NDSU/USDA-ARS

2005 Sclerotinia Initiative Annual Meeting

Bloomington, MN

January 18-20, 2005

National Sclerotinia Initiative

Canola

Chickpea

Dry Bean

Lentil

Soybean

Sunflower

2005 National Sclerotinia Initiative Annual Meeting

January 18-20, 2005

Holiday Inn Select

Minneapolis (Bloomington), MN

SCLEROTINIA INITIATIVE ABSTRACTS

Sclerotinia Initiative Annual Meeting 2005
Agenda

January 18, 2005

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

January 19, 2005

7:15 am Registration/Continental Breakfast

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

8:10 am Comments from ARS National Program Staff and Initiative Steering Committee

8:20 am Budget Update and Future Actions - **Dale Thorenson, U.S. Canola Growers, Washington, D.C.**

8:35 am Meeting Charge - **Larry Chandler**

8:40 am Industry Panel – How can we join efforts to fix this problem?

Moderator – Larry Chandler

Tim Eschbach – Frito Lay, Plano, TX
John Swanson – Croplan Genetics, Mentor, MN
Mike Hutter – Crop Consultant, Westhope, ND
Jean Liu – Pioneer Seeds, Johnston, IA
Additional panel members will be added.

10:00 am Break

10:30 am Research Updates

Moderator: Fred Muehlbauer, USDA-ARS, Pullman, WA

Bob Henson – North Dakota State University, Carrington, ND.
Development of mist nurseries to screen for sclerotinia resistance
Berlin Nelson – North Dakota State University, Fargo, ND. Innovative methods to identify resistance to Sclerotinia sclerotiorum
Howard Schwartz – Colorado State University, Ft. Collins, CO.
Cultural practices can play a key role in reducing white mold losses in dry bean and other crops.

noon — Working Lunch

Jean Liu – Pioneer Seeds, Johnston, IA. Current Sclerotinia Research on Soybean.

1:15 pm — Brochure/website update – **Kris Versdahl, Prairie Ag, Red Lake Falls, MN**

1:30 pm — Sclerotinia Best Management Practices – What do you Tell a Farmer Who Had a Great Crop of sclerotia?

Facilitator – **Larry Kleingartner, National Sunflower Association, Bismarck, ND**

Expert Panel:

Jim Steadman – University of Nebraska, Lincoln, NE
Craig Grau – University of Wisconsin, Madison, WI.
Tom Gulya – ARS, Fargo, ND
Art Lamey – North Dakota State University, Fargo, ND
Marty Draper – South Dakota State University, Brookings, SD

Discussion

3:00 pm — Break & Poster Session

4:00 pm — Research Updates: continued

Moderator: Shree Singh, University of Idaho, Kimberly, ID

Jim Myers – Oregon State University, Corvalis, OR. Mechanisms and transfer of resistance in common bean.
Phil Miklas – ARS, Prosser, WA. Marker-assisted breeding for white mold resistance in dry bean.

Discussion

5:00 pm — Adjourn

Dinner on your own

January 20, 2005

7:15 am — Continental Breakfast

8:00 am Research on Sclerotinia in Canada

Moderator: **Gerald Seiler, USDA-ARS, Fargo, ND**

Dwayne Hegedus – Ag. Canada Saskatoon, Saskatchewan. Changes in gene expression associated with Sclerotinia infection and resistance in Brassica napus.
Khalid Rashid – Agriculture and Agri-Food Canada, Morden, Manitoba.
Management of Sclerotinia in sunflower in Canada.
Dilantha Fernando – Univ. of Manitoba, Winnipeg, Manitoba.
Biocontrol bacteria vs. Sclerotinia: Biological warfare in the canola cropping system to control Sclerotinia.
Henry Huang – Agriculture and Agri-Food Canada, Lethbridge, Alberta. Research on Biology and control of Sclerotinia diseases in western Canada.

Discussion

10:00 am Break

10:30 am Research Updates: continued

Moderator: Henrik Stotz, Oregon State Univ.

Jeff Rollins – University of Florida, Gainesville, FL. Genomic analysis of gene expression in Sclerotinia.
George Graef – University of Nebraska, Lincoln, NE. Sclerotinia resistance approaches in soybean.
Steve Clough – ARS, Urbana, IL. Genomic responses in soybean stem tissue to Sclerotinia infection.

noon Working Lunch –
Update on International Sclerotinia Research Symposium – **Jim Steadman and others**
Funding Opportunity Discussions – led by Rich Wilson and/or Rick Bennett, ARS, Beltsville, MD

1pm Strategic Plan Updates – reports from each research priority area and developing a process to finish plan. – **Larry Chandler and Rich Wilson**

3pm Wrapup/Initiative Business - **Larry Chandler**

4pm Adjourn

Sclerotinia Initative Website:

http://www.whitemoldresearch.com/

http://www.whitemoldresearch.org

http://www.sclerotinia.com

http://www.sclerotinia.org

Sclerotinia Initiative Poster Session

January 18-20, 2005
Minneapolis, MN

Epidemiology & Disease Management

Poster No.	Title	Author
1	Validation of a Sclerotinia Disease-Warning Model for Canola in North Dakota	L. E. del Rio, R. Harikrishnan, C. A. Bradley, J. Rasmussen, H. A. Lamey, and G. Platford
2	Crop Rotation and Sclerotinia in Canola - 2004 Update	P. Porter and D. LeGare
3	Use of Crop Sequence and Biological Control to Minimize Sclerotinia on Canola, Chickpea, Dry Pea Lentils, and Sunflower, 2004	J. M. Krupinsky, D. L. Tanaka, M. A. Liebig, S. D. Merrill, J. D. Hanson, T. J. Gulya
4	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, and E. Z. Aberle
5	Eco-tillage, Biopesticide and Resistance Management of White Mold in Dry Bean	H. F. Schwartz and M. A. Brick
6	Control of Sclerotinia Head Rot in Sunflower	K. Y. Rashid

Pathogen and Host Genomics & Pathogen Biology and Development

Poster No.	Title	Author
7	Screening for Oxalate Sensitivity and Tolerance in Arabidopsis	H. U. Stotz and L. Meeks
8	Genetic Diversity of the White Mold Pathogen *Sclerotinia sclerotiorum* from a Single Lentil Field	W. Chen, J. N. Wamatu, N. J. Grunwald, and L M. Kohn
9	Dry Bean Transformation to Enhance White Mold Resistance	A. R. O. Armenia, R. F. Allison, J. D. Kelly
10	Mechanisms and Transfer of Resistance in Common Bean	J. R. Myers and H. E. Stotz

11	Microarray Analysis of Oxalate Oxidase Transgenic Soybean Challenged with *Sclerotinia sclerotiorum*	S. J. Clough, S. Hubbard, Z Zhang, A, Davidson, S. Rioux, B. Calla, and D. Simmonds
12	The Role of Light in the Soybean/*Sclerotinia sclerotiorum* Interaction	A. J. Peltier and C. R. Grau

Germplasm Enhancement and Variety Development

Poster No.	Title	Author
13	Evaluation of Brassica napus Accessions for Resistance to Sclerotinia Under Mist-Irrigation	C. Bradley, S. Khot, L. del Rio, and J. Rasmussen
14	Evaluation of Canola Cultivars for Resistance to Sclerotinia	R. Henson, P. Porter, C. Bradley
15	Resistance Improvement of Bean Through Multi-site Screening and Pathogen Characterization	J. R. Steadman, L. K. Otto-Hanson, and K. Powers
16	Introgression and Pyramiding of White Mold Resistance from the Secondary Gene Pool of Dry Bean	S. P. Singh and H. F. Schwartz
17	Validation and Introgression of White Mold Resistance from Andean into Middle American Germplasm	M. A. Brick, J. J. Maxwell, P. Byrne, and H. F. Schwartz
18	Evaluation of Lentil cultivars for Resistance to White Mold and of inoculation Techniques for Screening Resistance to White Mold in Peas.	W. Chen, J. F. Myers, K. E. McPhee, F. J. Muehlbauer, and N. J. Grunwald
19	Progress in Mapping Resistance to Sclerotinia White Mold in Lentil	F. Muehlbauer, W. Chen, and K. McPhee
20	Development of a Novel Method to Evaluate Soybean for Resistance to *Sclerotinia sclerotiorum*	Y. Chen, X. Guo, and D. Wang
21	Genetic Mapping of Genes Underlying Partial Resistance to Sclerotinia Stem Rot in Soybean PI391589B	X. Guo and D. Wang
22	Development of Sunflower Germplasm with Enhanced Resistance to Sclerotinia Head Rot and Stalk Rot	T. Gulya, J. F. Miller, and G. Seiler

Application of Genomic Technology to the Analysis of Gene Expression in *Sclerotinia sclerotiorum*

Jeffrey A. Rollins, University of Florida, Gainesville, FL and Martin B. Dickman, University of Nebraska, Lincoln, NE

Funded Plan of Work: Application of Genomic Technology to the Analysis of Gene Expression in *Sclerotinia sclerotiorum*

ABSTRACT:
Studies of the basic biology of *Sclerotinia sclerotiorum* to identify genes that regulate pathogenesis and multicellular development will reveal new targets for disease control. We have previously approached such studies through single, candidate gene strategies. We are now taking advantage of genomic technologies to obtain a global view of gene expression and to identify genes associated with pathogenesis and development. On such technology is Expressed Sequence Tag (EST) analysis which not only allows for identification of new genes and verification of open reading frames for whole genome sequencing, but also can be used as a tool to infer gene expression patterns. Two cDNA libraries have been constructed for this EST study. The first was derived from vegetative mycelia incubated at pH 7 for four hours. The second library was constructed from differentiating apothecial stipes exposed to UV-A light for 55 hours. These tissues represent culture conditions thought to mimic early stages of pathogenesis and multicellular differentiation required for ascospore production, respectively. Five thousand single-pass sequence reads will be produced from each of these two libraries. To identify stage-specific genes within these EST collections, we will make pair-wise comparisons between uni-genes and assembled uni-sequence contigs from the two libraries. Data provided from this project will identify new genes involved in these critical processes and will empower the development of more efficient expression profiling tools such as gene mircroarrays.

