National Sclerotinia Initiative

2003 Research Abstracts

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A Preliminary Molecular Marker Map for *Phaseolus coccineus*

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Our overall goal is to transfer partial white mold resistance from *P. coccineus* to common bean. Because of the difficulties in obtaining recombination during the interspecific transfer process, we are identifying molecular markers that can assist in transfer. We had originally intended to use bulked segregant analysis to identify markers for marker-aided selection to transfer resistance, but bulked segregant analysis does not lend itself well to manipulating multiple quantitatively inherited traits. Thus, we shifted to a quantitative trait locus (QTL) mapping effort using a population developed from the cross Wolven Pole x PI 255956. We chose this cross in part based on our investigations into the physiological basis of resistance. We examined the relationship between oxalate, an established virulence factor of *S. sclerotiorum*, and resistance. Wolven Pole was most sensitive to oxalate, and PI 255956 least sensitive. Oxalate concentrations were similar in infected stem tissues of the partially resistant line and lower than Wolven Pole. Conversely, oxalate oxidase abundance was higher in the more resistant lines than in Wolven Pole. Apparently, PI 255956 has at least two mechanisms of resistance. The Wolven Pole x PI 255956 map was constructed from an F_2 of 188 individuals. Plants screened with the straw test, revealed a bimodal distribution for reaction to *Sclerotinia* with peaks centered on moderate resistance (infection stopped at first node) and susceptibility (infection passed through first node). PI 255956 has partial resistance, as did 48 F_2 progeny while Wolven Pole and 144 F_2 were susceptible. Resistant individuals were retested and disease was allowed to progress for another four weeks to prevent escapes. From an initial screen of 600 RAPD primers, 111 were used in the total population. Twenty-four of the 111 primers were used to screen 188 individuals and the F_1 yielded 28 markers. Eighty-seven primers screened with 94 individuals and F_1 yielded an additional 92 markers. We also synthesized 10 bean-specific microsatellite primers three of which were polymorphic in the F_2 population. The map has 102 linked RAPD and microsatellite markers on 14 linkage groups for a total length of 395 cM. Ten markers are unlinked including one microsatellite. Interval mapping in QTL Cartographer revealed six statistically significant (LOD e 4.0) QTL on linkage groups 3, 7, 8, 11, 12, and 14. Percent additive genetic variation explained by QTL individually range from 9 to 12 %), while in combination they explain 63%. The two microsatellites mapped here have been placed on the *Phaseolus vulgaris* consensus map with our linkage groups 6 and 7c corresponding to consensus linkage groups 2 and 3, respectively.

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Cellular Basis of Oxalate Sensitivity and its Relationship to White Mold Susceptibility

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Oxalate's contribution to *Sclerotinia* diseases was documented by de Bary more than a hundred years ago. More recently, oxalate-deficient *Sclerotinia sclerotiorum* mutants and transgenic plants over-expressing oxalate oxidase genes provided evidence for a role of oxalate in pathogenesis. However, the cellular and molecular mechanisms, by which oxalate sensitizes plant tissue to become susceptible to *Sclerotinia* attack, are unknown. Our research shows that oxalate tolerance is a resistance factor in scarlet runner beans (*Phaseolus coccineus*). The white mold resistant *P. coccineus* accession PI 255956 is significantly less sensitive to exogenous oxalate than the susceptible cultivar 'Wolven Pole'. PI 255956 and the *Sclerotinia* resistant accession PI 535278 express more oxalate oxidase than 'Wolven Pole'. Thus, oxalate oxidase expression can only partially explain the elevated oxalate tolerance of PI 255956. We determined cellular mechanisms of oxalate action by exploiting oxalate-dependent changes in stomatal aperture. Exogenous oxalate increases potassium uptake and starch degradation, which result in an elevation of cellular osmotic pressure and opening of stomatal pores. Mutants of *Arabidopsis thaliana*, which have a defect in stomatal closure, are more susceptible to *S. sclerotiorum*. Thus, guard cell dysfunction not only enhances foliar transpiration and wilting but also disease susceptibility. We have initiated a mutant screen to identify genes that enhance oxalate tolerance based on oxalate-dependent decreases in seedling vigor.

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Characterization of Soybean Genotypes with Partial Resistance to *Sclerotinia* Stem Rot

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Funded Plan of Work: Characterization of Soybean Genotypes with Partial Resistance to Sclerotinia Stem Rot

Characterization of infection of a partially resistant (NKS 1990) and a susceptible soybean cultivar (MN 0301) by *Sclerotinia sclerotiorum* was examined with light and scanning electron microscopy (SEM). Petioles of plants at R1-R2 growth stages were inoculated in a humid chamber at 24E C using infested tissue paper placed directly on the petioles. Plants were sprayed with water to maintain a film of moisture over the tissues. Tissues were examined at 24, 48, 30, 72 and 110 hours post inoculation. Tissues prepared for SEM were fixed in 2.5% glutaraldehyde, sectioned with a Vibratome, dehydrated through a graded ethyl alcohol series, critical point dried and coated with gold. The method of placement of the inoculum on the tissue influenced infection. When the inoculum was gently placed on the petiole, such as what would occur in nature, it rested on the trichomes and the time to infect and reach the cortical tissues was increased When tightly appressed to the petiole, the mycelium came in direct contact with the epidermis and infection of cortex occurred earlier. The microclimate around the inoculum appears to be more variable when inoculum is gently placed on the petiole and therefore mycelial growth is more erratic. Infection occurred directly through the walls of the trichomes and the epidermal cells. Infection hyphae were generally swollen at the ends and often there were clusters of infection hyphae originating from one hypha. Infections began between 24 to 30 hours post inoculation, and the numbers of infections on a given area of the petiole were highly variable and appeared to depend on the amount of mycelial growth. Ramifying hyphae in the cortex were observed as early as 48 hours, but appearance in the cortex was highly variable, with some inoculated plants showing no hyphae in the cortex after 72 hours even though there was heavy mycelial growth on the exterior of the petiole. Intracellular hyphae were observed in host tissue before there was any gross evidence of host tissue degradation by pathogen enzymes or toxins. However, tissue breakdown in advance of hyphae was also observed. There were no apparent differences between NKS1990 and MN0301 in observations made up to 110 hours post inoculation. If organic debris colonized by ascospores, such as senescing petals, lands on a petiole, it will first come in contact with trichomes. In nature, therefore, trichomes are most likely the primary site of infection on petioles.

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Control of *Sclerotinia* diseases with Intercept in North Dakota

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Funded Plan of Work: Use of Intercept for control of *Sclerotinia* on dry beans, canola, and sunflower in North Dakota

Field experiments were installed at Carrington and Mapleton, ND in 2002 and 2003 to evaluate the efficacy of Intercept, a commercial formulation of *Coniothyrium minitans*, to control *Sclerotinia* diseases in canola, sunflower, and dry beans. Experimental plots, 20 x 15 ft were established following a split-split plot arrangement in a complete block design with four replications. Ten ft-wide alleys separated plots from each other. All plots were supplemented with field sclerotia to a final density of 60 sclerotia per sq m prior to Intercept application. Intercept was applied at rates of 0, 1, or 2 lb/A either in the fall of 2002 or the spring of 2003 and immediately shallow incorporated into the ground. At the end of each growing season samples were taken from the upper two inches of soil from each experimental plot. Sub-samples were baited with laboratory-produced sclerotia of *S. sclerotiorum* in order to detect the presence of *C. minitans*. In the 2002 season *Sclerotinia* wilt developed on sunflower plots at both locations and white mold developed on beans at Mapleton but not at Carrington. *Sclerotinia* stem rot did not develop on canola at any location. No statistical differences were detected between Intercept rates at any location or between times of soil infestation. When soil samples were evaluated for parasitic activity, over 60% of the sclerotia baited yielded *C. minitans*, an indication of high levels of parasitic activity at both locations. Even samples collected from untreated plots yielded *C. minitans*; however, soil samples collected from areas adjacent to the experimental sites did not yield *C. minitans*. Isolates retrieved from the sclerotial baits were morphologically similar to colonies produced by the Intercept isolate. In 2003 the same plots were planted to all three crops, but no additional Intercept was applied. *Sclerotinia* wilt epidemics developed in sunflower plots at both locations, but at significantly lower levels than in the 2002 season. Traces of white mold were detected in bean plots at Mapleton but not at Carrington. Although no disease was detected in canola plots at Carrington, disease incidence in neighboring fields was on average 14%. Soil samples collected at the end of the 2003 season yielded *C. minitans*, although at significantly lower levels than in the 2002 season. Residual parasitic activity was four times higher on Carrington samples than on Mapleton where less than 5% of the sclerotial baits yielded *C. minitans*. Soil pH from Mapleton was on average 8.5 compared to 7.7 of Carrington. Results of this study suggest that Intercept can actively parasitize sclerotia of *S. sclerotiorum* even at very alkaline soil conditions; however, the low residual activity detected in the highly alkaline soil indicates that repeated applications, maybe once a year, may be required to secure effective control.

