2007 National Sclerotinia Initiative Annual Meeting

January 17-19, 2007

Holiday Inn Select

Minneapolis (Bloomington), MN

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Sclerotinia Initiative Annual Meeting 2007
Agenda

January 17, 2007

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

January 18, 2007

7:15 am    Registration/Continental Breakfast  (Fireside Room)

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

8:05 am    ARS National Program Staff Update – USDA-ARS, Beltsville, CO


8:25 am    Meeting Charge – Bill Kemp, USDA-ARS, Fargo, ND

8:30 am    Commodity Research – Balancing Current vs. Long-term Needs – Mike Davis, Executive Secretary, National Barley Improvement Committee, Milwaukee, WI

9:30 am    Break  (Fireside Foyer)

                         Moderator – Todd Scholz – US Dry Pea & Lentil

10:00 am    Sclerotinia Grower/Commodity Panel – Current Concerns and Future Needs
(Fireside Room)

Eben Spencer, Agronomist, ADM Edible Bean Specialties, Inc., Oslo, MN

Craig Henkel, President of the Nebraska Dry Bean Growers Association, Bayard, NE

Frank Marcello, Owner of Frank’s Crop Watch, Mid. MI

John Swanson, Land O Lakes, Mentor, MN
11:00 am  Discussion

11:15 am  **Guest Speaker**  
Sclerotinia Blight Research in Peanut - **Hassan Melouk, USDA-ARS, Stillwater, OK**

Noon  Working Lunch *(Cortland Room)*

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

**Moderator – Jim Steadman, Univ. of NE**

1:10 pm  **Guest Speaker**  
Molecular Breeding Strategies for Enhancing Disease Resistance in Sunflower – **Steve Knapp, Univ. of Georgia, Athens, GA**

2:00 pm  Sclerotinia Resistance Enhanced by Accumulation of QTL and Transgenic Approaches – **George Graef, Univ. of Nebraska, Lincoln, NE**

2:20 pm  Genetic Architecture of Sclerotinia Stem Rot Disease Resistance in Soybean and Common Bean – A Synthesis of Review – **Chandra Paul and Glen Hartman, ARS, Urbana, IL**

2:40 pm  Improved Resistance to *S. sclerotiorum* in Pea and Lentil Through Breeding and Biotechnology – **Kevin McPhee, ARS, Pullman, WA**

3:00 pm  Discussion

3:15 pm  Break & Poster Session *(Cortland Room)*  

**Moderator – Henrik Stotz, Oregon State Univ**

4:00 pm  Mapping and Transfer of Sclerotinia Resistance From Scarlet Runner to Common Bean – **Jim Myers, Oregon State Univ., Corvallis, OR**


4:40 pm  Influence of Crop Rotation and a Cover Crop on Sclerotinia in Canola – **Paul Porter, Univ. of Minnesota, St. Paul, MN**

5:00 pm  Discussion

5:20 pm  Adjourn (Dinner on your own)
January 19, 2007

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

7:15 am  Continental Breakfast (Fireside Foyer)

Moderator – Tom Gulya, ARS, Fargo, ND

8:00 am  Epidemiological Studies on Sclerotinia Stem Rot of Canola – Luis del Rio, NDSU, Fargo, ND

8:20 am  Update on Sclerotinia Research in Carrington, ND – Blaine Schatz, NDSU, Fargo, ND

8:40 am  A Novel Approach to Develop Elite, Sclerotinia Resistant Canola Cultivars – Dan Phillips, Univ. of Georgia, Griffin, GA

9:00 am  A Search for Improved Resistance in Common Bean Through Multi-site Screening and Pathogen Characterization – Jim Steadman, Univ. of Nebraska, Lincoln, NE

9:20 am  Discussion

9:30 am  Break (Fireside Foyer)

Sclerotinia Research Activities – Progress on Strategic Plan (Fireside Room)

Moderator – Rich Wilson (NPS-Retired)

9:50 am  Germplasm Enhancement – Jim Myers, Oregon State Univ., Corvallis, OR

10:25 am  Pathogen Biology & Development – Berlin Nelson, NDSU, Fargo, ND

11:00 am  Epidemiology & Disease Management – Luis del Rio, NDSU, Fargo, ND

11:35 am  Pathogen & Host Genomics – Steve Clough, ARS, Urbana, IL

Noon  Working Lunch (Cortland Room)

1:15 pm  Strategic Plan Discussion – Refocusing needs – Rich Wilson (NPS-Retired)

2:00 pm  Wrapup/Initiative Business - Bill Kemp/Larry Chandler

3:00 pm  Adjourn
Sclerotinia Initiative Website:

http://www.whitemoldresearch.com/

http://www.whitemoldresearch.org

http://www.sclerotinia.com

http://www.sclerotinia.org
## Sclerotinia Initiative Poster Session

**January 17-19, 2007**  
**Minneapolis, MN**

### Epidemiology & Disease Management

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2005 and 2006 White Mold Fungicide Trials on Dry Beans in Michigan

G. Varner1 and K.A. Terpstra2

1Michigan Dry Bean Production Research Advisory Board, Saginaw, MI
2Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI

Abstract:
This study was undertaken to evaluate commercially available and experimental fungicides for control of white mold (Sclerotinia sclerotiorum) on dry beans (Phaseolus vulgaris) over a two year period. White mold is a serious, yield limiting disease of dry bean in the Midwest. Michigan dry bean producers can spend upwards of one million dollars annually on fungicides to control white mold. Evaluating fungicides provides growers with valuable information in determining the most effective fungicides to use. Similar fungicide trials have been conducted by the Michigan Dry Bean Production Research Advisory Board since 1982.

Treatments that included Endura, Omega, Formula A, both Switch treatments, and Topsin-M at 30 oz. out yielded the untreated check plot at a statistically significant level (p>0.05) in 2005. In 2006, only the Topsin M at 30 oz. and the Formulation A+B treatments out yielded the untreated check plot with statistical significance (p>0.05). White mold severity was much higher in 2005 than in 2006. In 2005, all treated plots except Proline showed a significant reduction in white mold incidence and severity as compared to the untreated check. In 2006, five treatments, including Proline, Formula A, and Formula B did not show a significant reduction in white mold incidence and severity as compared to the check. No phytotoxicity was observed with any treatments at either 2 or 10 days after fungicide application in either year.

Omega was consistently at or near the lowest disease incidence and severity of the fungicides tested and, along with both Topsin M treatments, showed a statistically significant decrease in incidence and severity in both years (p>0.05). Omega is not yet labeled for use in the United States on dry beans. Despite the superior performance of Endura, which is marketed as Lance in Canada, growers may remain reluctant to use Endura because of its relatively higher cost as compared to other available fungicides.

Contact Information: Greg Varner, Michigan Dry Bean Production Research Advisory Board, 3066 South Thomas Road, Saginaw, MI 48609, Phone: (989) 781-0260, e-mail: varnerbean@hotmail.com
Advancement of Pyramiding New Sclerotinia Stem Rot Resistant Genes from *H. californicus* and *H. schweinitzii* into Cultivated Sunflower

J. Feng¹, G. J. Seiler², T. J. Gulya², C. C. Jan²

¹North Dakota State University, Fargo, ND 58105
²USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105