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Crop Rotation and Sclerotinia in Canola – 2004 Update

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

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

ABSTRACT:
Research began in 2003 to better understand the effect of crop rotation and a rye cover crop on white mold development in canola. The primary objective was to evaluate sclerotinia incidence and severity in canola when grown in a number of cropping sequences with and without the presence of a fall-planted rye cover crop. A three-year field study was initiated in 2003 at one site (03CRye) and again in 2004 at a second site (04CRye). This update focuses on results of the second year of the 03CRye study. Treatments in 2004 involved canola and wheat following either 2003 canola or 2003 wheat with and without a 2003 fall-planted rye cover crop. Canola and wheat were planted on 4 May, and the rye was killed on 6 May. At that time, rye biomass on 2003 canola ground was 898 lb/ac, rye biomass and fall emerged volunteer spring wheat was 722 lb/ac, and biomass from fall emerged volunteer spring wheat alone was 325 lb/ac.

Previous crop (2003 season) had no influence on aboveground canola biomass in July. However, when rye was grown as a cover, the canola biomass was reduced by 39.7% compared with no cover crop (2372 vs. 1431 lb/ac). Previous crop influenced early-season wheat biomass: it was reduced by 12.8% with wheat following wheat (2048 vs. 1787 lb/ac). Early-season wheat biomass was reduced by 28.8% when grown following a rye cover (2240 vs. 1594 lb/ac). For early-season biomass there was no previous crop by rye cover interaction for canola, but there was an interaction for wheat (the wheat biomass decreased more after canola and a rye cover than after wheat and a rye cover).

Canola yield was not influenced by previous crop, but was reduced by 15.5% when rye was grown as a cover crop (1811 vs. 1531 lb/ac). Wheat yield was reduced by 9.1% when grown wheat on wheat compared with wheat on canola (3380 vs. 3072 lb/ac). Wheat yield following a rye cover was reduced by 9.0% compared with no cover (3378 vs. 3074 lb/ac. For grain yield, there was no previous crop by rye cover interaction for either wheat or canola.

The rye cover resulted in a reduced canola protein content in the seed (by 15%) compared with no cover (27.2 vs. 25.4%). Previous crop had no influence on protein content. Sclerotinia disease levels were quite low in 2004. The number of infected plants at swathing was less than 5%, yet there was some indication that infection levels were actually decreased following the rye cover crop compared with no cover crop.

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Dry Bean Transformation to Enhance White Mold Resistance

Ann Roselle O. Armenia, Richard F. Allison*, and James D. Kelly,
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Michigan State University, East Lansing, MI

Funded plan of work: Germplasm Enhancement/Variety Development

ABSTRACT:
White mold, caused by the aggressive fungal pathogen *Sclerotinia sclerotiorum*, is among the most destructive diseases to limit dry bean productivity. Previous studies have identified oxalic acid as the major pathogenic factor during white mold infection. Oxalic acid disrupts the integrity of the plant cell wall, facilitates lytic enzyme function, induces wilting and suppresses oxidative bursts within the host. The wheat germin gene *gf-2.8*, which encodes an oxalate oxidase that degrades oxalic acid into carbon dioxide and hydrogen peroxide, has previously conferred enhanced white mold resistance in both soybean and sunflower. Our study aims to: a) construct a transformation plasmid with both *gf-2.8* and *bar* genes; b) develop reproducible protocols for electrotransformation and particle bombardment of dry beans; c) evaluate effects of culture and transformation conditions on dry bean transformation efficiency; and d) develop dry bean cultivar(s) with enhanced white mold resistance. Plant transformation with the plasmid, pBKS*bar*/*gf-2.8*, is currently underway. Seedlings of two dry bean cultivars, Matterhorn and Olathe, were electrotransformed employing various pretreatments including hormones, lipofectine and ascorbic acid that were injected into the targeted apical meristems. To date, 1,040 seedlings have undergone electrotransformation. Among the T_1 progeny from 435 T_0 plants screened so far, eight have demonstrated resistance to the herbicide Liberty as would be predicted in plants expressing the *bar* gene. PCR analysis of two herbicide resistant T_1 plants revealed the integration of the *bar* gene in D20Matterhorn. Additionally, particle bombardment using the PDS-100 Helium Particle Delivery System with 2 rupture disk pressure levels is being directed to transform mature Matterhorn and Olathe embryos which are currently growing in tissue culture. Differences in embryo growth in terms of shoot and root production were observed between both cultivars treated with different cytokinin levels. Results from particle bombardment will be forthcoming.

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

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

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

ABSTRACT:

This project is investigating the roles of cultural practices (plant growth habit, plant spacing) and timely application of chemicals in reducing damage from *Sclerotinia sclerotiorum* to *Phaseolus vulgaris*. A field experiment was continued in 2004 to investigate the roles of plant growth habit (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, and boscalid) within an Integrated Pest Management context. Unfortunately, the regional drought effects of 2004 did not support appreciable disease development in this nursery in spite of our inoculation with white mold sclerotia prior to planting. The hot, dry conditions did favor a moderate outbreak of Mexican Bean Beetle after pod initiation. The field data were analyzed as a factorial, and plant spacing was the only significant main effect for yield (lb/acre). 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%, while the yield of the upright cultivar was increased by 50%. This field study will be repeated during 2005.

During the spring of 2004, a set of laboratory and greenhouse experiments systematically evaluated the efficacy of a standard (Topsin) and new (Endura) fungicide applied to foliage of susceptible cultivars. The rate of leaf colonization by the fungus was recorded over time (2 to 5 days post-inoculation and incubation at 23°C). The experiment was run 4 times, and data were combined over cultivars. Topsin provided 80 to 84% and Endura provided 97 to 99% control of white mold.

During the fall of 2004, another experiment evaluated efficacy of fungicides applied in varying gallonage to foliage of susceptible cultivar 'Montrose' before inoculation of leaf disks with the white mold pathogen. The experiment was run 2 times, and data combined over runs. Topsin applied in 5 to 250 gallons of water per acre, provided 84 to 96% control of white mold, while Endura provided 39 to 93% control. Both fungicides were more efficacious when applied in 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 25 gallons per acre are typically associated with aerial equipment. Additional greenhouse experiments with fungicide rain fastness will be conducted during Winter to Spring of 2005.

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Epidemiology and resistance to Sclerotinia head rot in wild sunflower species

Khalid Y. Rashid, AAFC, Morden Research Station, Morden, Manitoba
&
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, 2003, and 2004 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 2004, 400 accessions of the perennial wild sunflower species *Helianthus maximiliani* and *H. nuttallii* were evaluated using artificial inoculation 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 (August 16) and late flowering (August 30) stages, and were covered with light-brown paper bags. A few puffs of water were applied into each covering bag using a hand-held sprayer at the 2nd and 3rd day after inoculation to maintain high humidity and enhance the infection and disease development processes.

The level of infection in 2004 was Very high in this nursery in comparison with previous years. High levels of head and mid stem infections was widely spread in all commercial sunflower crops in the region due to the prolonged period of wet and cool weather during the 2004 growing season which created ideal conditions for Sclerotinia infections and development in all susceptible crops. In the wild sunflower, no signs of the typical soft rot and head disintegration as observed in commercial sunflower head rot symptoms. The stems were infected and showed typical symptoms of bleaching, shredding, and the formation of tiny cylindrical sclerotia inside the stems, while the heads were shriveled, dry with little or no seed setting. Most wild accessions identified with resistance in 2002-2003 remained resistant in 2004. However, a few accessions showed various levels of susceptibility in 2004 in spite of their resistant reaction in previous years.

The combination of ground Sclerotinia-infected millet inoculum and ascospores with paper bag covering in 2004 resulted in 93% infection in comparison with 88% infection in 2002 and 55% in 2003. Seven accessions remained healthy in the three 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.

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Evaluation of *Brassica napus* Accessions for Resistance to *Sclerotinia* under Mist-Irrigation

Carl A. Bradley, Sameer D. Khot, Luis E. del Río, and Jack B. Rasmussen, Department of Plant Pathology, North Dakota State University, Fargo, ND

Funded Plans of Work: Evaluation of *Brassica napus* Accessions for Resistance to *Sclerotinia* under Mist-Irrigation and Management of Sclerotinia on Canola in the Northern United States

ABSTRACT:
Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is a major yield-reducing disease of canola in North Dakota. Currently, the primary method of managing SSR of canola (*Brassica napus* or *B. rapa*) in North Dakota is through the use of fungicides. If SSR resistant canola cultivars were available, canola growers would be save the added cost of a fungicide application and be more profitable. Research was initiated in 2004 to evaluate *B. napus* accessions maintained by the National Plant Germplasm System for resistance to SSR. A total of 142 *B. napus* accessions were evaluated for resistance to SSR in a field study located at Fargo, ND in the summer of 2004. Plants were inoculated with *S. sclerotiorum* infected millet seed at the end of July and mist-irrigated to provide a conducive environment for infection and disease progression. Plants were rated for disease severity at seven different times after inoculation and an area under disease progress curve (AUDPC) value was calculated for each plot. The AUDPC values ranged from 0 to 72. Thirteen accessions had AUDPC values ranging from 0 to 30. These 13 accessions may have some level of resistance to SSR, but further evaluation is needed for confirmation.