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Development and Refinement of the Novel TRAP Marker Technique

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

We have developed a novel DNA marker technique termed TRAP, target region amplification polymorphism (Hu and Vick 2003, Plant Molecular Biology Reporter 21:289-294). The TRAP is a powerful, high-throughput PCR-based system useful for rapid genotyping of germplasm and genome mapping. It utilizes bioinformatic tools and the sunflower EST database information to design primers to detect polymorphic markers around the targeted candidate gene sequences. Total genomic DNA samples are used as templates in PCR reactions with two primers of 18 to 20 nucleotides long. The fixed primer is designed against an annotated EST, which is the putative candidate gene involved in governing the trait of interest. The arbitrary primer is of arbitrary sequences but with either an AT or GC rich core to anneal with introns or exons, respectively. Mismatching is allowed in the first five cycles of PCR amplification by annealing the primers with the template at 35 °C. The last 35 cycles are run with annealing temperature of 50 °C. To ensure the amplification of the fragments around the targeted sequence in PCR, the ratio of fixed primer and arbitrary primers is set at 30 to 1. It has been found that the fixed primer plays an important role in determining the amplification pattern. The arbitrary primers are 3' end-labeled with IR dye 700 or IR dye 800 for autodetection on a Li-Cor Global DNA Sequencer. For different plant species, each of the PCR reactions can generate about 50 scoreable fragments with sizes ranging from 50 to 900 base pairs, when separated on 6.5% polyacrylamide sequencing gel. The TRAP is being employed by researchers working with different crop species. We are using it to search for DNA markers associated with *Sclerotinia* tolerance in both cultivated and wild sunflowers.

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

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[Research Assistants: Barry Ogg, Dave Gent, Mark McMillan and Kris Otto]

12/19/03 Progress Report -- Year 2 of 2

ABSTRACT:
This project investigated the roles of cultural practices (irrigation interval, tillage at planting), timely application of chemicals, and partial plant resistance in reducing damage from *Sclerotinia sclerotiorum* to *Phaseolus vulgaris*. These objectives support the Sclerotinia Initiative areas of Epidemiology & Disease Management, and Chemical & Biological Control. Agronomic and chemical implications from this IPM approach will be applicable to other host cropping systems affected by foliar phases of white mold. Work was conducted in 2002 and 2003 to investigate the roles of irrigation interval (5 vs 10 days), tillage (deep ripping or not at planting to improve root health and water-use efficiency later in the season), plant resistance (susceptible pinto cultivar 'Montrose' vs partially resistant pinto cultivar 'Chase'), and timely application of chemicals (none, thiophanate methyl, and thiophanate methyl + systemic acquired resistance inducer -- Acibenzolar) within an Integrated Pest Management context.

The more frequent irrigation interval increased yield of cultivar 'Montrose' by 14 and 27% in 2002 and 2003, respectively. Planting-time ripping increased yield by nearly 40% in 2002, but decreased yield by 40% in 2003, apparently due to an interaction between Montrose and Fusarium Wilt. The highly susceptible cultivar 'Chase' showed a modest response to increased irrigation, but respond negatively to ripping both years; presumably as roots penetrated more deeply in the profile and encountered more disease problems with Fusarium wilt. White mold did not develop in the disease nursery due to the drought and high temperature conditions which persisted throughout 2002 to 2003, and there was no response to any fungicide treatment in the absence of disease.

During 2003, a set of laboratory and greenhouse experiments systematically evaluated the potential usefulness of conventional and experimental fungicides and biopesticides (including systemic acquired resistance inducers) applied to foliage of a susceptible cultivar 'Montrose' before inoculation of leaf disks with the white mold pathogen. Older white mold fungicides such as Topsin (thiophanate methyl) and Topsin + Blocker (PCNB) provided 82 and 91% control up to 4 days after inoculation. Newer chemistry such as Endura (Boscalid), Omega (Prochloraz) and Pristine (Pyraclostrobin) provided 98 to 100% control for this time period. Biopesticides such as Actigard (Acibenzolar) only provided 13% control after 4 days; suggesting that additional research is warranted with timing, rate and tank-mixtures with old and new chemistry to further evaluate the potential of promising materials for inclusion in IPM strategies for dry bean and other white mold - susceptible crops.

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Epidemiology and resistance to *Sclerotinia* head rot in wild sunflower species

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

Field trials were conducted in 2002 and 2003 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. Ninety-six accessions of wild sunflower species were evaluated using artificial inoculation with ascospores, fungal mycelia, and ground infected-millet seed. Plants were covered for 14 days after inoculation with light brown paper bags, sunflower pollination bags, and thin plastic bags. These treatments were repeated three times at 2-weeks intervals using different wild sunflower accessions every time. A few puffs of water were applied into each covering 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.

Very little infections have occurred to the wild sunflower heads, but the stems were infected and showed typical symptoms of bleaching, shredding, and the formation of tiny cylindrical sclerotia inside the stems. The ground infected-millet inoculum resulted in the highest level of infections followed by ascospores and fungal mycelia. The paper bags covering resulted in the highest infection levels followed by sunflower pollination bags. Plastic bags were not very effective covers.

The combination of ground infected-millet inoculum and the paper bag covers resulted in 88% infection in 2002 and 55% in 2003. Fifteen accessions remained healthy under the various artificial inoculation methods in both years of this study. Such accessions are believed/suspected to have genetic resistance to *Sclerotinia*. Future research will focus on studying the genetics of this resistance and the transfer of the resistance genes to sunflower hybrids.

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Evaluation of Canola Cultivars for Resistance to *Sclerotinia*

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

The objective of this project is to identify canola cultivars which are less susceptible to *Sclerotinia*. In 2003, field trials were conducted at the North Dakota State University Carrington Research Extension Center and an on-farm site near Red Lake Falls, Minnesota. Twenty canola cultivars, representing current production varieties and private breeding lines, were evaluated in a randomized complete block design with four replicates. Plot size was approximately seven 7-inch rows x 25 feet. At flowering, plots were inoculated with ascospores (foliar spray) and misted until physiological maturity to provide a favorable environment for disease development. Disease incidence and severity were evaluated, as well as plant height and lodging at maturity and grain yield, test weight, and oil concentration at harvest. Data were analyzed by standard statistical procedures and means were compared by F-protected LSD. Excellent disease pressure was attained at both sites, with incidence ranging from 90 to less than 51% at Carrington and 76 to 4% at Red Lake Falls. Significant differences among entries were observed for all parameters measured at both locations. Highly significant correlations existed among disease incidence, severity, and relative ranking of the entries within and across sites. In Carrington, disease incidence, but not severity, was negatively correlated with plant height. In Red Lake Falls, lodging was more pronounced than in Carrington and was positively correlated with disease incidence and severity. Although lower than normal for small-plot research, yields of some cultivars were quite good considering the level of disease pressure.

