Carrington except days to physiological maturity. However, the progression of disease did not allow evaluation of physiological maturity (and powdery mildew) in all plots. Several entries showed relatively high levels of susceptibility on the first evaluation date. The second evaluation may be the most useful for selection, since the disease had progressed sufficiently without dominating all entries and quite large differences were observed among commercial varieties. On the final evaluation date, Arvika (forage pea) and CDC Sonata showed the lowest disease occurrence. A highly significant negative correlation was observed between days to beginning and end bloom and Sclerotinia rating, but no relationship was shown to days to physiological maturity. Yield was highly negatively correlated to disease ratings and lodging. Genotypes with the greatest level of resistance will be used to develop genetic mapping populations for inheritance studies. 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 the binary vector, pART27A. The resulting plasmid is currently being used in transformtion studies to transfer the gene into pea and lentil germplasm. In addition, efforts are in progress to develop a twin binary plasmid which 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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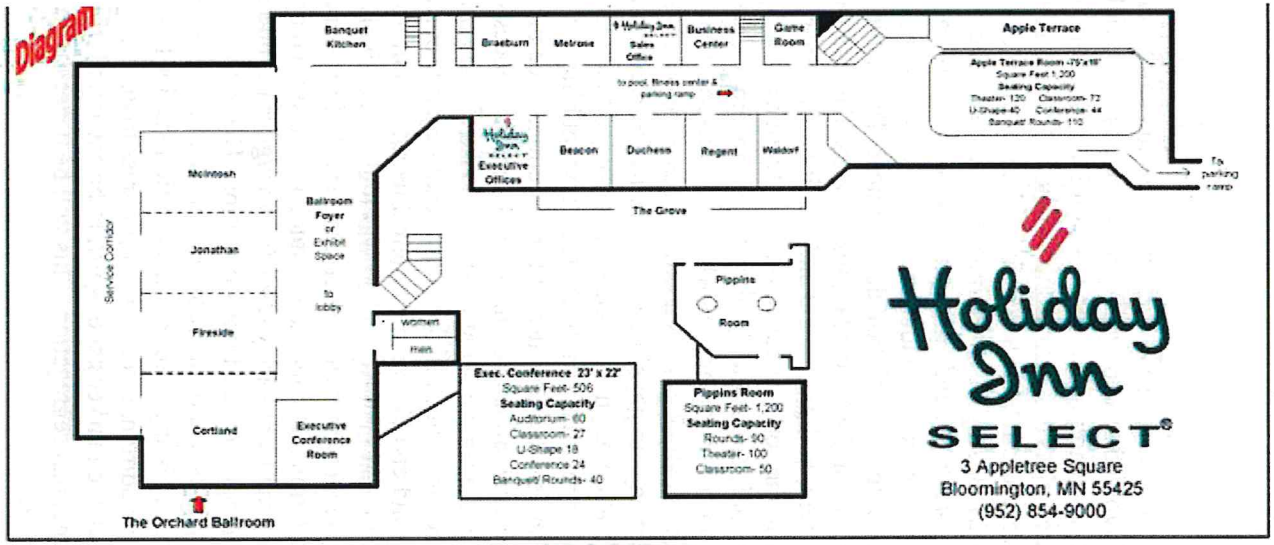

Meeting Room Specifications

	Orchard Ballroom					The Grove Room					The Empire			Apple Terrace	Executive Conference Room	Pippins Room	Meeting Foyer
	Cortland	Fireside	Jonathan	McIntosh	Combined	Beacon	Duchess	Regent	Waldorf	Combined	Braeburn	Melrose	Combined				
Dimensions	31' x 47'	28' x 47'	22' x 47'	22' x 47'	103' x 47'	25' x 20'	25' x 20'	25' x 20'	18' x20'	93' x 20'	25' x 25'	31' x 25'	56' x 25'	75' x 16'	23' x 22'	39' x 16' x 36'	
Square Feet	1,457	1,316	1,034	1,034	4,841	500	500	500	360	1,860	625	775	1,400	1,200	506	1,200	1,200
Seating Style	Seating Capacity																
Theater	175	150	125	125	600	60	60	60	30	210	70	80	160	120	60	100	N / A
Classroom	100	80	60	60	320	27	27	27	18	110	36	42	84	72	27	50	N / A
U-Shape	52	50	40	40	140	22	22	22	18	72	24	31	56	40	24	N / A	N / A
Hollow Square	62	54	48	48	172	30	30	30	28	78	34	40	66	72	30	40	N / A
Conference	44	44	44	44	176	18	18	18	14	68	20	30	52	44	18	24	N / A
Banquet Rounds	140	110	100	100	450	40	40	40	20	160	50	60	120	130	40	90	160
Exhibit Space 8x10	9	7	5	5	34	4	4	4	3	15	6	10	20	12	4	12	14
Reception (standing)	200	150	125	125	600	50	50	50	30	180	70	80	150	120	50	100	400
Ceiling Height	10'6"	10'6"	10'6"	10'6"	10'6"	8'6"	8'6"	8'6"	8'6"	8'6"	8'6"	8'6"	8'6"	10'	8'	10'	9'

Executive Conference Room is furnished with one removable 16 foot solid walnut table and 14 highback conference chairs.

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