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Evaluation of lentil cultivars for resistance to white mold and of inoculation techniques for screening resistance to white mold in peas

Weidong Chen, USDA-ARS, Pullman, WA; James R. Myers, Oregon State University, Corvallis, OR; Kevin E. McPhee, Fred J. Muehlbauer, USDA- ARS, Pullman, WA & Niklaus J. Grunwald, USDA- ARS, Corvallis, OR

Funded Plan of Work: Sources of resistance to white mold in the grain legume core collections

ABSTRACT:
Field experiments were conducted in 2004 in Corvallis OR to evaluate lentil cultivars for resistance to Sclerotinia white mold. Twelve cultivar-treatments were arranged in a randomized complete block with four replications. Each plot row was 20 ft long with 15 in. between rows. Seeds were planted on 23 June. Natural inoculum was abundant and no artificial inoculations were applied. Disease severity was rated on 4 November according to the following 1-to-9 scale: 1 = no infection, 2 = 1-3% infection, 3 = 4-10%, 4 = 10-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-90%, 8 = 90-97%, and 9 = 97-100%. ANOVA with a GLM (SAS) was used to determine the significance of treatment differences and protected LSD at $P = 0.05$ was used to separate treatment means. All of the cultivars are susceptible to white mold. Differences among the 12 test entries in response to white mold were observed. Mason and Pardina were clearly among the most susceptible cultivars, and should be avoided in fields where white mold is suspected to be a problem. CDC Sovereign and Pennel were among the cultivars showing tolerance to white mold. A general observation was that the more upright, thicker stemmed cultivars seemed more tolerant, which suggests that there is an architectural influence in white mold development in lentil. The results are in general agreement with previous greenhouse and field evaluations.

Greenhouse evaluation of peas for resistance to white mold is problematic because of a lack of suitable inoculation methods. Comparison of inoculation techniques for peas was conducted in the greenhouse at Pullman, WA. Three inoculation methods were evaluated: the colonized oat kernel method, the modified petiole inoculation method and the stem inoculation method. Among the three inoculation methods, it appeared that the stem inoculation method was more consistent in causing the disease on 10 pea lines. This stem inoculation method will be used on an extended collection of pea lines to determine the relative resistance to white mold among pea germplasm lines.

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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: Development of Sclerotinia Management Programs in Field Pea

ABSTRACT:
North Dakota ranked #1 in planted field pea acreage in 2004 with greater than 250,000 acres planted. Growers are including field peas in rotations that include other broadleaf crops susceptible to white mold (*Sclerotinia sclerotiorum*). Field peas are moderately susceptible to white mold compared to sunflower and canola. Preliminary studies conducted in North Dakota in 2002 and 2003 showed improved yield and reduced white mold incidence from fungicide applications. Additional studies were conducted in 2004 evaluating several promising fungicides, Bayer experimental JAU 6476 (prothioconazole), Endura (boscalid), and Topsin M (thiophanate methyl). The fungicides were applied at 10%, 40%, 100%, and 10 + 100% bloom growth stage to a determinate 'Integra' and an indeterminate 'Eclipse' flowering type cultivars. Sclerotinia incidence was different among cultivars and timings at both Carrington and Langdon. At Langdon fungicide applied at 100% bloom growth stage on 'Integra' was more effective than 10% growth stage, similar to results from Carrington that showed 10 + 100% growth stage application had less white mold incidence than 40% growth stage. In contrast, fungicide applications to 'Eclipse' were effective in reducing sclerotinia incidence at 100% growth stage compared to 10% growth stage at Carrington but no differences were measured at Langdon. Protein was reduced by applications of JAU 6476 at Langdon. Yields were increased by a 100% growth stage fungicide application of Endura at Carrington compared to a 40% growth stage application but decreased by the 100% growth stage application of JAU 6476 compared to 40% growth stage application.

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Genetic diversity of the white mold pathogen *Sclerotinia sclerotiorum* from a single lentil field

Weidong Chen, USDA-ARS, Pullman, WA; John N. Wamatu, Washington State University, Pullman, WA; Niklaus J. Grunwald, USDA- ARS, Corvallis, OR & Linda M. Kohn, University of Toronto, Ontario, Canada

Funded Plan of Work: Population structure of the white mold pathogen on pea and lentil in the US

ABSTRACT:
Experiments were conducted in 2004 to study genetic diversity of the white mold pathogen *Sclerotinia sclerotiorum* from a single lentil filed in eastern Washington. The research objectives were: 1. to determine MCGs of *S. sclerotium* collected from a single lentil field, and 2. to study the variation among strains in harboring dsRNA. Additionally the potential relationships of dsRNA with growth rates and sclerotia production were also examined. A total of 37 isolates of *S. sclerotiorum* were obtained from diseased lentil plants in a field near Colton, WA in 2003 by collecting sclerotia. Each sclerotium was collected from a different plant, unless otherwise indicated, at least 1 meter apart. Growth rates of the strains were measured by daily increase in colony diameter on PDA for the first three days, and production of sclerotia was measured by number and size of sclerotia produced in 10 day culture on PDA. MCGs of all isolates were paired in all possible combinations and determined on Modified Patterson's medium. Presence and relative concentration of dsRNA in total genomic DNA was determined using electrophoresis and confirmed by enzymatic digestions using RNase-free DNase and Ribonuclease A (RNase A) at different salt concentrations. Results showed that all strains that were from different plants were incompatible, although all strains were self compatible. Only four strains that were collected from the same plant were compatible. Thirteen of the 37 strains harboured nucleic acid bands with characteristics of dsRNA. They resisted DNase digestion and RNase digestion in high salt buffer, but were susceptible to RNase digestion at low salt buffer. Three dsRNA viruses ranged in sizes 0.7, 2.3 and 20.0 kbps were detected singly or in combination in strains of *S. sclerotiorum*. Strains showed significant differences in growth rate and in number, size and shape of sclerotium production. But the differences were not correlated with presence or absence of dsRNA. The results are very different from soybean and canola populations from the Midwest and Canada where a few MCGs dominated each population. The PNW may provide a unique environment that promotes genetic diversity in this fungus. The ecological roles of these dsRNAs remain to be determined.

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Genetic Mapping of Genes Underlying Partial Resistance to Sclerotinia Stem Rot in Soybean PI 391589B

Xiaomei Guo & Dechun Wang, Department of Crop and Soil Sciences, Michigan State University, MI

Funded Plan of Work: Development of Soybean Varieties or Germplasm Resistance to Sclerotinia Stem Rot

ABSTRACT:
Sclerotinia stem rot is one of the serious diseases in the northern U.S. Soybean plant introduction (PI)391589B showed partial resistance to the disease. The objective of this study was to identify putative QTL associated with resistance to the disease in PI 391589B. A population of 94 F_2 derived lines was developed from a cross PI 391589B x IA2053. The $F_{2:3}$ population was tested for resistance by artificial inoculation with *Sclerotinia sclerotiorum* at three different times in 2003. AUWPC (Area Under Wilt Progress Curve) was calculated for each plot. The $F_{2:4}$ population was tested for resistance by inoculation with drops of liquid suspension of *Sclerotinia sclerotiorum* mycelium on the top axilla of the main stem at six different times in 2004. The two parents of the mapping population were first screened with over 1200 SSR DNA markers. One hundred and nine SSR markers that were polymorphic between the two parents were used to genotype the entire population. The computer program JoinMap 3.0 was used to determine the linkage relationships among polymorphic SSR markers and the program WinQTLCart was used to test associations of the DNA markers with resistance to the disease. Both single marker analysis and composite interval mapping (CIM) methods were used in the QTL analysis. Single marker analysis revealed that marker Satt185, Satt212, and Satt651 on linkage group E, Satt153, Satt581, and Satt592 on linkage group O, and ungrouped marker Satt059 were significantly ($P<0.01$) associated with resistance to the disease. All the resistance alleles were from PI391589B except that the resistance allele of marker Satt185 was from IA2053. The CIM method identified two QTLs. One QTL with LOD score of 2.4 was near marker Satt651 on linkage group E and the QTL explained 10.1% of the total phenotypic variance. The second QTL with LOD score of 7.5 was near marker Satt581 on linkage group O and the QTL explained 34.0% of the total phenotypic variance.

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Genomic responses in soybean stem tissue to Sclerotinia

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Funded Plan of Work: Genetic Analysis of White Mold Resistance Using Microarrays

ABSTRACT:
We are using cDNA microarrays to aid in the search for genes related to defense against *Sclerotinia sclerotiorum* in soybean. The susceptible cultivar Williams 82 and the resistant plant introduction PI194639 were inoculated by applying agar plugs containing a fresh culture of *S. sclerotiorum* to freshly cut stems. The top 1.5 inches of inoculated or mock inoculate stems were collected at 0, 6, 18, and 48 hours post inoculation (hpi). This short time course indicated that the activation of enzymes involved in pathogen defense, such as phytoalexin production, had occurred within 18 hours. To identify differential expression at 18 and 48 hpi we used average fold change values of two biological repeats. Because differential gene expression at 6 hour was somewhat weak and less consistent, we increased our number of biological repeats to three independent inoculations and involved a statistical ANOVA calculation to assist in the identification of genes that were most significantly up or down regulated upon pathogen infection. The ANOVA of the 6 hour time point data revealed that about 100 genes out of 9,264 screened were significantly ($p \leq 0.01$) differentially expressed between PI194639 and Williams 82 including many with no known function. At 18 and 48 hpi we again noticed that a high percent (approximately fifty) of the differentially expressed genes had no known function. Select genes that showed a correlation with resistance, such as several signal transduction related genes including calcium associated and LRR containing, will be converted into molecular markers to determine if they are associated with known QTLs for resistance.

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Impact of preceding crops on incidence and severity of disease in canola

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A four-year rotation study was initiated in 2000 to determine the impact of preceding crops on disease incidence and severity in canola. Six rotations were evaluated and every phase of the rotation is present every year in a randomized complete block design replicated four times. The rotations consist of canola every one, two, three, or four years preceded by either canola, flax, or wheat. Half of each canola plot was treated with fungicide to prevent Sclerotinia stem rot (SSR). Plots were evaluated for SSR risk; SSR and blackleg incidence and severity; and yield and test weight.

Sclerotinia ascospore levels detected by petal and Steadman tests indicated very low disease risk in 2000. Low ascospore levels in 2000 were likely due to lack of inoculum in the area as the field history was cereal grains for twenty years prior to the initiation of the study.

In 2001, increased inoculum and favorable environment resulted in moderate disease risk, 65, and 100% incidence, detected by the early Petal and Steadman tests, respectively. However, little precipitation between the first and second evaluation caused a dramatic drop in disease risk. Risk is considered low at 0 to 45%, moderate at 45 to 95%, and high at 90 to 100% incidence on the petal test (Morrall and Thompson 1991).