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

**Abstract:**
*Sclerotinia* is a major disease in cultivated sunflower across the world and the present-day sunflower hybrids are considered lacking high resistance to *Sclerotinia*. In general, wild *Helianthus* species are known to possess a much wider genetic variability than that of the cultivated sunflower for Sclerotinia stem rot resistance. An abundance of wild *Helianthus* accessions have been evaluated as potential sources for Sclerotinia stem rot resistance with the perennial species having been found to be highly resistant, especially the higher ploidy level species. Interspecific hybridization of perennial hexaploid species *H. californicus* and *H. schweinitzii* with HA 410 were conducted in 2006 based on the BC₁F₁’s obtained in 2005. In total, 109 BC₁F₁ plants were grown in the greenhouse for backcrossing. The backcross BC₁F₁ progenies were triploids with a 2n chromosome number of around 51. Because of the unbalanced chromosome number in the triploid BC₁F₁, the BC₁F₁ pollen fertility ranged from 2.45% to 4.63%, and seed set was very low. In total, 59 BC₂F₁ seeds were obtained from 136,220 pollinated florets. After germination, only 24 BC₂F₁ plants were established with a chromosome numbers of 2n=40 to 49, and a pollen stainability of 34.2%. Based on these results, we are re-crossing the BC₁F₁ with HA 410 to produce more BC₂F₁ seeds to screen for resistance individuals. Our results clearly demonstrated that the major obstacle in interspecific gene transfer between hexaploid perennials and cultivated sunflower is the BC₁F₁ generation, with a chromosome number of 2n=51. We would strongly suggest using the embryo rescue technique to improve the establishment of the BC₂F₁ seedlings. Presently, we are crossing additional BC₁F₁ plants with HA 410 and culturing the BC₂F₁ embryos. We expect to produce a large population of BC₂F₁ progenies to provide a better foundation for further backcrosses as we approach the base 2n=34 chromosome of the recurrent parent HA 410. Identification of resistant progenies will be initiated as early as the BC₃F₁ or BC₄F₁ generation.

**Contact Information:** Dr. C. C. Jan, Sunflower Research Unit, Northern Crop Science Laboratory, P.O. Box 5677, State University Station, Fargo, ND 58105; 701-239-1319; janc@fargo.ars.usda.gov

T. J. Gulya and J. F. Miller. Sunflower Research Unit, USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58105-5677.

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

Abstract:
Sclerotinia diseases remain the most significant of all diseases on both oilseed and confection sunflower production in the U.S. Sclerotinia incidence was lower in 2006 due to a widespread drought throughout much of the U.S. sunflower production area. Sclerotinia stalk rot and head rot affected 16% and 10% of the fields in ND and SD, respectively, and affected 0.9% and 0.3% of the crop in 2006. These figures are in stark contrast to previous years, such as 2002, when stalk rot and head rot were found in 29 and 50% of surveyed fields and affected a combined 7.4% of the crop. Several germplasm releases of both oilseed and confection sunflower were made, culminating several years of effort to incorporate both head rot and stalk rot resistance. Five oilseed restorer lines (RHA 439, 440, 453, 454, 455) and three maintainer oilseed lines (HA 441, 451, 452), along with 8 confection genetic stocks were released in 2006. The confection germplasm releases are the first material incorporating head rot resistance into long-seeded confection types, which is the ideotype desired by private seed companies. Seventy-five commercial hybrids were evaluated at multiple, inoculated field sites for resistance to stalk rot, and the same material was tested by personnel of the Carrington, ND Research and Extension Center for reaction to head rot. Stalk rot levels on the 75 hybrids ranged from 2% to 42% infected plants (averaged over 4 locations) while head rot ratings on the same hybrids ranged from 20 to 97% infected plants (averaged over two locations). Three hybrids were identified which combined high levels to both head rot and stalk rot. A check hybrid produced with USDA lines (HA 412 x RHA 409), which was the most resistant entry in the 2005 stalk rot trials, was exceeded in 2006 trials by 13 commercial hybrids, indicating private breeders are succeeding in incorporating stalk rot resistance. A preliminary study was begun in 2006 to investigate the levels of calcium in various plant parts among sunflower hybrids with known Sclerotinia resistance. If a correlation between calcium content and Sclerotinia resistance can be established, this inexpensive test could be used as a selection criterion to achieve higher levels of Sclerotinia resistance.

Contact Information: Dr. Tom Gulya, USDA Northern Crop Science Lab, 1307 N. 18th. St., Fargo ND 58105-5677; 701-239-1316; gulyat@fargo.ars.usda.gov
A Role for Induced Defense Pathways in Resistance to *Sclerotinia sclerotiorum*

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

**Funded Plan of Work:** Genetic Basis of Oxalate Sensitivity in Relationship to *Sclerotinia* Diseases

**Abstract:**
Genotypic differences in susceptibility to *Sclerotinia sclerotiorum* have not been reported because *Arabidopsis thaliana* is highly susceptible to this fungal pathogen. We have established conditions to evaluate differences in susceptibility to *S. sclerotiorum* among *Arabidopsis* mutants and ecotypes. Oxalic acid is an important virulence factor and we have confirmed differential susceptibility of *Arabidopsis* to oxalate-deficient and wild-type *S. sclerotiorum*. In order to identify genes involved in oxalate tolerance, a library of *Saccharomyces cerevisiae* deletion mutants was screened and RIB4, encoding the riboflavin biosynthetic enzyme lumazine synthase, was identified as one of the genes implicated in oxalate tolerance. *COS1*, the Arabidopsis ortholog of RIB4, is involved in jasmonic acid (JA) signaling. The *cos1* mutant suppresses the *coi1* mutation in an F-box protein which is a central regulator of JA responses. We, therefore, compared susceptibility to *S. sclerotiorum* and oxalate sensitivity in these mutant and wild type plants. Two *coi1* mutant alleles conferred hyper-susceptible to *S. sclerotiorum*. JA signaling was disrupted because the plant defensin gene *PDF1.2* was no longer induced after challenging *coi1*-2 mutants with *S. sclerotiorum*. Unlike the Δrib4 mutant, the *coi1*-2/cos1 mutant of *Arabidopsis* did not increase sensitivity to oxalic acid. Thus, JA-induced resistance is independent of oxalate tolerance. Encouraged by these results, we also tested mutants in salicylic acid (SA) and ethylene (ET) signaling. Both *npr1* and *ein2* mutants, which are defective in SA and ET signaling, respectively, are hyper-susceptible to *S. sclerotiorum*. As expected, induction of *PDF1.2* and the pathogenesis-related gene *PR1* was reduced in *ein2* and *npr1* mutants respectively, clearly demonstrating that *S. sclerotiorum* activates all three defense pathways in *Arabidopsis*. Actigard, a commercial formulation of the systemic acquired resistance (SAR) inducer benzothiadiazole, enhanced resistance to *S. sclerotiorum*. This result is in agreement with the *npr1* mutant data and strongly suggests that SA contributes to resistance against *S. sclerotiorum*. Evidence was obtained for suppression of defense responses (H2O2 production and PR1 induction) by oxalic acid, but the effects were relatively small, suggesting that promotion of pectin degradation may perhaps play a more important role in virulence.

**Contact Information:** Henrik U. Stotz, Department of Horticulture, Oregon State University, Corvallis, OR 97331, 541-737-5468, stotzhe@hort.oregonstate.edu
Associations Between Ascospore Dispersal Gradients and Sclerotinia Stem Rot Incidence in Cando, North Dakota

Qandah, I. S., and L. E. del Río. Department of Plant Pathology North Dakota State University, Fargo 58105 ND

Funded plan of work: Epidemiological studies on Sclerotinia stem rot of canola

Abstract:
Seasonal and daily patterns of ascospore dispersal of *Sclerotinia sclerotiorum*, causal agent of Sclerotinia stem rot of canola (SSR), were studied in a canola field in Cando, N.D. during the month of July in 2005 and 2006. Viable *S. sclerotiorum* ascospores were collected on Petri plates containing the semi-selective Steadman’s Blue medium at various distances from an area source of inoculum. The plates, placed two inches above the soil surface, were exposed for two hours between 10:00 A.M and 1:00 P.M., and then incubated at 21°C for two days before colonies were counted. Most ascospores were trapped when the air temperature and relative humidity under the plant canopies were 13-21°C and >80%, respectively. A linear relationship was used to describe the relationship between the amount of ascospores trapped and the amount of diseased plant ($R^2 = 0.994$) in 2005. In 2006 the amount of ascospores trapped during the same period were less than 10% of what was observed in 2005, and SSR did not develop in the field. Inoculum concentration decreased with distance from the source ($R^2=0.977$). A regression analysis between the weather variables prevalent in the 3 days prior to the detection of high peaks of ascospore concentrations in 2005 indicated a significant association ($P= 0.023$) between relative humidity and spore concentrations. The information generated by this study will be used as part of a disease-warning program for Sclerotinia stem rot of canola.