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Evaluation of canola cultivars for resistance to *Sclerotinia sclerotiorum* using petiole and detached leaf inoculation in controlled conditions

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Funded Plan of Work: Evaluation of canola cultivars for resistance to *Sclerotinia*

Sclerotinia stem rot (SSR) can cause considerable economic damage to canola grown in North Dakota and Minnesota when conditions are favorable. Information on cultivar susceptibility to SSR is limited, and observations under field conditions can be inconsistent due to non-uniform disease pressure and differences in cultivar maturity and plant architecture. Twenty canola cultivars were tested under controlled greenhouse and growth chamber conditions for their level of resistance to SSR using a petiole inoculation technique (PIT) and a detached leaf assay (DLA). Significant ($P<0.05$) differences among cultivars were detected with the PIT under both greenhouse and growth chamber conditions. 'Hyola 357' had the lowest AUDPC in both the greenhouse and growth chamber. No significant differences among cultivars were detected using the DLA. Spearman and Pearson correlations between the PIT and the DLA were not significant. Results indicate that the PIT may be an efficient and reliable method to evaluate canola cultivars for their level of resistance to SSR.

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Evaluation of *Sclerotinia* Resistance and Defining QTL Regions in *Brassica napus*

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

The goal of our project is to identify genes for resistance to *Sclerotinia sclerotiorum* in oilseed *Brassica napus*, to verify the effects of the genes in different genetic backgrounds, and to apply this information to the improvement of canola, as well as other crop species.

Host-pathogen interactions were tested in a series of experiments including four *S. sclerotiorum* isolates and a large number of *B. napus* accessions. We adapted a Petiole Inoculation Technique (PIT) that had been developed for soybean to inoculate *B. napus* with *S. sclerotiorum*. Two criteria were applied to evaluate the responses of accessions: days to wilting (DW) and a lesion phenotype index (LP). The results showed no significant differences in virulence among the four isolates, and isolate-accession interactions were non-significant ($F=1.02$, $p=0.44$) for DW. However, highly significant differences ($p<0.0001$) were detected among the *B. napus* accessions for each experiment and for each evaluation criterion, and scores from the two evaluation criteria were highly correlated ($r = 0.86$). The Chinese lines were the most resistant and the Canadian lines were the most susceptible. We also found that most winter-type accessions exhibited higher levels of resistance than did spring-type accessions, and rapeseed accessions (high glucosinolates and high erucic acid content) showed slightly higher levels of resistance than did canola quality accessions within each growth habit type.

In order to map genes for *S. sclerotiorum* resistance and to dissect the association between growth habit and resistance, we screened a segregating population of DH lines for reaction to *S. sclerotiorum*. This population was derived from a cross between a resistant Chinese winter line (RV289) and a susceptible European spring line (P1804), and it had been analyzed previously with molecular markers to develop a linkage map. Seven QTL for *S. sclerotiorum* resistance were identified with individual effects explaining 8 - 19% of the variance. Some of these QTL were in the same genomic regions as QTL for flowering time, although the correlation between flowering time and *S. sclerotiorum* resistance was not high ($r = 0.52$). To gain further insight on resistance genes and their possible association with growth habit, we plan to evaluate additional segregating populations of DH lines and to test other methods of inoculating *B. napus* plants.

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Field Studies on the Effect of *Coniothyrium minitans* on Sclerotia Populations and Disease Incidence

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

Research was conducted in 2003 to evaluate a commercially available biological control organism for its ability to provide long-term suppression of *Sclerotinia sclerotiorum* in fields in New York. The cropping systems included potatoes (Wolcott), wax beans (Geneva), and an organic system with dry beans planted in 2003 (Hunt). The objectives were 1) to quantify the decline in populations of sclerotia in the soil following a single treatment of *Coniothyrium minitans* applied as the commercial product Contans (also called Intercept), and 2) to determine if applications resulted in a reduction in plant disease caused by *S. sclerotiorum*. Field populations of sclerotia declined over time in the Contans-treated areas and in the nontreated check. However, treating with Contans resulted in a more rapid decline and lower final sclerotia populations for several months following application at two locations (Geneva & Wolcott). At the third location (Hunt) the sclerotia populations were too low for valid comparisons. Disease incidence at the Wolcott location was statistically lower (P=0.01) in the Contans-treated versus the nontreated plots. At Geneva the trends were the same, but differences in disease incidence were not statistically significant due to extreme variability in disease occurrence in the field. The trials documented decline in soil populations of sclerotia following application of Contans. This result has implications for disease management, because fewer viable sclerotia in soil will result in fewer apothecia produced, and may result in reduced disease incidence. Our trials showed variability, possibly due to natural decline in sclerotia populations in soil, uneven distribution of sclerotia in soil, and the unknown survival rate of *C. minitans*. The research demonstrates that strategic use of *C. minitans* in various cropping systems may be an effective tool to reduce populations of sclerotia of *S. sclerotiorum* and subsequent disease incidence.

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Fungicides and Applications Timings for White Mold Disease Management in Field Pea, 2003

Scott Halley and John Lukach, 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

Research conducted in 2003 identified fungicides and application timings on field pea that have potential for use as management strategies to control white mold disease caused by the pathogen *Sclerotinia sclerotiorum* (Lib) de Bary. Fungicides, Ronilan (vinclozolin) and Topsin M (thiophanate methyl) improved yield at Langdon compared to Quadris (azoxystrobin). Fungicide application timings, 40% or 10 + 40% bloom growth stage, increased yield 4 bu/acre over a 10% bloom growth stage application. Bayer experimental fungicide JAU 6476 (prothioconazole) and Quadris applied at 40 or 10 + 40% bloom growth stage and Endura (boscalid) applied at 10 + 40% growth stage increased yield compared to the untreated at Carrington. Fungicides applied at 100% bloom growth stage produced yields that were not different from the untreated. Disease pressure was moderate level developing late in the season at Langdon and heavy at Carrington. Increases in 1000 seed weight were recorded at both locations as was test weight at Carrington by select fungicide applications. Blocker (pentachloronitrobenzene) and Ronilan did not increase yield at Carrington compared to the control and Quadris yield was less than Ronilan and Topsin M yield at Langdon. Further study is warranted to characterize differences between locations and environments in fungicide response and to further identify the most effective application timing.

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Identification of QTL for Soybean Resistance to *Sclerotinia* Stem Rot (*Sclerotinia sclerotiorum*) in the Merit x PI194639 Population

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Funded plan of work: Characterization of soybean genotypes with partial resistance to *Sclerotinia* stem rot

Sclerotinia stem rot (*S. sclerotiorum*) of soybean has become a major disease in soybean production areas of the Midwestern United States. Although thousands of soybean plant introductions (PI) have been evaluated for resistance to the disease, no soybean varieties or PIs have been identified as completely resistant to this fungal pathogen. Genetic studies have shown that the resistance is complex and quantitatively inherited. The objective of this study was to map quantitative trait loci (QTL) for the resistance to *Sclerotinia* stem rot in a population of recombinant inbred lines (RIL) derived from a cross between Merit (susceptible) and PI194639 (partially resistant). A total of 500 simple sequence repeat (SSR) markers distributing throughout soybean molecular linkage map were screened for genetic polymorphisms between the parents. Of these, 261 markers (~52%) were found to be polymorphic and were used to genotype 153 F4:5 RILs. The disease response of these lines was evaluated in a greenhouse under controlled environmental conditions using the cut stem inoculation method. Lesion length (cm) at 14 days after inoculation (DAI) was measured for each tested plant. QTL analysis was performed using JoinMap 3.0 and MapQTL 4.0. Single marker-trait correlation analysis using SAS 9.0 indicated that 20 markers were significantly, $P<0.05$, associated with lesion length at 14 DAI. The interval mapping showed that these markers mapped four putative resistant QTLs to molecular linkage groups (MLG) A2, D1b, J, and K. Although no major QTLs were detected for conferring partial resistance from PI194639, associations between lesion length and the markers on MLG D1b and J showed potential benefits for soybean improvement programs. It was particularly noted that one QTL on MLG J had the same markers, such as Satt244 and Satt596, which have shown to be involved in the resistance to brown stem rot in soybean. We anticipate that common marker loci conveying the resistance to various diseases can be detected and may be useful for the marker-assisted selection in soybean.