In 2002 and 2003, the petal test indicated very low disease risk at both evaluation dates. In 2004, petal tests indicated moderate SSR disease risk, 56 % incidence; however, actual SSR incidence remained very low.

SSR disease incidence on standing canola plants adjacent to canola swaths was too low to detect any significant differences throughout the study. SSR disease incidence peaked at 5% in canola on canola rotations in 2001, where risk test indicated the highest level of ascospores, but canola yield was not adversely affected. In 2000 through 2004, there was little risk or incidence of SSR, regardless of rotation or fungicide treatment, in this study.

To date, general observations on disease risk and incidence indicate it is more dependent on environment than rotation.

Section 1.01 Literature Cited

Morrall, R. A. A. and J. R. Thomson. 1991. Petal test manual for Sclerotinia in canola. University of Saskatchewan, Saskatoon, SK. 25pp.

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Innovative Methods to Identify Resistance to *Sclerotinia sclerotiorum*

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

ABSTRACT:
The objective of this research is to utilize the green fluorescent protein gene (gfp) as a tool to measure the amount of the pathogen in host tissue. Our hypothesis is that quantifying the amount of fungus biomass in host tissue could be used to detect resistance. Research continued on the transformation of isolates of *Sclerotinia sclerotiorum* with gfp. We obtained a variety of plasmid vectors, each with different promotors: pTEFEGFP (with the *EGFP1* gene); pCT74 (*SGFP* gene); gGFP and tGFP (*SGFP* gene); and pGV2 and pGV3. Plasmid DNA was produced via the transformation of competent *Escherichia coli* and extracted and purified according to the procedure outlined by Sambrook et al. (1989). We initially used the Bio-Rad gene gun and bombarded 3 day old cultures with DNA coated tungsten particles. However, due to difficulty in access to the gene gun we switched to protoplast mediated transformation. Protoplasts were generated and a standard protoplast-PEG (polyethylene glycol) transformation method (Liljeroth et al., 1993) was employed. We concentrated our efforts on two pDNA's, pCT74 and gGPF, and two isolates, ND 30 and ND 21. Selection for transformants was on media amended with 100 µg/ml Hygromycin B. An *Agrobacterium* mediated protocol (Bundock et al., 1995) is also currently being attempted. Putative transformants via the PEG method appeared on the surface of the selection medium in 7 to 12 days and were transferred to potato dextrose agar amended with 100 µg/ml Hygromycin B. Twenty hygromycin resistant transformants were obtained, and 8 expressed gfp. In two transformants the gfp expression was reasonably strong, but in the others it was poor. All 8 putative transformants were pathogenic on leaves of host plants. Transformation experiments are continuing. In preparation for the eventual examinations of interactions between gfp *S. sclerotiorum* and host tissue we examined host tissue under UV light to determine the autofluorescence of stem and petiole tissue of soybean (cv. MN0301), bean (cv. UI114), and canola (cv. Hyola 401) and the root tissue of sunflower (cv. HA 89). Autofluorescence could interfere with the measurement of gfp in host tissue, thus it is necessary to know where the tissues autofluoresce. The bases of trichomes strongly autofluoresced and some vascular tissue fluoresced in soybean, bean and canola while the vascular tissue and the endodermis in sunflower roots strongly autofluoresced. Time required for infection of host tissues was also examined to determine the optimum time to examine a *gfp S. sclerotiorum* in host tissues. Tissues were inoculated with a tissue paper + mycelium inoculum and maintained in a humid chamber. In general, to obtain strong development of mycelium in host tissue required about 100 hours post inoculation.

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Introgression and Pyramiding of White Mold Resistance from the Secondary Gene Pool of Dry Bean

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Funded Plan of Work: Introgressing White Mold Resistance from the Secondary Gene Pool of common bean

ABSTRACT:
Very low levels of white mold (WM) resistance exist in dry bean. In contrast, *Phaseolus* species in the secondary gene pool are highly resistant. Our goal is to introgress and pyramid WM resistance from the secondary gene pool. Specific objectives are to (i) screen introduced interspecific breeding lines in greenhouse in ID (petiole test) and CO (straw test), and in the field in ID and WA, (ii) develop a new group of breeding lines from crosses of susceptible 'Othello' and 'UI 320' and resistant *P. coccineus* PI 433246 and PI 439534, (iii) determine inheritance and tag resistance genes and QTL, and (iv) pyramid resistance from across the primary and secondary gene pools. Of 21 *P. coccineus* from Spain evaluated in greenhouse in Idaho ZJ 1198 (disease score 3.5) and PHA-0669 (score 3.4) were highly resistant. Approximately 400 breeding lines from crosses of 'ICA Pijao' with *P. coccineus, P. costaricensis,* and *P. polyanthus* were evaluated in greenhouse in ID. An average of six plants/breeding line were screened (scores ranged 1.0-9.0, where 1=symptomless and 9=severely diseased). Approximately 150 breeding lines were evaluated in greenhouse in Colorado. An average of 10 plants/breeding line were screened (scores ranged 1.7-9.0). Of these 19 were evaluated in replicated field trial at Paterson, WA (scores ranged 2.7-9.0). Thus, four interspecific breeding lines (IS115, IS198, WM32, and WM60) with moderate to high WM resistance were identified for further evaluation. Othello, UI 320, PI 433246, PI 439534, and their F_1, F_2, and backcrosses were evaluated in greenhouse in Colorado to determine inheritance of resistance. A single dominant gene controlled WM resistance in PI 433246 and PI 439534. Also, 562 F_3 from two single-crosses, 502 F_2 from two first backcrosses, and 290 F_3 from two second backcrosses of Othello and UI 320 with PI 433246 and PI 439534 were produced in Idaho. Four single-crosses (MO162/I 9365-25, ICA Bunsi/G122, I9365-25/BelDakMi-RMR-20, and USPT-CBB-1/CORN601) and their two double-crosses involving five different sources of WM resistance were made in Idaho to pyramid WM resistance. All interspecific breeding lines involving ICA Pijao will be evaluated in greenhouse in Idaho. Highly resistant genotypes will be screened in greenhouse in Colorado, and in the field in Idaho and Washington. Inheritance of WM resistance will be repeated in greenhouse in Idaho. Also, a total of 502 F_2 and 852 F_3 from the two single, two first backcrosses, and two second backcrosses will be advanced in greenhouse in Idaho. Recurrent selection for pyramiding WM resistance will be initiated in two double-crosses in Idaho.

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Marker-Assisted Backcrossing of Two White Mold Resistant QTL Into Susceptible Pinto Bean: III. QTL expression and agronomic characterization

Phillip N. Miklas, USDA-ARS, Prosser, WA

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

ABSTRACT:
Resistance to white mold [*Sclerotinia sclerotiorum* (Lib.) de Bary] in dry bean (*Phaseolus vulgaris* L.) is quantitatively inherited with low to moderate heritability. Evaluation of resistance is further complicated by environmental influences and expression of both avoidance traits and physiological mechanisms. The identification of quantitative trait loci (QTL) with major-effect on resistance provides an opportunity to use marker-assisted breeding to combine resistance sources and 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 (SCARs and RAPDs) were used to introgress the QTL into susceptible pinto bean cultivars 'Winchester' (B7 QTL) and 'Maverick' (B8 QTL) using up to two marker-assisted backcrosses. Four resulting populations consisting of 50 (Pop I), 38 (Pop II), 52 (Pop III), and 33 (Pop IV) $BC_3F_{4:5}$ 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 62% of the variation in reaction, respectively, for Pop I and II. The B7 QTL exhibited less effect in the field, nonsignificant and 13% 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% and 35% of the variation in disease reaction for Pop III and IV, respectively. The B8 QTL was also expressed in the field as measured for population IV (17%). Field data was missing for Pop III. Agronomic characteristics, primarily yield, seed size, and harvest maturity were evaluated. Population means did not differ significantly from the recurrent parents for yield and seed size, but did differ for harvest maturity ranging from 7 to 19 days later maturity than the recurrent parents. This study validates the effect of the B7 and B8 QTL for white mold resistance in different populations and genetic backgrounds and confirms the utility of the linked RAPDs and SCARs 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 beans possessing the B7 and B8 QTL for white mold resistance with acceptable maturity.

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Mechanisms and transfer of resistance in common bean

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Funded Plan of Work: Transfer of Total *Sclerotinia* Resistance from *Phaseolus coccineus* to *P. vulgaris;*

ABSTRACT:
Over the past five years, we have evaluated the nature of resistance in *P. coccineus* (runner bean) and developed the populations for introgression into *P. vulgaris* (common bean). Accessions within this species have the highest degree of resistance to white mold (*Sclerotinia sclerotiorium*) that is readily transferable into common bean. Some researchers have found resistance to be qualitatively inherited whereas others have found quantitative inheritance. A major qualitative factor appears to give resistance in interspecific crosses, while additional quantitative factors when resistant and susceptible *P. coccineus* accessions are crossed. Transfer of resistance requires a strategy that allows introgression of quantitative resistance genes. By combining the backcross-inbred method with molecular marker tagging of QTL, we can effect transfer of total resistance and pyramid with resistance from other sources already present within the common bean germplasm pool. Oxalate, secreted by *Sclerotinia,* affects a number of physiological processes in the host plant and facilitates pathogenesis. It has been hypothesized that oxalate may compromise host defenses in one of several ways: 1) oxalate shifts apoplastic pH so that pectolytic enzyme activities are enhanced; 2) oxalate is directly toxic to plant cells; 3) oxalate chelates calcium ions and thereby compromises calcium dependent plant responses; and 4) oxalate suppresses reactive oxygen species production thereby suppressing host defense responses. Tolerance to oxalate is closely associated with partial resistance to *Sclerotinia* in common and runner bean. In addition, certain accessions of runner bean are more highly tolerant than the most tolerant common bean accessions. Oxalate oxidase does not appear to play a significant role in inactivating oxalate in *P. coccineus*, but an unidentified resistance mechanism reduces the concentration of oxalate in tissues of partially resistant accessions. Identifying mechanisms of resistance will allow a more targeted approach to breeding for resistance in all susceptible crop species.