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Determining Species Identities of Sclerotinia isolated from Chickpea in the U.S.

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Funded Plan of Work: Sources of resistance to white mold in the grain legumes

Abstract:
In order to identify sources of resistance to white mold in chickpea, we need to ascertain all genotypes and even different species of Sclerotinia that cause the disease on chickpea. During investigating chickpea white mold, we discovered isolates of Sclerotinia in central California that exhibited morphological features inconsistent with the previous known pathogen of chickpea. Experiments were carried out towards determining species identities of these isolates. Phenotypic and genetic diversity of Sclerotinia isolates collected from chickpea plants showing collar rot symptoms in central California were studied and compared with previously identified isolates of S. sclerotiorum to determine their species identities. Isolates exhibited two growth rates, fast growing (~40 mm diameter in 24 hours) and slow growing (~20 mm diameter in 24 hours). Fast growing isolates showed strong oxalic acid production as detected in a pH indicator medium, whereas slow growing isolates produced little or no oxalic acid on the same medium. PCR was used on representative isolates from each group to detect the presence of an intron near the 3'-end of small subunit rDNA. An intron was detected in the slow growing isolates, but not in the fast growing isolates. PCR with single long primers also produced DNA polymorphisms separating these two groups. The fast growing isolates shared the same attributes of previously identified isolates of S. sclerotiorum. The slow growing isolates showed the reported characteristics of S. trifoliorum. The species identity of these two groups is being tested and confirmed by inducing carpogenic germination of sclerotia.

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Development of Sclerotinia Head Rot Resistant Germplasm Utilizing *H. maximiliani* and *H. nuttallii*

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

**Abstract:**
Sclerotinia is the most damaging disease with an incidence higher than other major sunflower diseases. Wild *Helianthus* species have played an important role in establishing sunflower as an important global oilseed crop. However, the present day sunflower germplasm is still represented by a relatively narrow genetic base, which greatly limits its future success as a competitive major oilseed crop. Interspecific hybridization of NMS HA 89 with Sclerotinia head rot resistant wild diploid perennial *H. maximiliani* and *H. nuttallii* accessions was successful using embryo rescue. A total of 162 F₁ hybrid plants were obtained after rescuing 228 embryos from 70,500 pollinated florets. Most F₁ plants had the expected 2n chromosome number of 34. A few F₁ hybrids had 2n=51 chromosome which was assumed to have resulted from fertilization of normal gametes with unreduced gametes. Most F₁ plants with 2n=34 chromosome had low pollen stainability of around 1%, and consequently low backcross seed set with only 85 seed from 506 pollinated heads. Backcrosses of 2n=34 F₁ plants with HA 441 produced BC₁F₁ progeny with 2n=34 or 35 chromosome. Sib-pollination among heads with colchicine-induced chromosome doubling also had low seed set with 250 seeds from 425 pollinated heads. These sib-pollinated seeds produced amphiploids with 2n=62 to 68 chromosomes. Backcrossing of 2n=34 to 35 F₁ plants with HA 441 is now in progress, as well as sib-pollination of the amphiploids for seed increase. The use of diploid perennial *Helianthus* accessions is expected to avoid the limitations of using higher ploidy *Helianthus* species or amphiploids by providing maximum genetic recombination in the F₁ generation, minimizing target gene loss, and shorten the breeding time to obtain adequate seed for replicated field disease evaluation.

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Dissecting Quantitative Trait Loci for Sclerotinia Head Rot Resistance in Sunflower

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

Abstract:
One hundred and twenty-three F2,3 families derived from a cross between HA 441 and RHA 439, both showing partial tolerance to *Sclerotinia* head rot, were used for the current study. A genetic map with 236 TRAP, 11 SSR, and 2 morphological markers was constructed in this F2 population with 123 individuals. The map has 18 linkage groups and spans 2328.3 cM. The F3 families (20-25 plants each) were planted in Carrington, ND, in a randomized complete block field design with two replicates. At flowering stage each head was inoculated with a suspension containing 5000 ascospores per milliliter and kept under mist irrigation for three weeks to create a microenvironment favorable for disease development. Five weeks after the inoculation, disease severity on individual heads was scored in a scale of 0 (no symptoms) through 5 (100% of head rotted). Disease tolerance was evaluated by disease incidence (DI) being calculated as the percentage of plants infected within a row and disease severity (DS) being measured by the mean disease score of the infected plants within each row. Six and seven QTLs were detected for DI and DS, respectively. These QTLs, each with a LOD score ranging from 2.1 to 18.1, were assigned to 10 of the 18 linkage groups. Although a positive correlation existed between the two disease indexes, the respective QTLs mapped to different chromosomal regions, suggesting a different genetic basis for the two indexes.

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Estimates of Yield and Economic Losses Due to White Mold on Rain-fed Dry Bean in North Dakota

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Abstract:
White mold (Sclerotinia sclerotiorum) of dry bean is an endemic disease in North Dakota and is considered the most important biotic factor affecting yield. Potential yield and economic impact assessments due to white mold in rain-fed dry bean were made by conducting a survey of 250 fields during 2003 to 2005 growing season in the three predominant dry bean producing counties. Two methods were used to estimate yield loss due to white mold incidence. In method one, white mold incidence across fields within each county were averaged per year and regressed against estimated yield for each county per year to evaluate yield loss associated due to white mold incidence while in the second method, instead of using yearly yield estimate of each county, we used a 10-year average yield for each county. To arrive at economic loss assessments, yield loss due to white mold incidence was used in conjunction with average price and harvested area as reported by National Agricultural Statistics Service for North Dakota. Based on regression analysis, method one ($R^2 = 0.50; P = 0.03$) resulted in a loss of 14 kg/ha for every unit of white mold incidence while method two ($R^2 = 0.54; P = 0.03$) resulted in a loss of 15 kg/ha for every unit of white mold incidence in rain-fed dry bean. Over the three-year period and across both methods of evaluation, yield loss due to white mold incidence averaged 280 kg/ha (range 42 to 780 kg/ha) while economic loss averaged $3.75 million (range $0.40 to 13.0 million). Although using 10-year average yield resulted in slightly higher yield loss per unit of white mold incidence, both methods evaluated produced similar yield loss estimates.

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

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

**Abstract:**
The ultimate goal of this project is to evaluate a broad range of wild *Helianthus* species for Sclerotinia stalk rot resistance. Wild sunflower germplasm is largely unexplored in terms of Sclerotinia stalk rot resistance, but wild *Helianthus* species are considerably more difficult to work with in field trials than cultivated sunflowers. Seed dormancy issues prevent direct seeding in the field, and thus seedlings need to be germinated, grown in the greenhouse, and later transplanted to the field. One of our objectives was to develop a reliable greenhouse screening method, so that susceptible material could be eliminated and only the most promising germplasm advanced to field trials. In growth chamber studies, incubation temperature was found to be the most important variable, as even modestly higher temperatures were detrimental to disease development. For example, at 21C, 100% of the plants of a susceptible variety wilted and died at an average of 11 days. At 25C, 40% of the plants had no symptoms, even after 21 days. Temperature was a more critical variable than pot size and shape, soil type, inoculum quantity, and inoculum placement in the pots.

Screening efforts will focus on annual diploid *Helianthus* species in the USDA sunflower germplasm collection, primarily the non- *H*. *annuus* taxa, such as *H*. *anomalous*, *H*. *argophyllus*, *H*. *bolanderi*, *H*. *debilis* (subspecies *debilis*, *cucumerifolius*, *silvestris*, *tardiflorus* and *vestitus*), *H*. *deserticola*, *H*. *neglectus*, *H*. *niveus* (subspecies *canescens* and *tephrodes*), *H*. *petiolaris* (subspecies *fallax* and *petiolaris*), *H*. *porterii*, and *H*. *praecox* (subspecies *hirtus*, *praecox* and *runyonii*). Our initial screening will focus on accessions known to have resistance to rust and/or downy mildew, as they offer the potential to pyramid resistance to several diseases. This will complement the emphasis in the USDA sunflower breeding program at Fargo, ND on developing germplasm with multiple desirable traits.

