National Sclerotinia Initiative

2002 Research Abstracts

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A Sclerotinia Risk Map for Canola

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Funded Plan of Work: Sclerotinia Control and Management in Canola

A Sclerotinia risk map, adapted from that already used in the prairie provinces of Canada, was initiated in 2001 for canola producers in North Dakota and northwestern Minnesota. The map was continued with modifications in 2002. Each day in 2002 data on maximum and minimum air temperature and rainfall from 57 North Dakota agricultural Weather Network (NDAWN) stations were automatically emailed to project consultants and a GIS map maker. Information submitted at the beginning of the year included soil moisture in the upper 4 inches of soil, estimated soil moisture as a percent of field capacity in the upper 4 feet, GPS locations, elevation, soil type and the 50% canola planting date for each NDAWN site. Upper atomospheric data, provided by Environment Canada, were used to calculate evapotranspiration and to run models for soil moisture in the surface soil and the sub soil. Crop consultants and extension personnel confirmed appearance of apothecia, expected after 10-14 days of saturated surface soil.

Risk maps, produced twice weekly from early June to late July, consisted of three maps, a map showing crop development based on accumulated growing degree days, a map showing upper soil moisture as percent of field capacity and a Sclerotinia risk map. The three maps were posted on the web site of the Northern Canola Growers Association and the NDSU Extension Service.

Risk map accuracy was evaluated from disease survey data of fields near NDAWN sites, together with planting date (to determine period of flowering by using accumulated degree days), crop rotation, cultivar planted and use or non-use of fungicide. The 2001 risk map appeared accurate except for slight underestimation in one location where apothecia appeared when expected but continued to appear longer than expected; this occurred under very heavy small grain canopies where the soil surface remained wet during three weeks without rain. The anomaly was addressed in 2002 by adding a second model for soil moisture under a small grain canopy to the existing model for soil moisture under a canola canopy. In 2002 disease incidence in most surveyed fields corresponded with the forecasted risk in maps when the field was flowering. Risk was generally low and Sclerotinia incidence was low, but in several isolated areas in Minnesota and North Dakota where the risk was high.

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Applicable management strategies for Sclerotinia stem rot of soybean and chickpea in South Dakota -- 2002 studies

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

Projects studying Sclerotinia diseases on three South Dakota crops, sunflower, soybean, and chickpea were conducted during the 2002 growing season. Sclerotinia stem rot or white mold is perceived to be one of the major diseases of soybean in eastern South Dakota and as an emerging problem on chickpeas grown in the central part of the state where high inoculum loads may be present due to a long history of sunflower cropping.

Soybean studies examined variety selection and row spacing to manage Sclerotinia stem rot and reduce losses and inoculum replacement. Chickpea studies were intended to screen varieties for disease response in a South Dakota environment with supplemental mist irrigation and to examine fungicide efficacy against Sclerotinia stem rot.

Two no-till on-farm soybean research and demonstration trials were conducted in an irrigated field in Brookings County and a dryland field in Brown County, South Dakota. Brown County is in the northeastern part of the state while Brookings County is east central. Soybeans are a major crop in each county. In 2001, Brown County led the state in soybean production. In each trial location extreme drought caused late canopy development that coincided with August rainfall in eastern South Dakota.

Mortality due to Sclerotinia at harvest time ranged from about 18-34% at Brookings and about 16-28% at Brown County. In Brookings County significantly less disease and greater yield resulted from wide (76 cm) row spacing. In Brown County, 76 cm rows resulted in significantly less disease and also lower yields, however, yields were extremely low at this site in 2002, less than half of the state yield average for soybean. At Brookings a significant Variety X Row spacing interaction occurred for plant mortality.

Due to the extreme heat and drought, chickpea growth and development was very poor, with no crop canopy development until August. Plots were inoculated twice each with a 10^4 suspension of ascospores and mycelial fragments in ground corn grain. Inoculations were unsuccessful during early flowering. Later in the season growth resumed and an outbreak of Ascochyta blight developed that would have confounded data collection if later Sclerotinia infection had developed. Poor results were also achieved with mist irrigated Sclerotinia studies on soybeans on the Brookings station.