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Microarray analysis of oxalate oxidase transgenic 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:

Oxalate is a major virulence factor of Sclerotinia sclerotiorum. Research involving fungal mutants as well as transgenic plants, has clearly shown that virulence of *S. sclerotiorum* is substantially reduced if oxalate is removed from the interaction. We are utilizing a transgenic soybean plant that constitutively produces the oxalate-degrading enzyme, oxalate oxidase (OxO), to study how soybean plants respond to oxalate and *S. sclerotiorum*.

Freshly opened flowers, inoculated between the standard and wing petals with 10 µL of ascospores (5000 ascospores/10 µL of 0.006% Triton X-100 in water), were incubated for 3 days in humid petri dishes and used for inoculum. The central leaflet of V4 leaves of the transgenic line 80(30)-1 and its parent AC Colibri were inoculated behind the first lateral vein with infected flowers.

Leaves were collected rapidly and frozen in liquid nitrogen within 30 seconds after removal from the plant. The lateral two leaflets were removed from the leaf, the remaining leaflet was removed from the plant by cutting the petiole close to the main stem, the infected flower was removed and the stage of invasion determined under a dissecting microscope. The leaflet was cut transversely 3 cm beyond its base and together with the petiole was frozen in liquid nitrogen. Two sample times were taken: 1. early appressorial formation (10-12 hour post inoculation) and 2. early vascular entry (18-20 hour post inoculation).

RNA from harvested tissue is being analyzed with soybean microarrays to determine how approximately 36,000 genes are responding to inoculation in the OxO transgenic line 80(30)-1 and the parent AC Colibri. ANOVA analysis of the microarray data will assign statistical significance to each gene as to whether or not its expression is differentially changing, and to what degree. Such knowledge promises to identify genes responding to *S. sclerotiorum* under normal and very low oxalate [in 80(30)-1] environment. This project promises to advance our understanding of the basic biology behind soybean's response to this serious pathogen and to contribute to our goal of developing crops with resistance to *Sclerotinia*.

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Population structure of *Sclerotinia sclerotiorum* in a pea field in the Pacific Northwest

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Funded Plan of Work: Population structure of *Sclerotinia sclerotiorum* in a pea field in the Pacific Northwest

ABSTRACT:
The population structure of *Sclerotinia sclerotiorum* on canola and on soybean in central Canada is part of one population of the pathogen that is mainly clonal with some local subdivision. The objective of our research is to describe the population structure of *S. sclerotiorum* in an intensively sampled field of pea. Fifty isolates were obtained from sclerotia collected from inside diseased stems or from plant tissue excised from the margins of stem lesions. AFLP, microsatellite markers and mycelial compatibility grouping were used to describe genotypes of the pathogen population. Two AFLP primer combinations were used and in one, 45 different fingerprints were identified, while in the other 50 fingerprints were observed. No common AFLP fingerprint patterns were detected between isolates retrieved from the WA pea field and 10 Canadian isolates derived from canola and soybean, used as reference isolates. Twenty-six different MCGs were observed and 6 of these were represented by more than one DNA fingerprint (between 2 and 6 fingerprints). Four clones based on MCGs and AFLP fingerprints were identified. Some isolates were compatible with isolates in several MCGs. Non-transitivity of MCG testing was observed in 10 cases. Using four microsatellite primer sets, 9 microsatellite profiles were identified in pea isolates and they differed from the reference isolates. The population of *S. sclerotiorum* in pea has a higher genotypic diversity in the Pacific Northwest than expected from previous studies.

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Producing interspecific hybrids for transferring Sclerotinia resistance genes from *Helianthus nuttalii* to cultivated sunflower

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Funded Plan of Work: Development and Use of DNA Markers for the Control of *Sclerotinia* in Sunflower

ABSTRACT:
We have obtained four interspecific F_1 plants with the long-term goal of transferring Sclerotinia resistance genes from perennial wild sunflower species to cultivated sunflower. Head rot resistant female parent, *Helianthus nuttalii* accession, N112, was pollinated with pollen of a cultivated sunflower inbred line, HA 89, and hybrid plants were obtained with the embryo rescue procedures. Four F_1 plants are confirmed by both morphological and DNA-based TRAP markers. The leaf morphology, plant height, and head diameter of the hybrids were intermediate between the two parents. Each of the these F_1 plants carried DNA markers from both parents. The segregation of the markers inherited from the female parent indicated that the female plant is heterozygous for many loci. These hybrids were highly sterile and embryo rescue procedures will be needed to produce backcrossed progeny. We plan to produce more hybrid plants to ensure the successful transfer of the resistance genes from *H. nuttallii* to the cultivated sunflowers.

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Progress in Development of Elite, Sclerotinia Resistant Canola Cultivars

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

PROGRESS DURING YEAR 1-The original project funded in 2004 followed 6 main stages: 1) Microspore culture and *in vitro* selection with oxalic acid; 2) Haploid plant development and screening by the leaf wilt test; 3) Doubled haploid production; 4) Screening of DH lines by the leaf wilt test and increase of seed; 5) Greenhouse inoculation on elongated stems during flowering; 6) Field testing for Sclerotinia resistance, agronomic traits and yield. (The greenhouse inoculation technique using petioles (Zhao, *et. al.*) will be added to stage 5. This will allow 2 inoculations, 1 on a petiole of a plant that will later be inoculated on the elongated stem during flowering.)
Progress on stages 1-4 (by Dr. Laima Kott, Univ. of Guelph, under sub-contract to this project) To assure seed in time for field testing in the Spring of 2005, the culturing began in Nov. 2003, prior to funding of the project.
1) Microspore culture and in vitro selection with oxalic acid - The 13 canola lines were divided into spring (10 lines) and winter (3 lines) groups because the winter lines take longer to process than the spring lines. Six donor plants of each of the 10 spring lines were cultured numerous times to provide large samples of immature pollen for mutagenesis and embryos for *in vitro* selection. After *in vitro* selection with oxalic acid, over 450 haploid embryos survived the mutagenesis and screening treatment. These were subsequently germinated on solid, rooting medium. After germination, haploid plantlets were transferred to soil. Culturing of the 3 winter lines is continuing.
2) Haploid plant development and screening by the leaf wilt test - Once the haploid plantlets reached the 3-4 leaf stage in soil, the leaf wilt test was done; just over 200 of the haploid plants had a score of 'good' at the 80 mM concentration of oxalic acid and were maintained for chromosome doubling.
3) Doubled haploid production - To date all plants have now been doubled and are at different stages of development. Of the over 200 lines generated, 64 have produced doubled haploid seed and have been threshed, while the rest are still drying down.
4) Screening of DH lines by the leaf wilt test and increase of seed - These 64 DH lines were replanted for increase and retested with the leaf wilt test at the 160 mM and 200 mM oxalic acid concentration levels. This procedure separates DH lines that can tolerate oxalic acid at the 'good' vs 'exceptional' levels.
5) -6) Greenhouse and field resistance testing - If the remaining 148 DH lines produce 'good' or 'exceptional' lines at the same rate as the 64 lines tested to date, there should be over 100 'good' to 'exceptional' lines for greenhouse inoculation this winter. The 75 best from greenhouse inoculations will be field tested for resistance in Spring 2005 in North Dakota and Minnesota and the best of these will be retested in Fall 2005 in Georgia.

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Progress in mapping 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:
Work was conducted in 2004 to advance our research to understand the genetics and to map resistance to Sclerotinia white mold in lentil. Resistance to white mold in lentil is quantitative. In order to map the quantitative trait loci conditioning the resistance in lentil, the first objective is to develop genetically defined populations of lentil that will segregate concurrently for resistance/tolerance to sclerotinia white mold and molecular markers. Nine populations from crosses between lentil lines with different levels of resistance to white mold have been advanced to F_4 by single seed descent to develop recombinant inbred lines (RILs). The nine populations consist of from 135 to 240 RILs in each for a total of 1752 lines. Polymorphic molecular markers were used to confirm that the populations are indeed from hybrid of the respective parents. It is anticipated that during the next 9 months, the populations will be ready for phenotyping their reactions to infection by *S. sclerotiorum*, and for genotyping polymorphic molecular markers. The phenotypic and molecular marker data will be used to generate a genetic linkage map of lentil for use in a quantitative trait loci analysis of white mold resistance.

Concurrent research efforts are to generate additional polymorphic markers in lentil. One approach was to transfer microsatellite markers from the model legume *Medicago truncatula* onto lentil. A total of 618 microsatellite markers of *M. truncatula* were tested on lentil and about 580 of the markers amplified by PCR. Twenty-one of the markers were co-dominant and four additional markers were found to be dominant. This suite of new polymorphic markers plus previously developed markers for lentil will allow us to generate a genetic linkage map for QTL analyses for white mold resistance.

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Progress in transferring *Sclerotinia* resistance from runner bean to common bean

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Funded Plan of Work: Transfer of Total *Sclerotinia* Resistance from *Phaseolus coccineus* to *P. vulgaris*

ABSTRACT:
Our overall goal is to develop white mold resistant dry and snap beans using highly resistant accessions of *Phaseolus coccineus* as a source. Resistance is quantitatively inherited within *P. coccineus*, while there appears to be a qualitative factor for resistance in interspecific crosses to *P. vulgaris*. In mapping efforts, we identified six QTL that collectively accounted for 62% of genetic variation for resistance within *P. coccineus*. This year, we focused on developing backcross inbred populations for six different interspecific cross combinations. The six populations have been chosen from a much larger set of initial crosses based on cross compatibility of the parents and fertility of the progeny. Backcross populations will be evaluated for resistance and for appropriate molecular markers. Initially, the population (OR91G x PI 255956) E BC_2F_2 has been evaluated for *Sclerotinia* resistance in the greenhouse using the straw test. Lines were evaluated four times at approximately one week intervals. The population showed a bimodal distribution that is associated with whether the infection passes through a node. We find that with *P. coccineus* derived materials, the standard eight day period used for evaluating common bean with the straw test in inadequate, with at least a two week interval needed for disease development after inoculation.