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Genetics and Mapping of Resistance to Sclerotinia White Mold in Lentil

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

Abstract:
The objectives of the project were to determine genetic variation, identify resistance in lentil germplasm, develop genetically defined populations from crosses that segregate for Sclerotinia white mold (SWM) resistance, and map and tag the genes for resistance. Sclerotinia white mold evaluations of lentil varieties and germplasm successfully identified useful sources of resistance. Crosses were made between resistant and susceptible parents and the populations were advanced to F6 derived recombinant inbred lines (RILs) to form genetic mapping populations. The mapping populations have been used to develop a genetic map of codominant markers that will be used to determine the loci that determine Sclerotinia white mold resistance in lentil. The genetic map of lentil now has 11 linkage groups with 54 codominant markers and covers a genetic distance of 643cM. Comparison of the linkage map with previously published maps of lentil and pea indicate linkage groups 1, 2 and 3 share common markers with three linkage groups of the Hamweih lentil map and the Loridon pea map. Currently, additional microsatellite markers are being developed to increase marker density on the lentil map. The recombinant inbred lines have been increased in the greenhouse and field and phenotyping for SWM resistance will be initiated in the winter of 2007 in the greenhouse and field in spring of 2007. Lentil breeding lines are also being evaluated for resistance under controlled conditions in the greenhouse.

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Genomic Analysis of Soybean Resistance to *Sclerotinia sclerotiorum*

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

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

**Abstract:**

*Sclerotinia sclerotiorum* is a necrotrophic fungal pathogen that infects soybean causing white mold disease. Oxalic acid is considered to be its major virulence factor. Plants from the oxalate oxidase (OxO) transgenic line (80(30)1) which showed resistance to the pathogen and its susceptible parent line (AC Colibri) were inoculated using infected flower buds. Samples of the leaflets were taken at two stages of the disease within 24 hours of inoculation. In a different experiment, fifteen-days-old soybean plants of a partially resistant variety (PI-194639) and a susceptible variety (Williams 82) were inoculated with actively growing mycelia utilizing the cut-stem technique and 1 inch sections were sampled at 8 and 14 hours post inoculation for microarray gene expression analysis. The data was obtained as the log2 of the normalized intensities for each gene and statistically analyzed using linear models in SAS. The genes with highest p-values (cutoff 0.05) for the difference between both varieties in the OxO experiment were assigned into functional categories and clustered using fuzzy-k-means. The results show that genes related to defense, oxidative stress and secondary metabolism are mostly down-regulated in the OxO transgenic compared to its parent. These preliminary results suggest that the susceptible line is actively trying to defend, whereas the transgenic is apparently depleting the oxalic acid levels at the beginning of the interaction minimizing the need for activation of defense genes. A cross comparison between the two experiments supports that the resistance in the OxO plants differs from that expressed in the stems of PI-194639.

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

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Funded Plan of Work: Variety Development/Germplasm Enhancement

Abstract:
Genetic resistance to white mold has been reported in both common (Phaseolus vulgaris L.) and scarlet runner (P. coccineus L.) beans. From funding received from the USDA Sclerotinia Initiative during the past three years, we were able to (1) develop a RIL population derived from a cross between CO72548, an elite Colorado State University pinto line and resistant common bean line G122; (2) evaluate the utility of a previously reported QTL for white mold resistance derived from G122, and (3) identify additional QTL for resistance in the RIL population. The objectives for this year’s research were to (1) quantify the effect of new QTL for resistance found in the RIL population, and (2) map resistant QTL via genome-wide composite interval mapping. The RIL population was evaluated for WM reaction in three greenhouse tests and one field environment and for molecular markers throughout the genome. Two RIL were identified with higher resistance levels ($P<0.05$) than the resistant parent G122 based on the straw test. The previously reported QTL (Miklas et al., 2001) on linkage group B7 was significant ($P<0.01$) in single-factor analysis of variance, but not with composite interval mapping. Five QTL with LOD>2.7 (on linkage groups B1, B2b, B8, and B9) were revealed through composite interval mapping for WM resistance in the greenhouse test. The QTL were contributed from both parents and together accounted for 48.4% of the phenotypic variation ($R^2$). For field resistance to WM, one QTL ($R^2=12.4\%$) on linkage group B8 was detected. These results confirm polygenic resistance to WM in common bean and suggest that marker-assisted selection may be useful to enhance WM resistance in common bean germplasm.

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Identification of Resistance to *Sclerotinia Sclerotiorum* in Peas

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**Funded Plan of Work:** Screening of the *Pisum* core Collection and Woody-stem Selections for Resistance to White Mold.

**Abstract:**
White mold, caused by *Sclerotinia sclerotiorum*, can be a serious disease in irrigated and dryland peas in the Pacific Northwest and is considered a serious potential threat to the expanding pea production in the Midwest of the United States. Due to poor economic returns to pea growers, expensive foliar fungicides used to manage white mold are cost prohibitive. Since there are currently no known sources of white mold resistance in peas, identifying resistant pea lines for breeding purposes is desired. Therefore, 498 pea accessions from the *Pisum* core collection located at the USDA-ARS, Regional Plant Introduction Station (RPIS), Pullman, WA and seven woody-stem pea lines from the John Kraft Germplasm Collection in Prosser, WA, were screened for resistance to white mold. Pea lines were inoculated with the white mold pathogen by applying a small agar plug removed from the leading edge of an expanding white mold colony growing on potato dextrose agar. The mini-agar plug was extracted using a dental amalgamator. Peas were inoculated with the mycelial plug at the 4th node immediately adjacent to the stem. The inoculated plants were then placed in a humidity chamber maintained at 20 to 25°C and 100% RH for three days. Of the pea lines screened, 5, 26, 77, 109, 91, and 197 accessions had lesion expansions between, 0 to 1, 1.1 to 2.0, 2.1 to 3.0, 3.1 to 4, 4.1 to 5, and >5 cm, respectively. Not a single *Pisum* lines was immune to the white mold isolate used to screen the pea lines. Of the 504 lines screened, 210 lines did not survive two weeks post-inoculation. Pea accessions with resistance to white mold based on the restriction of lesion expansion on the stem were identified.

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Influence of Crop Rotation and a Cover Crop on Sclerotinia in Canola

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

Abstract:

Two three-year field studies were initiated near Thief River Falls, MN in 2003 (03CRye) and 2004 (04CRye) to better understand the effect of crop rotation and a rye cover crop on growth and development of canola and wheat. In both 2005 and 2006 climatic conditions at flowering were not favorable for Sclerotinia development on canola, and infection was essentially non-existent. Attempts to monitor apothecia production from 'spiked' sclerotia were largely unsuccessful due to minimal apothecia counts.

Canola yields in the second year of the studies were reduced (15.5% in 03CRye and 10.7% in 04CRye), seed protein content was reduced (6.6 and 9.5%), seed oil content increased only in 04CRye (4.7%), and test weight was not influenced when rye was grown as a cover crop compared with no rye. Canola mid-season plant biomass and plant height at harvest were reduced when rye was grown in 03CRye, but were not influenced by the rye in 04CRye. Canola test weight, plant height, seed protein content, and sclerotinia disease incidence and severity were not influenced by whether the previous year’s crop was wheat or canola. Canola yield was reduced 9.0% in 04CRye when following canola compared with wheat, but was not affected by previous crop in 03CRye.