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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

Progress was made in three areas of research. The goal of the first area is to improve the sclerotinia stem rot resistance of elite soybean germplasm. This is being done through the mapping of quantitative trait loci (QTL) for resistance from plant introductions. The mapping is being done in three populations developed from crosses between partially resistant soybean plant introductions and partially resistant or susceptible varieties. These populations are being tested for resistance and with genetic markers. Population 1 was developed from crossing NKS19-90 with PI 153282. Lines from the population were evaluated for resistance during the summer of 2002 in three field locations. There was a modest level of disease at two locations and the lines were rated for disease severity. Lines from population 2 (Merit x PI194639) were grown in the greenhouse during the summer of 2002. Genetic marker testing of both population 1 and 2 has been initiated. Plants in population 3 (Kottman(2) x PI391589A) were grown during the summer of 2002 and are now being increased in Chile this winter.

In the second area of research, we are addressing whether resistant and susceptible soybean lines differ in anatomical and biochemical traits. To test this, an isolate of *S. sclerotiorum* transformed with the green fluorescent protein (GFP) and a second nontransformed isolate were used to challenge soybean plants with the petiole inoculation technique (PIT). Both isolates were equally aggressive and challenged and nonchallenged plants were examined using a fluorescence stereomicroscope. The transformed isolate fluoresced green but so did soybean tissue. Petioles of the partially resistant variety NKS19-90 and the susceptible variety M0301 were inoculated with *S. sclerotiorum* and examined at various times post inoculation to study mycelial development. Improvements have been made to the techniques to obtain good staining of the mycelium for viewing and for quantifying observations with light microscopy. No differences have been observed between cultivars in the first 48 hours after inoculation, but this work is continuing and observations at additional times are being made.

In the third area of research, methods used in the PIT were evaluated to determine if this method can be improved. Alternative sources of culture medium were evaluated that promote greater synthesis of oxalic acid and endopolygalacturonase, both accepted as pathogenicity factors produced by *S. sclerotiorum*. Rate of lesion expansion, total lesion length, rate of plant death, plant survival and pathogen reproduction were not affected by composition of media used to produce inoculum of *S. sclerotiorum*.

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Developing Sclerotinia Head Rot Resistant Sunflower Germplasm

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Early, medium and advanced generation oilseed sunflower breeding lines were evaluated for Sclerotinia head rot resistance under artificial inoculation using an automated mist irrigation system at Carrington, North Dakota, approximately 180 miles northwest of Fargo. Sources of resistance for the advanced generation lines were derived from USDA Plant Introductions, PI 548998, PI 497250, and Ames-3300. Medium generation lines were derived from European hybrids, Inedi, RO-12-13, and Dobritz demonstrated to have head rot resistance. The early generation materials consisted of two public lines from the French research agency INTA, 'SD' and 'PSC8', crossed with USDA high-oleic material. A severe August thunderstorm with winds of 90 k/h lodged a third of the rows prior to inoculation, but there were enough plants left to continue the evaluation of the cultivated sunflower germplasm. Resistant plants from each group will be advanced to the next generation, which will be evaluated for resistance to both Sclerotinia head rot and basal stalk rot. Sclerotinia head rot resistance was also evaluated in released interspecific hybrids, whose origins included 16 *Helianthus* species. Lastly, an inoculation method study was conducted on wild *Helianthus* using two annual (*H. debilis* and *H. praecox*) and two perennial species (*H. tuberosus* and *H. paucilflorus*). Deer preferentially ate all of the *H. tuberosus* flowers, leaving only three *Helianthus* species for testing. No classical head rot symptoms developed on any of the wild *Helianthus* species, presumably because of the small head. Future evaluations of resistance in wild *Helianthus* may need to be done on F_1 crosses with cultivated *H. annuus.* The loss of plant material due to weather and animal problems highlights the precarious nature of relying upon one field location for evaluation.