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QTL Analysis of Navy Bean-Derived Resistance to White Mold in Pinto Bean

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Funded Plan of Work: Identify and introgress molecular markers for white mold resistance in dry bean

ABSTRACT:
Pinto bean is the most widely grown dry bean (*Phaseolus vulgaris* L.) market class in the U.S., averaging 600,000 production acres annually. Pinto bean is extremely susceptible to white mold disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, which is rated the #1 disease plaguing dry bean production in the U.S.

Breeding pinto bean with resistance to white mold is difficult, due in part to the paucity of resistance sources in a related Middle American background. 'Bunsi' navy bean is a known source of resistance to white mold from the Middle American gene pool, which could be useful for improving resistance of pinto bean. Bulked segregant analysis was used to develop a partial linkage map of white mold resistance traits for the recombinant inbred population ($F_{5:7}$) derived from a cross between 'Aztec' pinto bean susceptible to white mold and ND88-106-04 navy bean with resistance to white mold derived from Bunsi.

The selective mapping approach identified four independent QTL conditioning resistance to white mold. One QTL (6-marker partial linkage group) had a major effect explaining 25%, 9%, and 8% of the phenotypic variation in disease score across three field environments. This QTL is derived from Bunsi and was previously identified by J. Kelly (Michigan State University) in two different populations Raven/Bunsi and Newport/Bunsi. This QTL also conditioned higher yield in two environments (9% and 8%), and is putatively placed on linkage group B2. Development of a SCAR marker to facilitate marker-assisted selection of this QTL is warranted. The three other QTL had lesser effect explaining 16% and 11% (6 marker partial linkage group), 14% and 7% (single marker), and 11% and 9% (single marker), of the variation in disease reaction across two field environments, respectively. Two of the QTL were also significantly associated with disease avoidance traits such as increased plant height and a more open plant canopy. The integration of these QTL in the core linkage map is ongoing. Markers linked with other Bunsi-derived QTL identified in Newport/Bunsi and Raven/Bunsi populations were mapped in the Aztec/ND88-106-04 by J. Kelly but were not found to be associated with resistance to white mold in more than one environment. A pinto bean line AN-37 directly obtained from the Aztec/ND88-106-04 RIL population has shown a promising level of white mold resistance in the international Bean White Mold Nursery for the past two years and will likely be released as a germplasm line in 2005.

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Quantitative Trait Loci and Gene Expression Changes Associated with Sclerotinia Resistance in Canola

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Funded Plan of Work: Genetics of *Sclerotinia* resistance in Canola

ABSTRACT:
Quantitative trait loci (QTL) involved in the resistance to Sclerotinia stem rot in canola were identified in two segregating populations of DH lines: the HUA population, derived from a cross between a partially resistant Chinese winter line (Hua dbl2) and a susceptible European spring line (P1804); and the MS population, derived from a partially resistant French winter cultivar (Major) and a susceptible Canadian spring cultivar (Stellar). A total of eight QTL affecting resistance were detected, seven in the HUA population and one in the MS population. Individual QTL explained 6 - 22% of the variance. The number of QTL detected varied from two to six among three evaluations of the HUA population. Four QTL were detected in multiple evaluations on N5, N12, N14, and N16. Another QTL on N2 was detected only in a single evaluation and it had the highest LOD score and R^2 value. Notably, QTL alleles on N2 and N12 were located in region of a non-reciprocal transposition. This suggests that genes in this homologous region may enhance the resistance through increases in gene dosage.

To determine the gene expression changes associated with Sclerotinia challenge, we used spotted oligo-gene microarrays designed from 26,000 annotated genes from Arabidopsis to investigate gene expression profiles in Hua dbl2 and Stellar in a time course of 12, 24 and 48 h post-inoculation. We found totally there were 840 and 1748 genes differentially expressed in Hua dbl2 and Stellar respectively. Most of these genes had a fold change of 2-10. There were a smaller number of genes differentially expressed at 12 h than at 24 and 48 h post-inoculation, and that few genes were consistently expressed at all times. We also found that more genes were up-regulated at the beginning of infection in the resistant parent than in the susceptible parent. The functions of differentially expressed genes and regulatory pathways involved in the resistance are being investigated. These results will be compared with the QTL mapping results to identify candidate genes for their potential use in the manipulation of crop resistance to white mold.
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Resistance Improvement of Bean Through Multi-Site Screening and Pathogen Characterization

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Funded Plan of Work: To examine putative sources of resistance in dry bean lines and to study the pathogen variation in multi-site bean production areas.

ABSTRACT:
No complete resistance to *Sclerotinia sclerotiorum*, the cause of white mold, has been reported in common bean, *Phaseolus vulgaris*. 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 measured by the detached leaf test (DLT), and molecular genotype. From the most recent complete field and greenhouse/lab rank correlation results, the following putative sources of resistance were identified: Dwarf Bees (*P. coccineus*), Cornell 601, G122, Cornell 501, AN 37, and Ex Rico (Bunsi). Sclerotia of nine *S. sclerotiorum* isolates used for screening in greenhouses/labs were collected from the following U.S. sites: ID, ND, WI, WA, NE, CO, MI, OR, and NY. These nine isolates were tested in a matrix for MCGs. No two greenhouse/lab isolates were compatible; each isolate was unique. Thus, there is no evidence of clonality among the greenhouse isolates. The most aggressive isolates used in greenhouse/lab screening were from ID, ND, and WI. The ID, ND, and WI isolates were significantly more aggressive than greenhouse/lab screening isolates from NE, WA, and CO. The variation in aggressiveness and MCGs may help explain why greenhouse/lab screening results often do not agree across different test sites. The 2004 field screening results were similar to the 2003 overall rank correlation. Beryl was again the most susceptible selection, while G122 was again one of the most resistant. However, AN 37 and Cornell 501 were ranked among the most susceptible compared to their rankings in 2003, while NO2 302 appeared to be more resistant in the 2004 field screen, which we expect is due to architectural escape in the field. We have shown that the internal transcribed spacer region (nuclear small subunit rDNA) can be used for interspecific separation of *S. sclerotiorum*, *S. trifoliorum*, and *S. minor*. A preliminary study of the nuclear large subunit rDNA failed to find intraspecific variation for the nine screening isolates. Further analysis of this region as well as molecular characterization by the use of Kohn's microsatellites is planned.

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Response of Canola Cultivars to *Sclerotinia sclerotiorum* in Field and Controlled Environments

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

ABSTRACT:
The objectives of this project are to identify canola cultivars which are less susceptible to Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, and to identify an efficient greenhouse or laboratory screening method that correlates with field reactions. In 2001 to 2004, field trials were conducted at the North Dakota State University Carrington Research Extension Center and an on-farm site near Red Lake Falls, MN. Field trials were conducted at sites with a history of *S. sclerotiorum*, were inoculated with ascospores, and were misted to promote disease infection and progression. A set of 19 canola cultivars were evaluated for resistance to SSR with a petiole inoculation technique (PIT), detached leaf assay (DLA), and oxalic acid assay (OAA) in a greenhouse, growth chamber, or laboratory. These same 19 cultivars (or a subset) were also evaluated in the ND and MN field trials from 2001 to 2004. Incidence of SSR ranged from 15 to 43%, 4 to 23%, 48 to 90%, and 0 to 5% at Carrington, ND in 2001, 2002, 2003, and 2004, respectively. Incidence of SSR ranged from 34 to 77%, 11 to 76%, and 1 to 25% at Red Lake Falls, MN in 2001, 2003, and 2004, respectively. Significant differences among cultivars occurred with the PIT and OAA, but not with the DLA. Significant ($P \leq 0.10$) negative correlations were detected between data from the PIT trial and canola yield data from Carrington in 2001, 2002, and 2003, and Red Lake Falls in 2001. This indicates that the PIT was the most efficient and accurate (compared with field data) of the greenhouse or laboratory methods used to screen canola cultivars for 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. Crosses were made to combine independent QTL into single soybean lines using SSR primers to mark the QTL regions. 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 during 2003 and 2004, we currently have F4-derived soybean lines that are homozygous for the desired marker alleles for the 8 QTL on 7 different linkage groups. A seed increase of the lines will be conducted during the 2004-05 winter season to be evaluated for resistance to *S. sclerotiorum* during 2005. Objective 2 is to determine if a novel antifungal synthetic peptide expressed in soybean will confer resistance to *S. sclerotiorum.* The T_1 herbicide-tolerant individuals derived from the transgenic soybean lines carrying the antifungal peptide D4E1 were evaluated from greenhouse-grown plants during the winter of 2003-04 using a detached leaf assay. Plants were harvested from the greenhouse in July 2004. The T2 plants are currently being grown in the greenhouse for generation advance to the T4 generation before the end of May 2005. Our goal is to obtain homozygous, stable lines in the T4 generation to derive lines that express the antifungal peptide. During the summer of 2005, we will have a field increase of seed of selected lines, and begin phenotypic evaluation for resistance to sclerotinia stem rot. 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 on germination of sclerotia. Development of Ca-cyanamide tolerant plants would allow post-planting application for more effective inhibition of apothecial development and ascospore release. Yield evaluation of ten transgenic *cah*-gene lines and their non-transgenic parent line was conducted during 2004 in two disease environments and one non-disease environment. Perlka treatments of 0 kg ha^{-1}, 100 kg ha^{-1}, and 400 kg ha^{-1} were applied just before the R1 stage. Yield of the Perlka-treated plots was greater than the control in the two disease environments. There was no difference in yield between the transgenic lines and their non-transgenic parent.

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Screening for Oxalate Sensitivity and Tolerance in Arabidopsis

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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*. For this reason, oxalate oxidase, which breaks down oxalic acid, has been used to genetically engineer sunflowers, soybean, and canola plants to counter some of the effects of *S. sclerotiorum*. Because the mechanisms of oxalate toxicity are poorly understood in plants we are using Arabidopsis (*Arabidopsis thaliana*) L. Heynh. as a model system to isolate genes that confer oxalate sensitivity and/or tolerance. Identification of these genes will provide the basis for mapping resistance to Sclerotinia, which can then be used for either genetic engineering or traditional breeding.