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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

Research began in 2003 to better understand the effectiveness of crop rotation and cover crop management on white mold development in canola. The primary objective of this project is to evaluate *sclerotinia* incidence and severity in canola when grown in a number of crop rotations with and without the presence of a cover crop. The crop rotations involve canola grown continuously for three years as well as after one year and two years of wheat. The canola is grown with and without the presence of a fall-planted winter rye cover crop in the cropping sequences. In addition to an evaluation of sclerotinia incidence and severity, canola yield and the economics of the cropping practices will be determined. The objectives will be accomplished by conducting a three-year field study that was initiated in 2003 at one site and again in 2004 at a second site. The field study involves eight cropping-sequence treatments the first two-years, after which the plots will be divided allowing for sixteen cropping-sequence treatments the third year:

Treatments in 2003 & 2004	**Crop Year**			**Treatments In 2005**
	2003	**2004**	**2005**	
1. W-C-C	W	C -&+ rye[1]	C	1. & 9.
2. W-W-C	W	W -&+ rye	C	2. & 10.
3. Wr-C-C	W + rye	C -&+ rye	C	3. & 11.
4. Wr-W-C	W + rye	W -&+ rye	C	4. & 12.
5. C-C-C	C	C -&+ rye	C	5. & 13.
6. C-W-C	C	W -&+ rye	C	6. & 14.
7. Cr-C-C	C + rye	C -&+ rye	C	7. & 15.
8. Cr-W-C	C + rye	W -&+ rye	C	8. & 16.

[1] The plots will be split following harvest in 2004, with half planted to the rye cover.

The results of this research will provide information on the influence of crop rotation and the use of a rye cover crop on the incidence and severity of sclerotinia in canola. If such practices are shown to reduce the incidence and severity of sclerotinia, and an economic analysis of the practices suggests such practices are cost effective, this research may lead to modifications in agronomic practices by canola growers. The results may not provide information that will be immediately beneficial to canola growers throughout the region. However, by conducting this sort of research our knowledge of sclerotinia management through the use of alternative cropping practices will be enhanced, which may benefit canola producers in the long run.

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Influence of Crop Rotation on Canola Diseases

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Funded Plan of Work: Impact of preceding crops on incidence and severity of disease in canola

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.

To date there has been little risk or incidence of SSR, regardless of rotation or fungicide treatment, in this study. There was no history of canola on this site prior to 2000, and weather conditions were not optimal for SSR infection in 2001 through 2003. Soil samples were collected in the fall of 2003 to establish a sclerotia baseline prior to the next cycle of the rotation.

Blackleg incidence has gradually increased each year. There was very little blackleg detected in 2000, the first year of the study. In 2001, blackleg incidence was up to 8% in canola on canola rotations. In 2002, the third year of the study, blackleg incidence was 37% in canola preceded by two years of canola, 24% in canola on canola, and less than 10% in first year canola or canola preceded by wheat preceded by canola (canola every other year). Although blackleg incidence in canola every other year was slightly higher than in first year canola, it was not significantly different. In 2003, canola once in four years and canola once in three years had similar blackleg incidence, 22%. Blackleg incidence was higher in canola preceded by three consecutive years of canola, 45%, which was similar to canola preceded by canola, and canola preceded by wheat preceded by canola (canola every other year). Blackleg severity did not increase with the occurrence of canola in the rotation and yield was not affected by blackleg incidence in 2002 or 2003. The lack of yield response to higher blackleg incidence is likely due to the blackleg resistance of the canola variety planted, as well as below-normal precipitation and high temperatures during flowering in 2002 and 2003. In fact, overall canola yields were down in 2002 and 2003 compared to 2000 and 2001.

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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*

Sclerotinia sclerotiorum is one of the most important pathogens of field crops in the North Central USA, causing economic losses in beans, soybeans, sunflowers, pea, canola and other crops. Incorporation of resistance to *S. sclerotiorum* into field crops is a desirable management strategy. The identification of, and screening for resistance, two procedures necessary to breed for resistance, has been impeded by the lack of consistently effective and rapid methods to detect and measure resistance. This research project is attempting to genetically transform isolates of *S. sclerotiorum* with the green fluorescent protein (GFP) gene using two transformation methods. The Bio-Rad gene gun is being employed to bombard 3 day old cultures with DNA coated tungsten particles while protoplasts are being transformed with the polyethylene glycol method. A variety of plasmid vectors are being used, each with different promotors: pTEFEGFP (with the *EGFP1* gene); pCT74 (*SGFP* gene); gGFP (*SGFP* gene); pNuc=Em2 (*EGFP* gene). Several other vectors were obtained and may be used if needed. These vectors should allow constitutive expression of GFP. The transformed isolates will be compared for expression of GFP and those with the highest expression will be selected for further study. Selected transformants will be grown on known susceptible and partially resistant genotypes of field crops (dry bean, soybean, sunflower and canola) and GFP will be used to detect and quantify the growth of the pathogen in host tissue. Our hypothesis is that quantifying the fungus biomass in host tissue can be used to measure resistance in young plants. Such methodology could provide an innovative way to detect resistance and advance the breeding of resistant crops. In addition, this technology would allow for new methods of studying the interaction between host and pathogen.

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

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

Yield losses from white mold (caused by *Sclerotinia sclerotiorum* Lib de Bary) in common bean (*Phaseolus vulgaris* L.) vary from 30% to 90%. Only low levels of resistance exist in common bean, and chemical and cultural controls alone are often inadequate. However, high levels of resistance exist in the secondary gene pool. Our goal is to introgress and pyramid high levels of resistance from across *Phaseolus* species to provide a sustainable solution to white mold. The specific objectives are to (i) screen inbred genotypes derived from interspecific populations in the field and greenhouse, (ii) screen known white mold resistant *P. coccineus* and common bean genotypes in the greenhouse, (iii) develop and screen a new group of recombinant inbred and inbred-backcross breeding lines from interspecific populations between susceptible pinto bean and recently identified white mold resistant *P. coccineus* accessions PI 433246 and PI 439534, (iv) determine the inheritance of white mold resistance found in PI 433246 and PI 439534, and (v) pyramid white mold resistance from across *Phaseolus* species. Four hundred thirty-three F_2, inbred-recurrent, and inbred-congruity backcross derived breeding lines from 10 interspecific populations of 'ICA Pijao' with the three species in secondary gene pool (*P. coccineus, P. costaricensis,* and *P. polyanthus*) were evaluated in the field in Idaho and in greenhouse in Colorado in FY2002. Approximately 75 breeding lines resistant (disease scores e3) in both field and greenhouse screenings and an additional 325 interspecific breeding lines were planted in the field at Hazelton, Kimberly, and Rupert, Idaho for white mold evaluation in FY2003. Single-row plots 10 ft long with 5 to 9 replications were used for evaluation. Despite multiple inoculations during flowering, and use of solid-set sprinkler system to maintain humidity no white mold infection of any consequence occurred at any site due to prolonged hot and dry weather. The greenhouse evaluation of these interspecific breeding lines using the straw-test is in progress at Fort Collins, Colorado. Twenty-one previously known white mold resistant *P. coccineus* and common bean accessions along with susceptible cultivar Bill Z were screened using the straw test in greenhouse at Fort Collins, Colorado in FY 2002. Our results were similar to those reported by earlier researchers. Of these two *P. coccineus* accessions, namely PI 433246 and PI 439534 were crossed and backcrossed twice with pinto Othello and UI 320, respectively. Two inter-gene pool single crosses and one double-cross were made to pyramid white mold resistance from across *Phaseolus* species. All interspecific breeding lines will be evaluated in fields in Idaho, Washington, and/or Wisconsin in 2004. Inheritance of white mold resistance in PI 433246 and PI 439534 will be determined in the greenhouse at Fort Collins, Colorado. At least one multiple-parent cross will be made for pyramiding white mold resistance. Resistant genotypes from all experiments will be tested nationally and information shared with bean growers, researchers, and other clientele. Research results will be published in refereed journals.