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Eco-Tillage, Biopesticide and Resistance Management of White Mold in Dry Bean 12/16/02 Progress Report -- Year 1 of 2

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[Research Assistants: Barry Ogg, Dave Gent & Kris Otto]

This project during 2002-2003 will investigate 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 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 both cultivars by 10% and seed weight by 2%. Planting-time ripping with less frequent irrigation increased yield by nearly 10% for cultivar 'Montrose', but decreased yield by 40% for cultivar 'Chase' due to its susceptibility to Fusarium Wilt. White mold disease did not develop in the disease nursery due to the drought and high temperature conditions which persisted throughout the experiment.

During 2003, a set of laboratory and greenhouse experiments will systematically evaluate the potential usefulness of conventional and experimental fungicides and biopesticides (including systemic acquired resistance inducers) applied to foliage and blossoms of a susceptible cultivar 'Montrose' before inoculation with the white mold pathogen. Data will include incidence and rate of leaf and blossom colonization. In addition, the field experiment will be repeated during 2003.

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Engineering Resistance to White Mold in Dry Beans

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Funded Plan of Work: Combining conventional and contemporary approaches to develop white mold resistance in dry bean (*Phaseolus vulgaris*).

During infection pathogenic fungi like *Sclerotinia* produce copious amounts of oxalic acid that plays a primary role in pathogenicity and ability of the pathogen to infect a wide range of host plants such as dry bean. Theoretically, if the oxalic acid produced by the fungus was degraded in the plant tissue, fungal infection could be prevented or limited. The Germin oxalate oxidase (G-OXO) from wheat that degrades oxalic acid to water and hydrogen peroxide has been cloned and has been used successfully to control white mold in transgenic soybean. We are engineering resistance to infection by *S. sclerotiorum* through the transformation of the Germin gene (*gf-2.8*) into the dry bean genome. We obtained the *Agrobacterium* TDNA plasmid pRD400/35S-*gf-2.8* with kanamycin resistance (nos-*nptII*-nos) from Dr. Daina Simmonds, Agriculture and Agri-Food Canada, who used the same vector to successfully transform soybean. In this plasmid the Germin gene (*gf-2.8*) is driven by an enhanced CaMV 35S promoter and also contains the 35S polyA terminator.

In order to avoid potential problems of detecting transformants with *nptII* resistance and not to introduce antibiotic resistance genes into dry bean, we redesigned the constructs as follows: a recombinant DNA construct was made by removing the antibiotic resistance gene within the T-DNA borders of the pRD400/35S-*gf-2.8* plasmid and replaced it with the herbicide-resistance reporter gene, *bar*. Only the T-DNA portion of the plasmid would then be used to transform *P. vulgaris* seedlings. Instead of using traditional transformation methods, for example particle bombardment, to integrate the T-DNA into the host plant's genome, a novel electro-transformation procedure will be used. Since transformation efficiency is low, the *bar* gene, which conditions resistance to the herbicide glufosinate, will provide an efficient screen to detect putative transformants by spraying with Liberty ®. Screening for tolerance to white mold will be conducted in the greenhouse using either the oxalate test, where bean seedlings are evaluated for wilting response in a solution of oxalic acid, or using the straw test where plants are inoculated with a mycelium plug (straw) of *S. sclerotiorum*. Dry beans engineered to express the G-OXO gene may offer a unique opportunity to control the oxalic acid generated by the pathogen during the process of infection and colonization and result in increased levels of resistance to white mold.

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Enhancing sclerotinia resistance using modern and traditional methods

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Funded Plan of Work: Enhancing sclerotinia resistance using modern and traditional methods

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. The QTL come from different sources and represent 8 QTL on 7 different linkage groups. Objective 2 is to determine if a novel antifungal synthetic peptide expressed in soybean will confer 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. 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.

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 7 different linkage groups in soybean. For the transgenic work, expression of the antifungal peptide was accomplished in 2002 and testing can begin in 2003. Homozygous lines with the *Cah* gene were grown in the field in 2002 for a seed increase and preliminary testing.