We have established conditions to select Arabidopsis seedlings for oxalate sensitivity/tolerance. The effects of oxalate on seedling growth and development are specific. Of seven dicarboxylic acids tested, oxalic acid was the most toxic and significantly more toxic than malonate and oxaloacetic acid (succinate, fumarate, malate, and glutamate were not toxic). Oxalate causes significant changes in root and shoot growth over a narrow range of concentrations. Interestingly, $CaCl_2$ reverses the effect of oxalate toxicity on seedlings, suggesting that calcium oxalate precipitation limits availability and toxicity of oxalate. At all concentrations tested, the cations Fe^{2+}, Mg^{2+}, Mn^{2+} did not provide the same amelioration and were toxic at much lower concentrations. Two transgenic Arabidopsis lines that constitutively express a wheat oxalate oxidase gene were obtained from Dr. William Powell (Cornell University). The roots of both transgenic lines grew significantly less than the parent, Col-0, in the absence of oxalate, but when grown in the presence of oxalate, one of the two transgenic lines, had the same growth rate as Col-0.

Thus, we are currently screening ethylmethane sulfonate (EMS)-induced mutants for oxalate sensitivity and tolerance. Four classes of oxalate-sensitive mutants were identified after screening 9,536 EMS-mutagenized seedlings. As a control, EMS mutants were also grown in the absence of oxalic acid. One of the four classes of mutants turned brown in the presence of oxalate, but this class was not present when seedlings were grown without oxalate. The other three classes of chlorotic, dark green, and purple mutants were present in plates with or without oxalate, but were more frequent when EMS mutants were grown in the presence of oxalic acid. A portion of these seedlings recovered when transferred to oxalate-free medium. We are currently analyzing the progeny of the surviving hypersensitive mutants for segregation of oxalate sensitivity. We are also in the process of screening over 28,000 EMS mutants for oxalate tolerance, and analyzing their progeny for segregation of oxalate tolerance.

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South Dakota studies on management strategies for Sclerotinia head rot of sunflower and stem rot of chickpea and soybean.

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Funded Plan of Work: Sclerotinia resistance and other management strategies among susceptible South Dakota crops.

ABSTRACT:

Projects studying Sclerotinia diseases on three South Dakota crops, sunflower, soybean, and chickpea were planted during the 2004 growing season.

Sunflower studies were designed to examine head rot resistance reactions of hybrids, efficacy of fungicides and directional application of fungicides in suppressing Sclerotinia head rot. Planting was delayed to improve the chances of flowering during a cooler period. Studies were planted in a misted nursery site in Brookings, SD. All sunflower trials were lost due to jackrabbit depredation. Some of the trials have been replanted for greenhouse screening over the winter months.

Soybean studies were planted in Aurora and Bath, SD to examine variety selection, row spacing, and plant population to manage Sclerotinia stem rot and reduce losses as well as reduce inoculum replacement. The Bath site is a dryland, no-till field while the Aurora site is a conventionally tilled, irrigated field. Soybeans are a major crop in each county. No white mold developed in either plot, thought the fields where the studies were planted did have areas with moderate disease.

Chickpea studies were intended to screen varieties for disease response in a South Dakota environment with supplemental mist irrigation and to examine fungicide efficacy against Sclerotinia stem rot. This is the third year of studying chickpea in the same general area. Each year, Ascochyta blight has become more and more severe. This year, Ascochyta was severe by early July, causing defoliation during flowering. Ascochyta was so severe that despite inoculation with ascospores, the canopy was thinned sufficiently to prevent the development of Sclerotinia stem rot. These studies will be repeated in the greenhouse over the winter months. Greenhouse studies last year showed that a modified straw test has potential for screening genetic resistance, however, attempts to develop a method for fungicide screening Sclerotinia suppression on chickpeas in the greenhouse was not successful.

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Sunflower Head Rot Screening Nursery

Bob Henson, North Dakota State University Carrington Research Extension Center

Funded Plan of Work: Sunflower Head Rot Screening Nursery

ABSTRACT:
Sclerotinia head rot (*Sclerotinia sclerotiorum*) is a devastating disease of sunflower (*Helianthus annuus* L.) and no resistant commercial hybrids are available. A long-term germplasm screening nursery was established in 2000 at the North Dakota State University Carrington Research Extension Center. Entries consist of production hybrids and experimental lines submitted by private breeding programs. Individual heads are inoculated with ascospores and plots are misted at half-hour intervals to provide favorable conditions for disease development. After several weeks of misting, inoculated heads are evaluated for head rot symptoms. To date, substantial progress has been made in developing the infrastructure (water delivery and misting systems) and methodology (inoculation and evaluation procedures) for conducting a head rot screening nursery. 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. The best of the 82 entries in the first screening nursery in 2000 was used as the resistant check in subsequent years. 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. Further testing is needed to confirm these observations and to evaluate new materials. However, variability of disease reaction within plots is still quite high and more research is warranted on the methodology of conducting a head rot screening nursery to optimize labor and land inputs and to increase the precision of the results. Also, more site-years of data are needed to verify previous results and to release the information to growers. Work was also conducted in 2004 to evaluate fungicide treatments and application strategies for head rot control. The products and rates tested did not significantly reduce head rot incidence or severity, regardless of whether applied to the face or the back of the head.

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The role of light in the Soybean / *Sclerotinia sclerotiorum* interaction

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Funded Plan of Work: Improvement of resistance to Sclerotinia stem rot in soybean.

ABSTRACT:
The occurrence of Sclerotinia stem rot (SSR) of soybean in the field is highly dependent upon prevailing weather conditions. Researchers often have difficulty relating interaction phenotypes observed in controlled environments to field performance. Light intensity is regarded as important to the outcome of host- pathogen interactions and is a potential source of variation among experiments. The objectives of this study are to 1) determine the role of light in the environmental sensitivity of soybean accessions, 2) determine whether light influences phenotypes observed in controlled environments and the ability to predict phenotypes in field environments, and 3) compare statistical methods to achieve maximum resolution in planned pair-wise comparisons. Experiments were conducted in a growth chamber and in an SSR nursery at the West Madison ARS. Five individual light environments (266- 393 µmol m-2 s-1) were created with white shade cloth. The cut petiole method was used to inoculate plants in controlled environments and lesion length and days to wilt were assessed ten days post-inoculation. A modified disease severity index was used to evaluate disease severity and yield loss in the field. The lesion length interaction phenotypes of two resistant accessions W04.1000 and W04.1002 proved stable across light environments. ANOVA-type statistics (ATS) was able to detect more subtle phenotypic differences between accessions than the traditional LSD statistical comparison of means, with W04.1002 performing better than NK S19-90, the standard resistant check. Spearman's rank correlations of field and light environment phenotypes indicate that light influenced the ability of inoculation in controlled environments to predict field performance. Soybean accessions should be evaluated for reaction to *S. sclerotiorum* in environments that provide 303 to 337 µmol m-2 s-1 of light. The implications of these results and future experiments are discussed.

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Two Novel Methods to Evaluate Soybean for Resistance to *Sclerotinia Sclerotiorum*

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

ABSTRACT:
Several greenhouse inoculation methods are available to evaluate soybean [*Glycine max* (L.) Merr.] for resistance to *Sclerotinia Sclerotiorum* (Lib.) de Bary. Most of these methods are tedious and often produce inconsistent results among the tests. The objective of this research is to develop a low-cost and high-efficiency greenhouse inoculation method that can generate a consistent result. We developed a spray-mycelium method in which mycelia were cultured in liquid potato dextrose broth and homogenized before spraying on the leaves of soybean and a drop-mycelium method in which a drop of homogenized mycelium suspension was dropped on the tips of main stems. Inoculated plants were incubated in a greenhouse chamber with about 80% relative humidity. Disease incidence and AUWPC (Area Under Wilt Progress Curve) were used to measure disease severity. Eighteen soybean genotypes, including a partially resistant variety NKS19-90 and a susceptible variety Resnik, were employed in this study. The spray-mycelium method and the drop-mycelium method were compared with the cut-petiole method in the greenhouse. The experiment was a randomized complete block design with three replications. Twenty four plants per genotype in each replication were inoculated at V3 growth stage in the greenhouse. Significant differences ($P < 0.05$) in the disease incidence and AUWPC were found among the 18 tested genotypes. No significant differences were found among the replications for the three inoculation methods. The results obtained with the spray-mycelium and drop-mycelium inoculation methods were highly significantly ($R > 0.73$, $P< 0.01$) correlated with the results obtained with the cut-petiole inoculation method for both of the disease incidence and the AUWPC. The spray-mycelium and the drop-mycelium method used less than one fifth of inoculation time used by the cut-petiole method and cost less than one third of the cost of the cut-petiole method. Both of these new methods are low cost, efficient, and reliable methods and they can be valuable for large scale evaluation of germplasm and breeding lines for resistance to Sclerotinia stem rot in a greenhouse or other similar facilities.