Wheat yields in the second year of the studies were reduced (9.2% in 03CRye and 19.1% in 04CRye) when rye was grown as a cover crop compared with no rye, as were wheat test weights (0.5 and 1.6%), mid-season biomass (29.0 and 66.4%), and plant heights at harvest (4.5 and 8.9%). In 04CRye, wheat protein content and 1000 kernel weight were decreased (8.1 and 10.5%) when rye was grown, but this did not occur in 03CRye.

The presence of a rye cover crop prior to canola in the third year of the studies reduced canola yield by 30.6% in 03CRye and 9.1% in 04CRye, decreased seed protein content (9.2 and 7.2%), but increased seed oil content (5.6 and 3.1%) compared with no rye. The presence of canola prior to canola as compared with wheat prior to canola in the third year of the studies decreased canola yield by 31.0% in 03CRye and 9.2% in 04CRye. In 03CRye canola prior to canola increased protein 6.3% and decreased oil 4.9% compared with wheat prior to canola, but had no influence on protein or oil content in 04CRye.

These results document the negative influence of a rye cover crop preceding canola and wheat, as well as the importance of rotating wheat with canola. The influence of rye on the life cycle of Sclerotinia could not be assessed due to lack of disease development.

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Integrated Pest Management of White Mold on Common Bean in Colorado and Idaho

Howard F. Schwartz, Colorado State University, Shree P. Singh, University of Idaho, and Mark A. Brick, Colorado State University

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

Abstract:
This 3-year project will investigate the importance of cultural practices (plant spacing), and timely application of multi-action pesticides in reducing damage from Sclerotinia sclerotiorum to Phaseolus vulgaris cultivars with varying levels of resistance (plant architectural-disease avoidance, within common bean and interspecific physiological resistance). Replicated field trials in white mold-infested and inoculated nurseries will investigate the importance and value of cultural practice modification (spacing of breeding lines with upright plant architecture or recently transferred interspecific sources of resistance) within an Integrated Pest Management context that compares cultivar, plant density and fungicide effects. The study will be conducted during 2006 to 2008 at one site each in Colorado (furrow irrigated) and Idaho (sprinkler irrigated).

During 2006, the Colorado experiment was adversely affected by drought conditions during flowering to pod fill stages of growth, and only a trace amount of white mold could be induced. Agronomic responses of treatments revealed that there was a noticeable increase in plot yield when plant population was increased 50% from 1 row to 2 rows. The % increase in yield when comparing 1 to 2 rows was 74%, 55% and 26% for Montrose, Vision and Matterhorn, respectively. With an average yield of 20 cwt/A (2240 kg/ha) and average grower price of $0.20/pound ($0.44/kg), the increased plant population (2 rows) could provide an addition return of 11 cwt valued at $220/A (1232 kg valued at $542/ha) for a upright Type II cultivar like Vision in the absence of white mold or if the cultivar was resistant to the white mold.

The Idaho experiment was planted later and avoided the effects of high temperatures during critical phases of flowering and pod set. Three inoculations with white mold were successful and fungicide treatments reduced infection by 50% or more for each cultivar and each plant population. White mold incidence in non-fungicide treated plots was comparable for all three cultivars and either population; Montrose, Vision and Matterhorn % disease intensity ratings with 1 and 2 rows were 94 and 99, 12 and 18, and 10 and 8, respectively. The Type II Vision - 2 lines had 18% white mold with no fungicide protection, but only 1.25% with fungicide. Assuming this modest disease control and associated yield gain with an upright, susceptible cultivar like Vision, 1 fungicide application, and 2 rows per bed, a grower could net an additional $190/A or $512/ha at a cost of $30/A or $74/ha for the fungicide application in the presence of white mold.

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

Shree P. Singh, University of Idaho, Kimberly, ID & Howard F. Schwartz, Colorado State University, Fort Collins, CO


Abstract:
White mold (WM) is a devastating disease of common bean. Only low levels of resistance occur in common bean. But, Phaseolus species of the secondary gene pool possess higher resistance. The goal is to introgress WM resistance from the three Phaseolus species of the secondary gene pool. The specific objectives are first, to pure-line three interspecific breeding lines (IBL) derived from the congruity-backcross of ‘ICA Pijao’ with P. coccineus G 35172 and one each derived from the recurrent-backcross with P. costaricensis S 33720 and P. polyanthus G 35877. Second, determine the inheritance, and tag and map the WM resistance genes from the three Phaseolus species. Third, screen a new group of IBL derived from crosses of ‘Othello’ and ‘UI 320’ with highly WM resistant P. coccineus PI 433246 and PI 439534. Forty-seven IBL derived from the three Phaseolus species had survived selection in FY2006. The 47 IBL and susceptible and resistant checks were evaluated in FY2007. The greenhouse in Colorado and Idaho and field in Idaho were used. White mold pressure in all three environments was severe. Thus, five WM resistant IBL were selected. But, all were variable for WM reaction. Twenty-four WM resistant plants from each of the five IBL will be screened in the greenhouse in Colorado and Idaho and in the field in Idaho. The IBL with the highest WM resistance from each of the three Phaseolus species will be crossed with susceptible ‘Grand Mesa’. The respective backcrosses and F2 will be produced for each set. These will be screened in the greenhouse in Colorado and Idaho and in the field in Idaho to determine inheritance of WM resistance. For tagging and mapping WM resistance genes and QTL, development of approximately 150 F5-derived F6 recombinant inbred lines (RIL) from each of the three single-crosses will be initiated for subsequent screening in the greenhouse in Colorado and Idaho and in the field in Idaho. Screening of the new set of 482 IBL derived from crosses of Othello and UI 320 with P. coccineus PI 433246 and PI 439534 will be initiated in the greenhouse and field. Subsequently, true-breeding IBL with the highest levels of WM resistance will be identified. Thus, higher levels of WM resistance will be available from all three Phaseolus species of the secondary gene pool to broaden the genetic base and combat WM problems nationwide. These IBL will be evaluated in the National Bean White Mold Nursery, used for development of resistant cultivars, and for further genetics and breeding studies. They will be used in Bean Field Days for growers, extension educators, researchers and other clientele, and results also disseminated through refereed papers, bulletins, news paper, TV, radio, electronically, and other means. One highly WM resistant germplasm line (A 195) was released and one refereed paper and one research note were published in FY2007. The PI presented an invited (all expenses paid) paper in the IV Phaseomics International Conference in Salta, Argentina.

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Management Tools for White Mold Disease of Sunflower

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

Abstract:
Field trials were conducted in 2005 and 2006 to determine the resistance levels of several germplasms to Sclerotinia head rot. The lines tested were part of a larger set of germplasms previously evaluated at Carrington. The environment at Langdon was very conducive to the development of head rot in 2005 and less conducive in 2006. Differences in resistance were determined in both years. The resistant check was included with a group of lines that had the least amount of head rot and the susceptible check was included with a group of lines with the greatest amount of head rot in both years. These results and results from other centers conducting similar studies indicate that progress is being made identifying lines that will provide additional management tools to producers to combat Sclerotinia head rot.

A second set of trials were conducted to evaluate fungicides for efficacy against Sclerotinia head rot and determine if yield could be increased with a fungicide application. The studies were conducted adjacent to the trials previously described. Nineteen fungicide and fungicide/adjuvant combinations applied at one or more application timings were evaluated for efficacy of head rot on a confection type cultivar in each year. Despite a range yield increases (nearly 600 lbs over the untreated) and disease incidences and severity reductions, no statistical differences were determined. Further study will be necessary to determine the most effective time to apply fungicide relative to the initial time of infection. The orientation of the face of the sunflower makes the application of fungicide to the face very difficult with standard spray equipment. Studies are needed to determine if application to the face will curb disease development or if application to other areas of the head are also necessary.