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Mapping of QTL for Resistance to *Sclerotinia* Stem Rot (*Sclerotinia sclerotiorum*) in Soybean

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Funded plan of work: Characterization of soybean genotypes with partial resistance to Sclerotinia stem rot

Current soybean varieties express only partial resistance to Sclerotinia stem rot, with one of the highest levels shown in the cultivar NK S19-90. Our objectives were 1) to develop elite germplasm with increased resistance to this disease and 2) to identify quantitative trait loci (QTL) for resistance that could be used in marker-assisted breeding. In order to accomplish these objectives we crossed NK S19-90 to plant introduction (PI) 153.282, which had previously been identified for its high resistance and agronomic similarity to NK S19-90. One hundred and seventy four F_4-derived lines from this cross were field tested over two years at three locations: East Lansing and Sandusky, Michigan and Urbana, Illinois. Data were taken on agronomic traits and resistance to sclerotinia stem rot. Resistance was rated on 30 plants per plot and recorded as a disease severity index (DSI) ranging from 0 = no symptoms to 100 = most severe symptoms. The disease occurred in 2002 only in Sandusky and Urbana with a mean DSI of 1.7 and 1.3, respectively, and in 2003 only in East Lansing and Urbana with a mean DSI of 6.3 and 30.7, respectively. Disease ratings from 2002 were disregarded due to low DSI values. For both 2003 environments, genotypic variance for DSI was significant (alpha = 0.05) within each environment and across environments. There was a significant genotype x environment variance (alpha = 0.05). The heritability estimates were 31% in East Lansing, 68% in Urbana, and 35% across both environments. Yield was determined in Urbana, 2002, since it was a test with low disease incidence indicated by mean DSI. The genotypic variance for yield was significant (alpha = 0.05) and the heritability was 76%. Out of the 27 lines that were identified as more resistant than NK S19-90, 15 lines had yield similar to NK S19-90 at an experimentwise alpha of 0.1. Among these 15 lines, there was one line that was more resistant than NK S19-90 across tests. This suggests that we were able to increase resistance to sclerotinia stem rot without significant yield drag.

The entire population is being genotyped using simple sequence repeat (SSR) markers to map resistance QTL. Currently a total of 273 polymorphic SSR markers have been identified and results of the QTL mapping study will be presented.

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Mapping QTL Associated with White Mold Resistance in Common Bean

James D. Kelly and Marcio Ender, Michigan State University, East Lansing MI

Funded Plan of Work: Improving White Mold Resistance by Transforming Dry Bean with the Germin Oxalate Oxidase Gene

White mold, caused by *Sclerotinia sclerotiorum*, is a serious disease of common bean (*Phaseolus vulgaris*) that results in substantial yield loss and reduced seed quality. Resistance to white mold in bean is a quantitative trait, complexly inherited, and highly influenced by the environment, making selection for resistance difficult. The present work was undertaken to identify and map QTL linked to white mold resistance in common bean. This study was conducted in a population of 98 recombinant inbred lines (RIL) derived from the cross between Bunsi and Raven. Bunsi is an indeterminate navy bean, with physiological resistance and a porous plant canopy. Raven is a susceptible indeterminate black bean with upright architecture. The phenotypic data was collected in naturally infected field plots in 2001($F_{4:7}$) and 2002 ($F_{4:8}$) and the 2003 data was used to confirm markers linked to putative QTL. Bulked segregant analysis and AFLP analysis with 256 primer pair combination (EcoRI + ANN and MseI + CNN) were first tested on the parents and resistant and susceptible bulks to detect useful associated markers. Candidate markers associated with QTL controlling resistance to white mold were identified in the RIL population on bean linkage groups B2, B5, B7 and B8. Our data confirmed previously reported QTL on B2 and B7 and the presence of new QTL for white mold resistance on B5 and B8. The potential use of these QTL in marker-assisted selection for resistance to white mold in common bean is discussed.

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Marker-Assisted Backcrossing of Two White Mold Resistant QTL Into Susceptible Pinto Bean: II. Generation Advancement and Marker Assessment

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

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

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 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 expression in both the greenhouse straw test and field, derive from different sources: G122, a large-seeded landrace from India, and NY6020-4, a snap bean breeding line from Cornell University. The QTL from G122 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 three marker-assisted backcrosses. Four populations (Pop-1, Pop-2, Pop-3, Pop-4), consisting of 50, 38, 52, and 33 BC_3F_2 plants have been advanced to $BC_3F_{4:5}$ lines. The BC_3F_4 plants have been assayed for the QTL linked markers. For each population tested, a 1:1 expected segregation ratio for presence and absence of the markers among BC_3F_4 plants was observed. The next and final step will be to compare by regression the marker genotype with disease reaction phenotypes obtained for $BC_3F_{4:5}$ lines in replicated greenhouse and field tests to be conducted in 2004. Comparison of agronomic traits (yield, growth habit, seed quality, maturity) between advanced BC inbred lines and the recurrent pinto parents will enable linkage drag effects to be determined. Together, the regression and agronomic trait analyses will determine whether marker-assisted backcrossing for the B7 and B8 QTL will be useful for improving white mold resistance in susceptible pinto bean.

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Minimizing *Sclerotinia* on Canola, Dry Pea, Sunflower, Chickpea, and Lentil Using Crop Sequence and Biological Control, 2003

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Funded Plan of Work: Management practices, including a biological control agent, and Sclerotinia on canola, dry pea, chickpea, lentil, and sunflower.
The effects of crop sequence, management practices, and biological control on *Sclerotinia sclerotiorum* disease were evaluated in three experiments in 2003.

1) A multi-disciplinary team of scientists is conducting a Crop Sequence Project, which is a multi-phased project to develop guidelines for diversified crop production systems and to provide producers with management flexibility for developing their own cropping systems and managing disease risk. The Crop Sequence Project 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 in 2003. Sclerotinia basal stalk rot was present on sunflower and increased during four evaluations but because of the low number of sunflower plants infected, disease severity could not be statistically related to the crops grown in 2002. During the third and fourth evaluations of sunflower, insect/disease problems, which caused premature wilting of plants, seriously impacted the sunflower-following-sunflower plots. Evaluations will continue in 2004 on chickpea, canola, dry pea, lentil, and sunflower at another crop by crop residue matrix site.

2) The use of *Coniothyrium minitans* (Intercept WG®) to reduce the risk of Sclerotinia disease was evaluated. Treatments included the timing of Intercept WG® applications, tillage or no-till, the use of a non-host crop (spring wheat) for one season (2002), and the use of a sunflower indicator crop to determine the presence of Sclerotinia (2003). Because of the dry conditions and higher than average temperatures in July and August in 2003, low numbers of sunflower plants were infected with Sclerotinia basal stalk rot making it difficult to statistically relate disease levels to treatments.

3) An experiment to evaluate the combination of crop sequence and the application of *Coniothyrium minitans* (Intercept WG®) to reduce the risk of Sclerotinia disease was established. Treatments included the uniform application of sclerotia, the growing of susceptible and resistant crops, and varying the timing of Intercept WG® applications. Plots were evaluated for Sclerotinia, soil water, and surface soil properties in 2003, and will be evaluated again in 2004. Influence of crop sequences and management practices on development of Sclerotinia will be evaluated with an indicator crop, which will be direct seeded over the residue of the previous crops.