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Using Crop Sequence and Biological Control to Minimize Sclerotinia on Canola, Chickpea, Dry Pea, Lentils, and Sunflower, 2004

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Funded Plan of Work: Minimizing Sclerotinia on canola, dry pea, chickpea, lentils, and sunflower using crop sequence and biological control

ABSTRACT:
1) A multi-disciplinary team of scientists is conducting a Crop Sequence Project, which includes a crop by crop residue matrix to evaluate the impact of previous crops (buckwheat, chickpea, corn, lentils, proso millet, grain sorghum, canola, dry pea, sunflower, and wheat) and crop residue on Sclerotinia diseases of chickpea, canola, dry pea, lentil, or sunflower. With the exception of Sclerotinia basal stalk rot on sunflower, Sclerotinia diseases were not detected because of the dry conditions during the growing season in 2004. Sclerotinia basal stalk rot was present on sunflower and increased during the three evaluations (3,000 plants per evaluation) but because of the low number of sunflower plants infected, the incidence of disease could not be statistically related to the crops grown in 2003. A susceptible sunflower crop will be seeded over a crop by crop residue matrix site (100 crop sequence treatments) in 2005 and 2006 to determine if the incidence of Sclerotinia stalk rot can be related to the chickpea, canola, dry pea, lentil, and sunflower crops grown earlier.
2a) The use of *Coniothyrium minitans* (Intercept WG®) in reducing the risk to Sclerotinia disease was evaluated in a Biological Control Project at site one in 2004. Treatments after the uniform application of sclerotia included: the growing of susceptible and resistant crops, and varying the timing of Intercept WG® applications. Influence of crop sequences and biological control on development of Sclerotinia was evaluated twice with sunflower, an indicator crop (9,500 plants per evaluation). Because of dry conditions, low numbers of sunflower plants were infected with Sclerotinia basal stalk rot making it difficult to statistically relate disease levels to treatments. Plots will be reseeded to a susceptible crop in 2005 for further evaluation. Soil coverage by crop residue indicated that higher values were associated with small grain species. Minor differences were detected among plots with soil water measurements and analyses of soil properties indicating that the site was relatively uniform. 2b) At site two, treatments will be evaluated with a susceptible crop in 2005. 2c) At site three, another experiment was established to evaluate management practices in reducing the risk to Sclerotinia disease.
3) A Sclerotinia Inoculum Project was established to evaluate the impact of sclerotia density on Sclerotinia disease severity and possible economic losses under dryland no-till field conditions.

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Validation and Introgression of White Mold Resistance from Andean Into Middle American Germplasm

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Funded Plan of Work: Germplasm Enhancement and Variety Development

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*. By pyramiding resistance genes from different sources, resistance should be more durable across a range of environments and pathotypes of white mold. The specific objectives for this research are to: 1) Validate the utility of the *Phs* SCAR marker linked to the QTL for resistance found in G 122 using a newly created recombinant inbred line (RIL) population, 2) Map the RIL population using molecular markers, especially regions flanking the G 122 QTL and identify new markers linked to resistance, and 3) initiate pyramiding of resistance genes from G 122 with those found in *P. coccineus* by making crosses between the most resistant lines from the CSU RIL Population and white mold resistant *P. coccineus* accessions identified by Gilmore and Myers.

To date, we have screened the CSU RIL Population using the straw test and in field trials. We found that some RILs possess resistance superior ($P<0.05$) to G 122. We have also determined that presence of the T *Phs* SCAR marker, linked to the G 122 QTL for resistance, accounted for approximately 8% of the resistance in the CSU RIL population based on the straw test, and 9% of the resistance based on a field evaluations in North Dakota. The resistant QTL in our studies accounted for lower levels of resistance that the 38% originally reported by Miklas et al. To date, we have identified 278 polymorphic bands in the CSU RIL population based on RAPD, AFLP and SSR markers. Seventy eight of the 278 have been evaluated for associations with resistance and used to develop a preliminary linkage map. Among the 78 polymorphic loci, seven have shown a significant ($P<0.05$) level of association with physiological resistance. A preliminary linkage map using the 78 markers provided 21 linkage groups with several anchors from the core common bean map, including the *Phs* SCAR marker on linkage group B7. Three additional markers were found on this linkage group, however only one was significantly ($P < 0.05$) associated with resistance, probably because it was also linked to the *Phs* SCAR marker. We are currently completing analysis of the remaining 200 polymorphic markers to locate and map new QTLs for resistance. We anticipate that some of the new QTLs for resistance will be from the CSU pinto parent used to develop the RIL population. Crosses between the best CSU RILs and the two resistant *P. coccineus* lines have been initiated and should be completed by the end of the budget year.

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Validation of a Sclerotinia disease-warning model for canola in North Dakota

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Funded Plan of Work: Validation of a Sclerotinia forecasting system for canola in North Dakota and Minnesota

ABSTRACT: A survey that estimated Sclerotinia stem rot (SSR) prevalence and severity on 60 canola fields was conducted in late August 2004 as a means to validate information generated by a disease-warning model. The model estimates the probability of apothecia production as a measure of risk of SSR development. Surveyed fields were arbitrarily chosen for their proximity to weather stations of the North Dakota Agricultural Weather Network located in Cavalier, Towner, Rolette, Bottineau, Ward, and Renville counties. At each field a total of 100 plants in 10 stations were scouted for disease presence. Results of the survey indicated that SSR was present in every scouted field (100% prevalence); however, the proportion of infected plants within each field (incidence) ranged from 1 to 58%. Fields that were still flowering by the time the survey was conducted had significantly lower SSR incidence (6%), independently of where the fields were located, compared to fields that had reached maturity (22%). SSR incidences higher than 35% were observed only in fields located in counties where the model predicted at least 9 continuous days of moderate to high -risk warnings at the beginning of the flowering period. The only exception was Ward County, were the highest incidence was 24% and the average incidence for the location was 6%. Rolette County had the shortest warning duration (three days of moderate risk) and the second lowest SSR mean incidence (14%). The broad range of SSR incidences observed among fields within the same county is an indication that differences in growth stage and inoculum availability played decisive roles in epidemic development. At the time of survey 50% and 60% of the fields scouted in Rolette and Cavalier counties, respectively, were still in the flowering period. According to the model the flowering period should have ended in late July. The unusually cold weather at the beginning of the growing season and at flowering time may have accounted for such large discrepancy. The relative importance of inoculum availability is represented by the broad range of SSR incidence detected among fields, within the same county, that were at maturity when the survey was conducted: 1 to 56% in Cavalier County, 8 to 55% in Towner County, 5 to 58% in Bottineau County, and 9 to 48% in Renville County. The model evaluated does not include inoculum availability as a factor; however, it is evident from this survey that growers should include it in their decision making process. In general, only 20% of the fields scouted in these counties had incidences higher than 35%. Environmental factors that may affect the development of SSR epidemics once apothecia are formed should also be included in the model. Efforts toward the latter are being planned.

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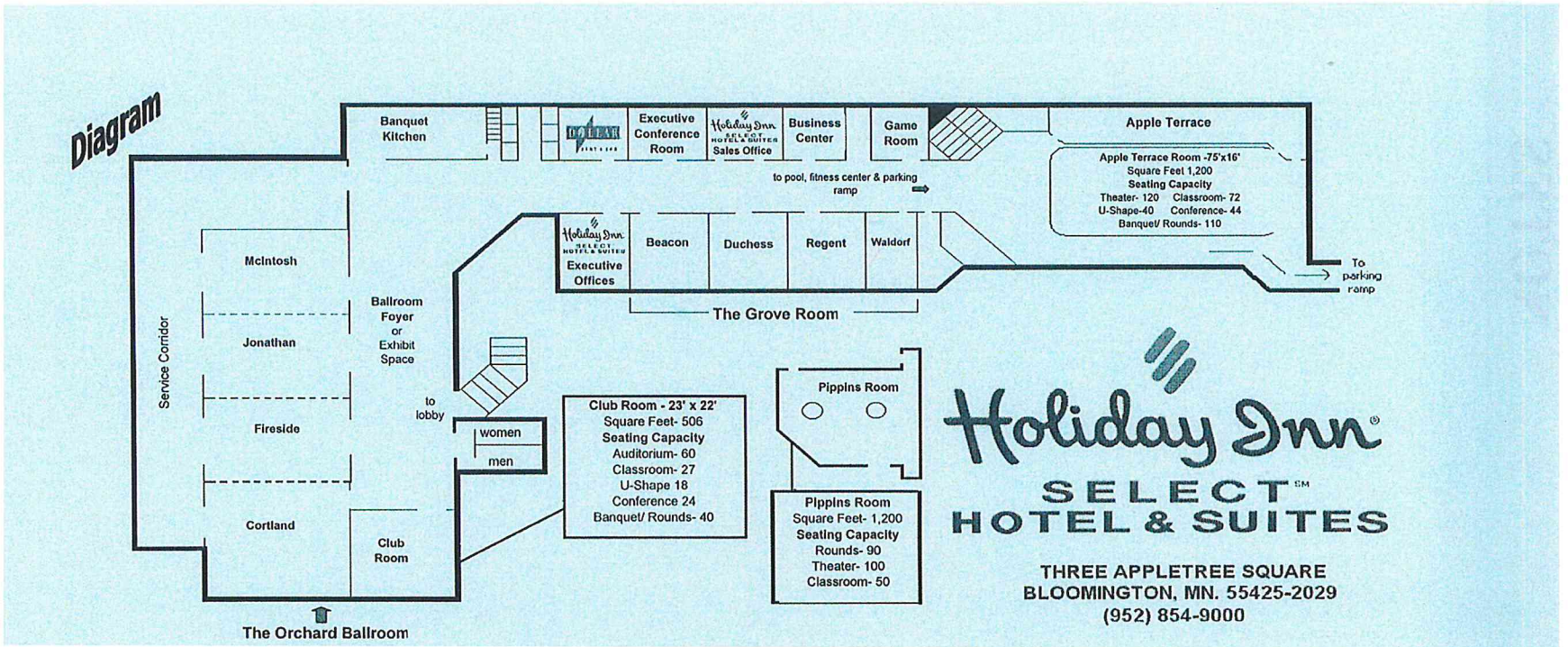
Diagram
Banquet Kitchen
Executive Conference Room
Holiday Inn SELECT HOTELS & SUITES Sales Office
Business Center
Game Room
Apple Terrace
Apple Terrace Room -75'x16'
Square Feet 1,200
Seating Capacity
Theater- 120 Classroom- 72
U-Shape-40 Conference- 44
Banquet/ Rounds- 110
to pool, fitness center & parking ramp
Holiday Inn SELECT HOTELS & SUITES Executive Offices
Beacon
Duchess
Regent
Waldorf
To parking ramp
The Grove Room
McIntosh
Jonathan
Fireside
Cortland
Service Corridor
Ballroom Foyer or Exhibit Space
to lobby
women
men
Club Room
Club Room - 23' x 22'
Square Feet- 506
Seating Capacity
Auditorium- 60
Classroom- 27
U-Shape 18
Conference 24
Banquet/ Rounds- 40
Pippins Room
Pippins Room
Square Feet- 1,200
Seating Capacity
Rounds- 90
Theater- 100
Classroom- 50
The Orchard Ballroom
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