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Mapping and Transfer of *Sclerotinia* Resistance from Scarlet Runner to Common Bean

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

Abstract:
In 2006, we continued generation advance of four backcross-inbred populations developed to analyze and incorporate high levels of white mold resistance from *P. coccineus* into *P. vulgaris*. The populations are: 91G/PI 255956, consisting of 115 BC$_2$F$_7$ lines; and 91G/PI 433251B, G122/PI 433251B, and MO162/PI433251B populations each contain greater than 200 BC$_2$F$_3$ lines. The 91G/PI 255956 population was tested three times with the straw test, once with the oxalate test, once in the field as families in 2005 and once in the field as individual lines in 2006. Each straw test was read three times and area under the disease progress curve was calculated. While the straw tests and field trials are significantly correlated, the oxalate test results had no correlation to the others. This supports the hypothesis that white mold response in this cross is conditioned by multiple factors, one of which may be oxalate tolerance. Mapping efforts to date have characterized 71 microsatellite markers in the 91G/PI255956 population. Most microsatellite markers have known position on the bean consensus map. While 71 markers are not enough to construct a substantial scaffold map, we have been able to identify some potential QTL through single marker analysis. Four markers map to B02 in a 20 cM region with significant effects in two or more tests. Twelve additional markers show a significant effect in one test with locations on B02, B03, B06, and B07. Four markers, with two also on LG B02, are associated with segregation for oxalate tolerance. These preliminary results need to be confirmed with interval mapping. Nevertheless, we are very interested in the fact that the location of some of the QTL found in our most recent mapping efforts may be in regions reported on other maps. In particular, the Bunsi/Newport and Bunsi/Raven maps revealed a QTL on B02 that maps to a similar region.

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Pathogenicity and fluorescence of GFP transformed *Sclerotinia sclerotiorum*

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**Abstract:**
*Sclerotinia sclerotiorum* is an important pathogen of a wide variety of crops. To obtain a genetic marker to study the interaction of the pathogen with its hosts, isolates ND 21 and ND 30 were transformed using constructs pCT74 and gGFP both containing genes for the green fluorescent protein (gfp) and hygromycin B phosphotransferase. Protoplasts were generated and a protoplast-PEG (polyethylene glycol) transformation method was employed. Hygromycin resistant putative transformants appeared on the surface of the selection medium in 7 to 12 days. Seven transformants were selected for further study based on stability of the gfp gene and intensity of fluorescence. The sgfp gene was detected in these seven stable transformants using sgfp specific primers and Southern analysis detected a single copy of the sgfp gene in the genome. The pathogenicity of four ND 30 and three ND 21 transformants were evaluated on canola, dry bean, soybean, and sunflower by measuring the diameter of leaf lesions. On dry bean and soybean, ND 30 wild type had significantly higher lesion diameter compared to the transformants. However, on sunflower and canola one ND 30 transformant ND30-Y41 was as pathogenic as the wild type. Lesion formation by ND 21 wild type and the transformants was slower than ND 30. All ND 30 transformants were pathogenic on the four hosts, but pathogenicity of ND 21 transformants varied depending on the host. The fluorescence of these seven transformants was evaluated by directly examining the fluorescence of the mycelium and protein extractions from mycelium. Fluorescence of mycelium on agar plugs was quantified using a Synergy HT multi-detection microplate reader with an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm. Fluorescence values of transformants ND30-41, ND30-Y41, ND21-17 and ND21-14 were significantly higher compared to the wild types. In addition, protein extracts from mycelium of ND30-43 and all transformants of ND 21 had higher fluorescence values compared to the wild types. Infected tissues were sectioned and examined on a Leitz Wetzlar epifluorescence microscope, or a Nikon E600 CARV Confocal cell imaging system, both equipped with filters for gfp excitation and emission. Hyphae of transformants fluoresced in host tissue and could be distinguished from the plant cells. These stable gfp transformants are useful tools for studying the biology of this important pathogen, especially when examining the interaction with plants or other microorganisms.

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QTL Underlying Tolerance to Sclerotinia Stalk Rot in a Sunflower Recombinant Inbred Line Population

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

Abstract:
The stalk rot caused by Sclerotinia sclerotiorum (Lib.) de Bary is a serious disease of sunflower (Helianthus annuus L.) and cannot be effectively or economically controlled through the application of fungicides. The development of resistant inbred lines and hybrids has been an important goal of the seed industry; however, resistance is polygenic, the heritability of resistance is low, and immune germplasm has not been identified. Comparative mapping of quantitative trait loci (QTL) for resistance to Sclerotinia should facilitate the development of marker-assisted selection strategies for enhancing resistance. The goal of our study was to identify QTL for resistance to Sclerotinia in a recombinant inbred line (RIL) population developed from a cross between a completely susceptible confectionery inbred line (RHA280) and a moderately resistant oilseed inbred line (RHA801). RHA280 x RHA801 RILs (n = 91) were artificially inoculated using mycelium-bearing millet seeds in greenhouse and field (Fargo and Grandin, ND) trials in 2006. Using a scale from 0 (highly resistant) to 9 (susceptible), disease severities ranged from 1.7 to 8.3 among RILs in the greenhouse. Disease incidence was investigated in field tests in Fargo and Grandin, and it ranged from 0 to 27.5% in Fargo and from 0 to 52.5% in Grandin. Phenotypic correlations for RIL disease severity between tests were moderate (r = 0.21, 0.25, and 0.26) but significant (p = 0.01-0.04). We identified five QTL on four linkage groups with moderate additive effects (2.0 to -2.9 for disease incidence and 0.59 to -4.9 for disease severity). Individual QTL explained 11.3% to 26.3% of the phenotypic variability. Using the software of QTLMaper 1.60, six, three, and six digenic epistatic QTL were detected for stalk rot tolerance in the greenhouse trial and in the two field trials in Fargo and Grandin, respectively. Totally, the QTL explained 30.0% to 49.9% of phenotypic variability which is much larger than that explained by single locus QTL. Thus, it seemed that digenic epistasis was more important in controlling stalk rot tolerance in sunflower. Replicated tests of the RILs (n = 171) are planned for 2007 and should shed more light on the locations and effects of Sclerotinia stalk rot resistance QTL segregating in RHA280 x RHA801.

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Reaction to Oxalate of Selected Common Bean Lines
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Funded plan of work: Mapping and Transfer of Sclerotinia Resistance from Scarlet Runner to Common Bean

Abstract:
Oxalate is employed by Sclerotinia sclerotiorum to prepare host tissue for invasion by the fungus. This organic acid is a major factor in causing disease because when it is absent, Sclerotinia is only weakly pathogenic. Additionally, crop plants with an oxalate oxidase transgene are partially resistant to Sclerotinia. Oxalate has multiple effects on plant tissues. It lowers the pH of infected host tissues and increases the activity of polygalacturonase and other hydrolytic enzymes that destroy plant cells. Oxalate is also a strong chelator and able to remove calcium from pectin. Oxalate has also been shown to suppress the oxidative burst of host plants. It opens stomata ahead of mycelial growth, causing wilting and allowing mycelial invasion of the interior of plant organs. Certain genotypes of common bean and scarlet runner bean which have partial Sclerotinia resistance have been shown to be more tolerant of oxalate. However, most of the bean accessions with partial Sclerotinia resistance have never been characterized for oxalate tolerance, and it is not known whether oxalate tolerance is a common feature of Sclerotinia resistance. In the present study, lines selected for testing were those identified as possessing partial resistance to white mold, or had been used as parents in recombinant inbred populations used to identify QTL for white mold resistance. Thirty-two bean accessions were grown in the greenhouse to produce cutting for the oxalate test. Cuttings with three to four nodes were arranged in a randomized complete block and were subjected to 20 mM oxalate (pH 4.0) for 12 h in the dark. Wilting was measured on a 10 point scale where 0 is no effect and 9 indicates complete collapse of the plant. The test was repeated three times. With a few exceptions, partially Sclerotinia resistant varieties were more tolerant of oxalate than susceptible varieties, and recombinant inbred parents were ranked as would be expected based on their disease resistance. The most oxalate tolerant accession was Cornell 605. Oxalate tolerance and white mold resistance do not appear to be absolutely correlated. For example, NY6020 sublines and OSU5630 have similar levels of oxalate tolerance, but differ in white mold susceptibility. Other lines with partial Sclerotinia resistance but that had very little oxalate tolerance included Ascher DR, and I9365-31. Our results suggest that factors other than oxalate tolerance may influence white mold resistance.