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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

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 well known source of resistance to white mold from the Middle American gene pool which could be useful for improving resistance of pinto bean. The objective for the second year of this project was to develop a molecular linkage map 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 linkage map is being used to identify quantitative trait loci (QTL) conditioning resistance to white mold as measured across four field trials conducted in North Dakota and Washington. So far, 102 markers have been mapped in the Aztec/ND88-106-04 population: five genes -- *Ur-3*, *I*, *P*, *C* and *Znd*; four sequence characterized amplified regions (SCARs) -- SW13, SK14, SAP6, and BC409; 75 random amplified polymorphic DNA (RAPDs), and 18 target region amplified polymorphisms (TRAPs). Of the 102 markers: 47 were located across linkage groups B2, B7, B8, B10 and B11 of the core linkage map (UC-Davis); 21 were located across partial linkage groups, and 34 were unlinked. The partial linkage map generated to date has revealed QTL ($P < 0.01$) associated with disease avoidance traits and physiological mechanisms of resistance. Three independent QTL conditioning 14, 13, and 10% of the phenotypic variation for open plant canopy (disease avoidance trait) were identified with stable expression across environments. A single QTL for plant height (disease avoidance trait) explaining 12% of the phenotypic variation was identified. For physiological resistance mechanisms, two independent QTL for stay-green stem trait, explaining 25 and 15% of the variation were identified. A QTL controlling late maturity (26%) was associated (tightly linked or pleiotrophic) with the major QTL for stay-green stem trait. Seven QTL conditioning resistance (lower disease score) to white mold in the field, explaining from 9 to 18% of the phenotypic variation, were expressed in single environments. Only one QTL (11%) was expressed across multiple environments. Additional DNA markers (~100) will be mapped in the Aztec/ND88-106-04 population to ensure identification of the major-effect QTL, and to improve resolution of existing QTL. Pinto bean lines with promising levels of white mold resistance obtained in this study are undergoing advanced stages of testing for potential release.

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Quantitative Trait Loci for Resistance to *Sclerotinia sclerotiorum* in PI391589A

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Funded Plan of Work: Characterization of soybean genotypes with partial resistance to Sclerotinia stem rot.

Sclerotinia stem rot continues to impact soybean yields in many parts of the north central region. The best means to manage this disease is by planting soybean cultivars with high levels of partial resistance to *Sclerotinia sclerotiorum*. Two previous studies identified QTLs in soybean cultivars associated with partial resistance to *S. sclerotiorum*. In addition 68 plant introductions were identified that have higher levels of partial resistance than what is currently available in commercial soybean cultivars. There are several questions that have arisen from these findings. Are the QTL's associated with resistance within the plant introductions all the same or different? And are the QTL's in the plant introductions different from those that have been previously identified in the current soybean cultivars? If there are different QTLs involved, then there is the potential to combine resistance from different sources, possibly leading to cultivars more resistant than any now available. This research was the focus of a larger project led by University of Illinois entitled "Characterization of soybean genotypes with partial resistance to Sclerotinia Stem Rot" funded by the USDA Sclerotinia Initative. The objective of our study is to: Identify the QTLs associated with partial resistance to *S. sclerotiorum* in PI391589A, one of the PI's identified earlier. A population of Kottman (susceptible) x PI391589A (resistant) consisting of 230 $BC_1F_{4:6}$ lines was made. These lines were screened for resistance to Sclerotinia and genotyping with SSR markers is in progress. Mappping Sclerotinia resistance QTLs and development of resistant germplasm to manage this pathogen meet the goals of the Sclerotinia Initiative. . Using molecular techniques greatly expedites discovery of economic solutions for Sclerotinia stem rot, ie: resistant cultivars.

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Sampling and AFLP fingerprinting of white mold isolates from pea and lentil in the Pacific Northwest

Niklaus J. Grunwald, USDA ARS, Prosser, WA, Linda M. Kohn, University of Toronto, Toronto, Canada & Weidong Chen, USDA ARS, Pullman, WA

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

Understanding the population structure of the white mold pathogen is crucial to several aspects of disease management including breeding for resistance and monitoring durability of control practices. The population structure of *Sclerotinia* on cruciferous crops in the US and Canada and on soybean in central Canada is very well elucidated. Canadian field populations of *S. sclerotiorum* on canola and on soybean are part of one population of the pathogen with some local subdivision; this population is mainly clonal. The population structure of *S. sclerotiorum* existing in the U.S. on pea and lentil has not been described, but we would expect to find a similarly clonal population where a few clones dominate a single agricultural field and there exist a large number of clones in different regions of the U.S. The objective of our research is to describe the population structure of *S. sclerotiorum* in pea and lentil growing regions of the US to improve management of the disease. We will use AFLP markers, microsatellite markers and mycelial compatibility groups to describe genotypes and pathogenicity and fungicide resistance assays to describe phenotypes of the pathogen population. To date, a total of 123 isolates have been collected from pea and lentil from WA and ID. Sampling of isolates from other areas including OR, ND and MN is ongoing. Several AFLP primer combinations have been screened on a subset of isolates and AFLP primer combinations EcoRI-AT/MseI-CT and EcoRI-AG/MseI-CC were selected for further studies. These primer combinations show that pea isolates from WA within a single field characterized to date can be clonal, but that many isolates are unique. A total of 16 and 34 loci were analyzed with the primer combinations AG/CC and AT/CT, respectively. Work using microsatellite markers and mycelial compatibility groups to describe genotypes and pathogenicity and fungicide resistance assays to describe phenotypes of the pathogen population is in progress. In the mycelial compatibility test, 26 isolates of 51 were compatible with at least one other isolate.

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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

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. The goal is to obtain F4-derived lines that are homozygous for up to 8 of the identified resistance QTL identified on 8 different linkage groups in soybean. During 2003 we screened over 1,200 F2 plants from the crosses to identify individual F2 plants that had the desired genotypes for resistance at important QTL based on microsatellite (SSR) markers. We grew F2-derived F3 progeny rows in the field during the 2003 season and sampled leaf tissue from each F3 plant in selected F2:3 rows to identify F3 plants with the desired marker genotypes. We used 22 SSR markers that identify QTL for resistance to sclerotinia stem rot on 8 different linkage groups in soybean. We are completing genotype analysis of the F3 plants. Each F3 plant was harvested individually for a total of ca. 1,000 plants. From selected F3 plants with the desired marker genotypes, we will grow F4 progeny rows (F3:4 lines) to obtain F4 plants that are homozygous for the desired marker alleles at more loci. The F3:4 lines will be grown from December 2003 to May 2004 in the greenhouses. Phenotypic analysis of resistance to *S. sclerotiorum* will be conducted on F4-derived lines during the 2004 growing season. Objective 2 is to determine if a novel antifungal synthetic peptide expressed in soybean will confer resistance to *S. sclerotiorum.* To date, 18 primary transformant lines have been established in the greenhouse. T1 seeds were harvested from the lines and progeny analysis was begun in the greenhouse during October 2003. We will identify plants expressing the inserted gene construct by screening with gluphosinate herbicide. During the winter, we will conduct a detached leaf assay and petiole test on the tolerant lines, and wild-type controls including the parental line Thorne to evaluate resistance to *S. sclerotiorum*. The second goal is to improve the use of calcium cyanamide as a control option for *S. sclerotiorum*. Objective 1 is to field-test Ca-cyanamide tolerant transgenic plants. Development of Ca-cyanamide tolerant plants would allow post-planting application for more effective inhibition of apothecial development and ascospore release. A preliminary yield evaluation of the transgenic cah-gene lines was conducted. Objective 2 is to determine the lowest levels of Perlka" (granular Ca-cyanamide) needed to control ascospore release and effectively reduce white mold severity in soybean. A greenhouse experiment is in progress, and a field evaluation during 2004 is planned.

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Sclerotinia sclerotiorum resistance screening and disease management demonstration trials on sunflower, soybean, and chickpea in South Dakota in 2003.

Martin A. Draper, Kay R. Ruden, and Shannon M. Schilling, South Dakota State University, Brookings, SD

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

South Dakota trials were conducted at Brookings, SD on sunflowers and chickpeas and in Groton, SD on soybeans. Additional Sclerotinia trials associated with a North Central soybean trial were also conducted at Brookings, but are not reported as a part of this study. The sunflowers and chickpeas were both planted in mist irrigated nurseries, but the on-farm soybeans demonstration plots received only natural precipitation.

Seven hybrid oilseed sunflower genotypes, submitted by seed companies, were screened for resistance to Sclerotinia head rot. The entries were planted in a randomized complete block with three replications. Ten heads per plot were selected for similar developmental stage and inoculated with a suspension containing about 15,000 ascospores/each in 0.01% Tween-20 and sterile water applied R-5.5. Mist irrigation was initiated several hours following inoculation. In a related study, a confection sunflower hybrid was protected with nine fungicides (benomyl, thiophanate-methyl, vinclozolin, boscalid, azoxystrobin, pyraclostrobin, trifloxystrobin + propiconazole, fluazinam, and prothioconazole) at standard rates and the following day were challenge inoculated with Sclerotinia ascospores, as described above. In all sunflower studies, disease developed, but disease incidence was erratic and no significant differences were detected. In the genotype study, the resistant and susceptible standards were the most resistant and susceptible entries, respectively. The susceptible check had significantly more disease than all other entries, but the resistant check was not significantly better than the commercial submissions. No fungicides reduced disease in the trial in 2003 due to extreme variability in disease incidence. While none of the products in the trial can be completely eliminated from future consideration, based on severity alone, some products appear to show greater promise. In a related trial, 24 genotypes were screened against the basal stalk rot/wilt phase of the disease. Those results are not reported here.

Similar obstacles were encountered in the chickpea trial. Little disease developed following ascospore inoculation in either a fungicide trial (products listed above) or on any of six chickpea varieties, Dwelly, Sanford, Yuma, Amit (B-90), Chico, and Anna.

In the on-farm soybean demonstration, four cultivars were planted three row widths (19, 38, 76 cm) with 312,500 or 625,000 plants/ha, in a factorial design with three replications. No Sclerotinia stem rot (white mold) developed on that site.

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Searching for defense-related gene candidates from soybean that confer partial resistance to *Sclerotinia*

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

We are using cDNA microarrays to aid in the search for genes related to defense against *Sclerotinia* disease in soybean. We have completed two rounds of inoculations using the susceptible cultivar Williams 82 and the resistant plant introduction PI194639. Inoculated and uninoculated resistant and susceptible plants were analyzed at 0, 6, 18, and 48 hours post inoculation. This short time course indicated that the activation of enzymes involved in phytoalexin production was already going strong by 18 hours. Therefore, to identify genes involved in early signally of defenses, we focused more deeply on the 6 hour time point. Because differential gene expression at 6 hour was somewhat weak and less consistent, we increased our number of reps to 3 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. To summarize our current microarray results, we have identified several hundred genes as candidates for marker development and analysis of association with resistance in PI194639. The ANOVA of the 6 hour time point data revealed that 58 genes were significantly (pd0.001), differentially expressed between PI194639 and Williams 82. These changes in expression at 6 hour post inoculation were seen when comparing PI194639 inoculated to not inoculated (26 genes), PI194639 inoculated versus Williams 82 inoculated (27 genes) and PI194639 not inoculated versus Williams 82 not inoculated (22 genes). Due to overlap, the total number of differentially expressed genes at 6 hours post inoculation was 58, which can be roughly broken down into: 27 unknown genes, 8 DNA or RNA binding factors, and 23 miscellaneous genes.

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Searching for DNA markers associated with *Sclerotinia* tolerance in cultivated and wild sunflowers

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

Using the in-house developed target region amplification polymorphism (TRAP) technique, we are aggressively searching for DNA markers associated with *Sclerotinia* tolerance in sunflower. The TRAP technique uses a fixed primer designed against an annotated EST (expressed sequence tag) in combination with a fluorescently labeled arbitrary primer to amplify DNA fragments from the genomic DNA. For the current project, we designed the fix primers from the sunflower ESTs homologous to the identified plant disease resistance gene components such as kinase, leucine rich repeats (LRR), and nucleotide binding sites (BNS). The arbitrary primers were labeled with infrared dyes, and the PCR products were analyzed with a Li-Cor DNA Analysis System. For cultivated sunflower, we used three tolerant and three susceptible lines released by our unit to conduct the preliminary marker-trait association study. The identified markers will be mapped in an F_2 population of 190 plants segregating for *Sclerotinia* tolerance and plant heights. This population was derived from a three-way cross of HA412/SD//Romania *Phomopsis* Resistant B-line. The published sunflower SSR markers will be used to anchor the linkage groups. To screen for markers associated with *Sclerotinia* tolerance in wild sunflowers, we used two perennial species, *H. maximiliani* and *H. nuttallii*, grown at the AAFC Morden Manitoba Research Center. Twenty resistant accessions with a disease score of 0 (no symptom) and 20 susceptible accessions with a disease score of 5 (severe disease) were selected based on two years of evaluation for marker-trait associations. The identified markers will be useful to monitor the introduction of the favorable genes from the wild species into cultivated sunflower via interspecific hybridization.

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Sources of resistance to *Sclerotinia* white mold in lentils

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

Field and greenhouse experiments were conducted in 2003 to identify sources of resistance to Sclerotinia white mold in pea, chickpea and lentil. Twenty four chickpea cultivars, 12 lentils cultivars and 12 chickpea cultivars and advanced breeding lines were evaluated in the filed at two locations Pullman and Prosser, WA. The 12 lentil cultivars were also evaluated in the filed at Corvallis, OR. The weather conditions in the 2003 growing season were extremely dry in the Palouse region with only 2.20 inches of rain during the months of May, June, July and August. Minimum development of white mold was observed in the field plots at the Pullman and Prosser locations. However at the Corvallis location, moderate levels of white mold developed. The cultivars that developed least amount of disease include Athena (9%), Pennel (25%), Richlea (10%), Sovereign (5%) and Crimson (20%). The most susceptible cultivars include Brewer (62%), Pardina (78%), and Merrit (80%). The data have a good correlation with those we observed in 2002. In 2002, we observed that Pennel was among the resistant and Pardina among the susceptible. However, cultivar Merrit was considered as resistant but showed high levels of incident in the 2003 field trial.

In the greenhouse we completed screened part of the core collection of lentil. There are about 280 accessions in the lentil core collection. Two hundred of them were screened in the greenhouse using colonized oat kernels as inoculum. Inoculated plants started to wilt three days after inoculation. The number of plants wilted out of total inoculated plants was recorded at three-day intervals. Most of the lentil accessions screened so far are susceptible to white mold. A few accessions were observed to be resistant. The resistant accessions include ILL 669, ILL 1878 and Precoz. These resistant accessions remained green and no signs of wilting 12 days after inoculaton. In comparison, the susceptible lines like Giza 9 and Redchief were wilted three days after inoculation. The difference is believed to be physiological because signs of infection at the base of the plants was observed three days after inoculation in all inoculated plants. The identified resistant accessions are being used in crosses with susceptible lines in order to study the inheritance pattern of the resistance to white mold in lentil.

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Sunflower Germplasm Development with *Sclerotinia* Head and Stalk Rot Resistance

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Work Plan: Development of Sclerotinia head rot resistant sunflower germplasm.