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Sclerotinia Resistance Enhanced by Accumulation of QTL and Transgenic Approaches

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

Abstract:
This project has two goals involving research on germplasm enhancement and variety development, including biotechnology. The first goal is to increase the level of resistance to *Sclerotinia sclerotiorum* in soybean. Objective 1 is to combine quantitative trait loci (QTL) that were previously mapped and identified with the resistance phenotype into single breeding lines. Three different populations were developed that combine resistance QTL from different sources. After screening over 4,000 plants through the F2, F3, and F4 generations we obtained F5-derived soybean lines that are homozygous for the desired marker alleles for the 8 QTL on 7 different linkage groups. During 2005, we identified 10 F4 families that had significantly smaller average lesion size compared with the most resistant parent in the cross. We identified 40 F5:6 lines with the smallest lesion size that were evaluated during 2006 for reaction to *S. sclerotiorum* in 12 replications of a lattice design using the detached leaf test (DLT). Nineteen of the lines had a lesion size equal to or smaller than the best parent in the cross, and better than the resistant check NKS19-90. The 19 selected F5:7 lines will be evaluated again during 2007 using the DLT, as well as in multi-location yield tests to evaluate agronomic characteristics. Objective 2 is to determine if a novel antifungal synthetic peptide expressed in soybean will confer resistance to *S. sclerotiorum*. We developed new transformed plants with a codon-optimized gene-expression cassette for the antifungal peptide that contains the barley alpha-amylase signal sequence to export the peptide to the apoplast. The T1 populations from seven independent transformation events containing the lytic peptide in the new codon-optimized gene expression cassette are growing in the greenhouse during winter fall/winter 2006-07. We will run the DLT on T2 populations during summer 2007.

The second goal is to improve the use of calcium cyanamide as a control option for *S. sclerotiorum*. Our previous results indicate that the *cah* gene has no negative effects on yield in the transgenic lines vs. the non-transgenic control. Furthermore, Perlka application reduced germination of sclerotia and increased yield. It is unlikely, however, that the results for sclerotinia reaction alone will be sufficient to justify regulatory approval expenses for a transgenic event. Perlka has been shown to affect other pathogens as well as nematodes, and it has herbicidal activity. These effects together could make an attractive disease management package for producers. Objective 1 is to evaluate effects of Perlka™ (granular Ca-cyanamide) on soybean cyst nematode. Results from 6 replications at two locations during 2006 show no consistent yield trend.

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**Sclerotinia Stem and Head Rot Resistant Germplasm Development Utilizing Interspecific Amphiploids**

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

**Abstract:**
How to control *Sclerotinia*, a major fungal disease in cultivated sunflower, has always been a major concern for sunflower producers, breeders, and researchers. A considerable effort has been made to discover resistance genes in wild species and transfer them into the present-day hybrids, which are considered to possess insufficient resistance genes to *Sclerotinia*. Interspecific amphiploids of crosses between wild perennial *Helianthus* species and cultivated line P21 have been produced and used to provide resistant genes to a newly evolved race F of broomrape (*Orobanche*) in Spain. Similarly, these amphiploids, with their good backcross seed set, can quickly be utilized for pyramiding of *Sclerotinia* resistance genes, if they prove to be resistant. In 2005, seven interspecific amphiploids were evaluated at Fargo, ND, and all were found to be highly resistant to *Sclerotinia* stem rot compared to the tolerant check HA 410. In 2006, we repeated the evaluation of the same amphiploids for their resistance to *Sclerotinia* stem rot at Mapleton, ND, and head rot at Fargo, ND, using artificial inoculation in the field. Meanwhile, resistant amphiploid plants were crossed to stem rot tolerant HA 410 and head rot tolerant HA 441 for stem and head rot resistance gene pyramiding, respectively. Interspecific amphiploids have been confirmed as useful sources of resistance genes for both *Sclerotinia* stem rot and head rot based on our evaluation over two years, 2005 and 2006. The interspecific amphiploids include crosses of wild perennial *Helianthus gracilentus*, *H. hirsutus*, *H. strumosus*, *H. grosseserratus*, *H. maximiliani*, and *H. nuttallii*, crossed with P21, plus one intercrossed amphiploid involving *H. divaricatus* and *H. grosseserratus*. The result indicated that most amphiploids have better stem rot and head rot resistance than the tolerant check HA 410 and HA 441, respectively. The good F\(_1\) seed set between the amphiploids and HA 410 or HA 441 provided a sufficient number of plants for further backcrossing and chromosome reduction toward the 2n=34 chromosome number of cultivated sunflower. Based on our earlier success of transferring resistance genes from amphiploids for the broomrape resistance, we believe similar results can be achieved by using these amphiploids to develop sunflower germplasms superior to HA 410 and HA 441 for stem rot and head rot resistance, respectively.

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Symbiotic Bacteria Associated with *Sclerotinia sclerotiorum* from Pea

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

**Abstract:**
Research conducted in 2005-2006 focused on sequencing expressed sequence tags of *Sclerotinia sclerotiorum* strain WMA1 and *Pisum sativum*. Three cDNA libraries were generated from culture-grown mycelium on PDB and YPG and transformed into *E. coli*. Sequences from each of these EST libraries and most clones had similarity to bacterial sequences from *Pseudomonas* spp. despite no evidence for contamination of the growth medium. When the growth medium was changed to YPG, the ratio of sequences with similarity to bacterial genes relative to that of fungal sequences was reduced. This result suggests the presence of symbiotic bacteria in *S. sclerotiorum* strain WMA1. We attempted to detect the occurrence of bacteria in several *S. sclerotiorum* strains by PCR with bacteria-specific primers. Primers (63F and 1378R) were designed to bacterial 16S ribosomal DNA. This primer set amplified a fragment only from WMA1 and not from WMA1 and 1980 from pea and bean. Strain WMA1-5, obtained by sub-culturing WMA1 on gentamicin, and strain 1980, provided by Jeffrey Rollins, University of Florida, were bacteria-free based on PCR. Sequences of fragments amplified by the bacteria-specific primers had the greatest similarity to *Ralstonia* spp. These results suggest that strain WMA1 may harbor symbiotic bacteria which are absent from strain 1980 and subcultured strain WMA1-5. Additional experiments are being performed to verify the presence of bacteria including the use of the LIVE/DEAD Baclight bacterial viability (Molecular Probes) assay and fluorescent in situ hybridization with bacteria-specific probes.

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Unraveling Mechanisms Associated with Resistance: Soybean Stem Lignin Concentration and Susceptibility to *Sclerotinia sclerotiorum*

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**Funded plan of work:** Unraveling the genetics of resistance in soybean to *Sclerotinia sclerotiorum* using multiple evaluation criteria.

**Abstract:**
The goal of this project is to identify genetic resources using multiple evaluation criteria to develop soybean accessions that express complete and environmentally stable forms of resistance to *Sclerotinia sclerotiorum* (Ss). We propose to revise objectives based on research findings in year 1 of the project. Although preliminary results appeared promising, plant reaction to oxalic acid was found to be inconsistently correlated with interaction phenotypes observed in controlled and field environments. Oxalic acid, therefore, cannot be used to differentiate soybean accessions for reaction to *Ss*.

Conversely, lignin content of stem and node tissues was correlated with interaction phenotypes among a set of soybean accessions inoculated with *Ss* in both controlled ($r_s = 0.83 - 0.94; P = 0.05 - 0.005$) and field environments ($r_s = 0.90 - 0.94; P = 0.04 - 0.005$). Specifically, lignin content of internode and node tissues was positively correlated with disease severity caused by *Ss*. W04-1002, a breeding line that has expressed a high level of partial and environmentally insensitive resistance, was found to have a lower lignin content compared to less resistant and susceptible soybean accessions. W04-1002 is considered a novel source of resistance to *Ss*.