Several lines of study to develop sunflower germplasm tolerant to Sclerotinia head rot or stalk rot were continued or initiated during 2003. Following successful field tests in 2002, three sunflower inbred lines (RHA 439, RHA 440, HA 441) with high levels of head rot resistance were released in the spring of 2003. USDA breeding material was again evaluated for head rot tolerance at the mist field plots in Carrington, ND. Sclerotinia infection was low with susceptible checks averaging only 36% infected heads and a DSI of 2.7 on a 5-point scale. High winds and hot weather following ascospore inoculation were suspected to be the main factors causing the low disease severity. Modifications for 2004 to improve infection include switching from mist nozzles to micro-sprinkler heads, and installing leaf wetness and relative humidity sensors to determine when the mist duration is insufficient. Future modifications may include use of a data logger coupled with the sensors, programmed to activate the mist system instead of a simple time controller. In studies to evaluate wild sunflower germplasm, eight annual *Helianthus* species were tested for the second year at Carrington, ND. As observed in the first year, the primary response of wild *Helianthus* species to ascospore inoculation was peduncle necrosis rather than head rot. Only in *H. annuus,* with the largest heads, (approaching 3 cm in diameter) were typical head rot symptoms observed, and then only after cut heads were incubated in moist chambers in the laboratory. Additionally, only 35% of the 500+ transplanted wild sunflowers produced flowers in time for inoculation. The consensus after two years of experience is that evaluation of wild *Helianthus* species for *Sclerotinia* resistance would be most efficient after crossing with cultivated sunflower, and testing the F_1 interspecific hybrid, which should have agronomic characteristics approaching that of cultivated sunflower. A second mist irrigation nursery at Fargo was initiated to supplement the Carrington site. Sunflower, canola, and dry bean pathologists from North Dakota State University will use the four-acre site cooperatively. A two-year stalk rot inoculation method study at two locations was completed. Sclerotinia grown on either oats or millet and placed in a row-side furrow one month after planting gave consistent and high levels of stalk rot. Sclerotia, in contrast, produced low levels of disease. Twenty commercial hybrids were evaluated at four locations using the oat/Sclerotinia inoculum. Disease incidence across hybrids ranged from 22 to 56% at the four locations, with the resistant check hybrid averaging 17% infection compared to 55% for the susceptible check hybrid. Mechanizing this inoculation procedure by dispensing the inoculum with a granular chemical applicator mounted on a tractor-drawn cultivator should enable us to efficiently test a large number of entries. With artificial inoculation procedures for both head rot and stalk rot, our program should be able to screen for both diseases in our attempt to incorporate resistance to both Sclerotinia diseases in the same germplasm.

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Validation and Introgression of Resistance from Andean to Middle American Germplasm Using Marker Assisted Selection

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Funded Plan of Work: Validation and Introgression of The Resistance Gene found in Andean Germplasm Into Middle American Germplasm Using Marker Assisted Selection

The long-term goal of this project is to combine genes that contribute to white mold resistance found in the Andean common bean line G 122 with resistance from related species P. coccineus. We are using two recombinant inbred populations (RIL) that we developed from crosses between pinto lines and G 122 to validate the effect of resistance gene(s) found in G 122. We are also seeking markers closely linked to the QTL for resistance in G 122 reported by Miklas et al. (2001). We are currently fine mapping the region around the QTL to identify markers more closely linked to the QTL. The specific objectives of this research are to: 1) validate the effect of resistance genes found in G 122 using a RIL population, 2) identify molecular markers that can be used to saturated the linkage map in the region of the QTL found in G 122, 3) determine if linkage between the QTL in G 122 and T phaseolin can be broken.

Currently, we have completed the initial screening of the two RIL populations for reaction to white mold using the straw test. Because the QTL reported by Miklas et al. was linked to a SCAR marker that identifies T phaseolin (T Phs), we also characterized both populations for presence of the T Phs SCAR marker. We anticipated that the T Phs SCAR marker (linked to the QTL for resistance) would provide a marker to identify resistant RIL lines. Based on the initial evaluation for white mold reaction, we did not find a relationship between white mold score and the T Phs SCAR marker. We initially thought that the lack of association may have been due to environmental effects that prevented a precise evaluation of lines based on the straw test. We are now repeating the straw test on both populations. To date, 26 RIL lines have been re-screened for reaction based on the straw test, and based on this limited number of lines, we again have not found a relationship between phaseolin type and white mold reaction. Among the 26 lines, 14 had S phaseolin with a mean ASI of 6.5, and 12 had T phaseolin with a mean ASI of 6.4. Because these results were contrary to what we expected, we were concerned that the SCAR marker was not effectively evaluating phaseolin type. Consequently, we characterized seed proteins among a subset of the RIL populatin using electrophoresis (Gepts et al., 1992). These results validated that the T Phs SCAR marker correctly identified the Phs allele that conditions T phaseolin in the lines. We are now focusing our efforts on mapping the region around the QTL. To facilitate genome-wide mapping of our white mold populations, we will utilize known markers in the region of the Phs allele and utilize primers for the 100 microsatellite loci that were recently mapped by Blair et al. (2003). These markers will be extremely useful for linking our results to previously published maps.We also anticipate conducting field evaluations on a subset of RIL Population One during summer 2004 to validate the straw test data and utility of markers found in year 1 funding. If we receive year 2 funding, approximately 65 of the lines from RIL Population 1 will be evaluated in a white mold field nursery in both Idaho and North Dakota.

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Validation of a white mold forecasting system for dry beans and canola in North Dakota and Minnesota

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Funded Plan of Work: Validation of a white mold forecasting system for dry beans and canola in North Dakota and Minnesota

A forecasting system that estimates the risk of development of white mold epidemics was evaluated in the 2003 season for use in canola and dry beans. Electronic maps depicting estimated soil moisture content for the upper 4 inches of soil, canola growth stages, and areas with high, moderate, and low risk of disease development were produced twice a week during the months of June and July. Soil moisture content and risk maps were also produced for dry beans. Nylon mats, containing 50 sclerotia, were placed at five fields planted to canola and four fields planted to dry beans in early June. These fields were visited once every week to observe apothecia formation and to record growth stages. A survey that covered 116 canola fields and 90 dry bean fields was conducted at the end of the growing season to estimate disease prevalence and severity in areas within the three risk levels. Marginal conditions for disease development occurred throughout the canola growing areas. Sclerotinia stem rot developed in less than half of the fields identified as being in areas of high and moderate risk. However, epidemics were more severe, incidence within fields, in the high-risk area than in at the moderate risk area. No disease was detected in fields in areas at low risk. White mold epidemics developed more uniformly across the surveyed dry bean growing areas. Three areas were identified as being consistently at high, moderate, or low risk, but no statistical differences in disease prevalence, proportion of fields with Sclerotinia stem rot. However, disease incidence, proportion of infected plants, within fields was significantly greater (P=0.1) in areas considered at high risk than in areas considered at low risk. Factors other than those initially included in the forecasting model may be playing important roles in disease development and will be investigated in upcoming seasons. Some of these factors include canopy development, air temperature, availability of initial inoculum, and fungicide usage.

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White Mold Resistant Dry Bean Lines Selected at Multiple Nursery Sites in the U.S.A. and Using Laboratory / greenhouse Tests.

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Funded Plan of Work: Resistance improvement of bean through multi-site screening and pathogen characterization of *Sclerotinia sclerotiorum*.

In 2003, six sites ranging from states on the west coast to the midwest had sufficient white mold severity in field nurseries to allow ranking of putative partial resistance sources. Great Northern Beryl was consistently ranked as the highest in disease severity. Two lines, G 122 and Cornell 501, ranked as the lowest in disease severity in the field as well as in the greenhouse/laboratory oxalate and straw screening tests. Cornell 601 was in the middle of the field rankings, while exhibiting the lowest mean disease severity in greenhouse/lab tests. AN-37 was also in the lower mean disease severity rankings in both the field and greenhouse/lab tests. The extreme variation in disease reaction found in field nurseries is demonstrated with Cornell 501 which ranged from the lowest disease severity ranking to the second highest, depending on the test site. The variation in disease reaction of specific lines between nursery sites could be due to overall disease severity differences reflecting weather and microclimate differences. However, the local pathogen variation in virulence and in other characteristics from site to site may also contribute to this range of reaction rankings. The pathogen variation will be tested in 2004. The same lines will be tested in field nurseries in 2004 because five sites did not have white mold in 2003. Once all of the sclerotia are collected from all sites, they will be used to study within field and between field isolate variation as well as variation in the screening isolates using phenotyping and genotyping methods.

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