We propose to apply to the soybean and *Ss* system the concepts and methods used to study lignin in forage crops. In alfalfa, lignin is regarded as the most environmentally stable of measurable traits associated with forage feed quality. If the same is true for soybean, and the link between lignin concentration and reaction to *Ss* is confirmed, this method could have great value in evaluating plants for resistance in years not conducive for disease development.

Although cell wall lignin concentration and disease severity were positively related, our data does not support a conclusion that lignin has a direct effect on the reaction of soybean to *Ss*. It does however, lead us to hypothesize that specific QTL confer resistance to *Sclerotinia* stem rot and are associated with low lignin content in internode and node tissues. We propose experiments designed to test these hypotheses. A greater understanding of these traits will facilitate breeding for a superior form of resistance to *Ss*. In addition, a combination of assay methods that involve both quantification of stem lignin and plant reaction to *S. sclerotiorum* will facilitate the identification of new and superior sources of physiological resistance.

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Use of Yeast to Discover Mechanisms of Fungal Pathogen-Plant Interactions

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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 produced by various phytopathogenic filamentous fungi, including Sclerotinia sclerotiorum. In order to better understand the specific effects of oxalic acid on plant cells, a Saccharomyces cerevisiae library consisting of 4,800 deletion mutants was screened for oxalate sensitivity. Our premise was that genes whose loss resulted in sensitivity normally provide protection in yeast, and that orthologs in Arabidopsis may provide similar protection, and thus, modulate the outcome of the fungal pathogen-plant interaction. A total of 105 mutants were identified, 25% of which were sensitive to oxalate acid concentrations as low as 1 µM to 2 mM. Among the 28 mutants found to be highly sensitive to oxalate acid, four have deletions in genes whose homologs in filamentous fungi or plants are involved in host-microbe association. Significantly, none of these genes had previously been implicated in oxalate-associated plant diseases. Two of these genes, RIB4 and PTC1, encode lumazine synthase, an enzyme in the riboflavin biosynthetic pathway, and protein phosphatase 2C, respectively. The Arabidopsis homolog of RIB4 is COS1 which is involved in jasmonic acid signaling, a key mediator of plant defenses. ABI1 and ABI2 are Arabidopsis homologs of PTC1, which control abscisic acid signaling, transpiration, and interactions with S. sclerotiorum.

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

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

Abstract:
The project has two goals. The first is to identify white mold (WM) resistance in adapted common bean lines. Data from multi-site field tests in eight locations supported the separation of field resistance in ten lines with three of the lines exhibiting levels significantly higher than the resistant standard, G122. In greenhouse straw tests, nine lines exhibited resistance levels similar to G122. The field and greenhouse test data allowed us to identify WM 55 as having partial resistance similar to Bunsi, i.e. more escape/avoidance than resistance to the pathogen. The second goal is to determine if there is variability in the WM pathogen Sclerotinia sclerotiorum. The first objective under this goal uses mycelial compatibility grouping (MCG) as a method of determining clonality of isolates used to screen for WM resistance in the straw test. Six MCGs were identified; three MCGs of unique single isolates and three multiple isolate MCGs. The second objective is to determine differences in aggressiveness using the straw test. Straw test results were influenced by the host plant genotype used, e.g. Bunsi as the host resulted in higher overall mean ratings compared to use of G122 as the host. Also, the aggressiveness rankings differed depending on the host line used. There are significant differences between greenhouse screening isolates for aggressiveness and between MCGs and aggressiveness. Thus, choice of screening isolate may influence the separation of resistant and susceptible lines. Aggressiveness of the 155 field screening nursery isolates is not completed; however, MCGs for the 155 field isolates have been identified. The 155 isolates were highly variable with 64 MCGs, indicating many unique isolates, and only six MCGs had clonality between locations. The third objective involves use of microsatellites to determine isolate variability. Ten primer sets found to have the most informative polymorphisms when the ten greenhouse screening isolates were tested are currently being sequenced. After sequencing, five to seven of the most informative primer sets will be used to evaluate the 155 field screen isolates.

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White Mold Resistance in Dry Bean Derived from 

*P. vulgaris x P. coccineus*

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

**Abstract:**
Scarlet-runner bean (*Phaseolus coccineus* L.), a representative species of the secondary gene pool of common bean, is a potential source of white mold resistance for improving dry bean. I9365-31, I9365-25, and VA19 are dry bean lines that possess resistance to white mold putatively derived from scarlet-runner bean. The objective of this research was to characterize resistance of these lines to white mold in mapping populations tested across multiple field and greenhouse environments. Recombinant inbred populations consisting of F5-derived lines were developed from the crosses Raven/I9365-31, Montrose/I9365-25, and Benton/VA19. ‘Raven’ and ‘Montrose’ are commercial black and pinto bean, respectively, susceptible to white mold. ‘Benton’ is a susceptible snap bean. Separate R and S bulks for field and greenhouse reactions to white mold were used in bulked-segregant analyses to identify markers associated with resistance in the Raven/I9365-31 population. There were four independent quantitative trait loci (QTL) expressed across field environments, explaining from 9% to 24% of the phenotypic variation for disease score. Two major independent QTL conditioning resistance (22% to 37%) in the greenhouse were stably expressed across five separate straw tests. Integrating the QTL from this population on the core map has been difficult and is still in progress. Disease reaction for the Benton/VA19 population has been obtained from multiple field and greenhouse environments. Preliminary assay of the Benton and VA19 parents with SRAP markers indicate adequate polymorphism for mapping traits in the corresponding RIL population. The Montrose/I9365-25 population has been tested for disease reaction in only one field and one greenhouse environment, thus far. The narrow range for reaction to white mold observed among RILs suggests this population may not be suitable for mapping QTL conditioning resistance.

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White Mold Resistance in Pea and Lentil through Breeding and Biotechnology

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

Abstract:
Two approaches to develop resistance to *Sclerotinia sclerotiorum* in pea and lentil have been initiated. The first approach involved screening 36 pea genotypes under field conditions at Carrington, ND and 24 and 12 genotypes of pea and lentil, respectively, at Pullman, WA. Research plots at Carrington consisted of seven rows spaced 18 cm apart and 7.6 m long and were arranged in a randomized complete block design with 4 replicates. During the flowering period, all plots at both locations were inoculated with ascospores. Immediately after inoculation a misting system was employed to maintain a humid environment to favor disease development. The misting system was run for 2-4 minutes every half hour, 24 hours/day, for 4 weeks. Disease was scored periodically and growth, development, and grain yield and quality data were recorded. Disease scores at Carrington in 2006 were not as severe as 2005 due to dry and unfavorable conditions. In 2005, statistically significant differences were observed in all parameters measured at Carrington except days to physiological maturity where disease progression did not allow accurate assessment of physiological maturity (and powdery mildew) in all plots. Several entries showed relatively high levels of susceptibility on the first evaluation date and on the final evaluation date, ‘Arvika’ (forage pea) and ‘CDC Sonata’ showed the lowest disease occurrence in 2005. In 2006, ‘Admiral’ (0.5) and ‘Carneval’ (1.0) had the lowest disease ratings and Arvika had a score of 2.8 compared to 3.8 in 2005. A highly significant negative correlation was observed between yield, days to beginning and end bloom and disease rating. Genotypes with the greatest level of resistance will be used to develop genetic mapping populations for inheritance studies. The second approach involved introducing the oxalate oxidase gene from barley (*Hordeum vulgare* L.) into pea and lentil through *Agrobacterium tumefaciens*-mediated transformation. The oxalate oxidase gene was successfully cloned from barley cDNA and incorporated into a twin binary vector. Two pea cultivars, ‘Mukta’ and ‘Joel’, and one lentil cultivar, ‘Pardina’, have been transformed and explants are currently on selection media. The twin binary vector will allow the selectable marker gene, *nptII*, to be separated from the oxalate oxidase gene through natural Mendelian segregation. This will be beneficial if deregulation of the transformants is pursued.

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