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Epidemiology of 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

Work was conducted in 2002 to understand the epidemiology of the Sclerotinia infections to wild sunflower heads and stems and to establish methodology for assessing germplasm accessions of wild sunflower species under field conditions. Ninety-two accessions of wild sunflower species were evaluated. Artificial inoculation of the plants was conducted using ascospores, fungal mycelia, ground infected millet seed, and a non-inoculated control. Plants were covered for 14 days after artificial inoculation with light brown paper bags, sunflower pollination bags, thin plastic bags, and uncovered control plots. These processes of artificial inoculation and head covering was 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 a high humidity and enhance the infection and disease development processes.

The preliminary results for this study indicate that very little infection appears to have occurred on the wild sunflower heads, but the stems were infected and showed typical Sclerotinia symptoms with sclerotia bodies inside the stems. The infected ground millet inoculum resulted in the highest level of stem infection followed by fungal mycelia and ascospores. The paper bags covering of heads and stems resulted in the highest infection levels followed by sunflower pollination bags. Plastic bags were not very effective as covers.

The combination of infected ground millet for artificial inoculation and the paper bag covers after inoculation resulted in 88% infected wild sunflower accessions. A few accessions remained healthy under the various artificial inoculation methods and covering materials, and are suspected to have genetic resistance to Sclerotinia. These results will be confirmed in a repeated trial in 2003.

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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

Uniform cultivar trials were initiated in 2001 with funding from a USDA-ARS grant to the University of Minnesota. In 2001, 17 current production cultivars were evaluated at the NDSU Carrington Research Extension Center and nine at an on-farm site near Red Lake Falls, MN. In 2002, 20 cultivars were screened at both sites. The plots were inoculated with commercially-available ascospores and misted periodically during flowering to keep the plants wet and favor infection. Sclerotinia incidence in 2001 ranged from 15 to 43% in Carrington and 13 to 73% at Red Lake Falls. In 2002, plots at Red Lake Falls were lost due to a combination of excessive rainfall and hail. In Carrington, infection was disappointingly low, ranging from 4 to 22%. Despite the misting system, hot weather between inoculation and petal fall several days later may have reduced the survival of ascospores. The initial findings show that cultivar differences do exist. Relative rankings for disease incidence are generally in agreement, however, exceptions were observed. With the experience gained in inoculating and misting, an increase in our understanding of cultivar differences is anticipated as this research continues.

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Evaluation of Lentil Cultivars for Resistance to White Mold

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Funded Plan of Work: Sources of resistance to white mold in the pea and lentil core collections

Greenhouse and field experiments were conducted in 2002 to evaluate cultivars of lentil for resistance to Sclerotinia white mold. Sixteen entries (15 cultivars and one advanced breeding line) were evaluated at the Spillman Farm of Washington State University in Pullman, WA. The treatments were arranged in a randomized complete block design with four replications. Each plot was 8 ft square with a 4-ft alley between plots. Entries were double planted in two directions to create a microenvironment favoring development of white mold. The plots were inoculated twice. First inoculation was on 12 June using cold-treated sclerotia recovered from dry pea screenings. The pre-treatment consisted of placing sclerotia inoculum in a 4°C cold room for nine weeks. Four hundred fifty milliliter of the sclerotia was hand spread over each plot. A second inoculation was carried out using colonized oat kernels on 1 July. Two liters of 2-wk old colonized autoclaved oat kernels were thoroughly mixed with greenhouse soil, and the mixture was evenly spread over the plot area (each plot received the same amount of inoculum equivalent to 25 ml colonized oat kernels). Disease severity ratings were taken on 9 July and again on 25 July. Because of the dry summer, white mold disease severity was generally low. Nevertheless, differences among the 16 test entries in response to white mold were observed. Cultivars Athena, Mason, Palouse and Pardina were clearly among the most susceptible cultivars. Seven of the tested entries (6 cultivars and one breeding line) showed very little disease and appeared to be relatively resistant to white mold. None of the test entries were immune to white mold.

The 16 lentil entries plus four winter lentil lines were evaluated for relative susceptibility to white mold in the greenhouse. Two plants were grown in each pot and three to six replications were used for each entry. Initial trials included two inoculation techniques: the petiole inoculation developed for soybean at University of Wisconsin and colonized oat kernels. The petiole technique worked very well for the lentil lines that branch early in growth habit, and the disease progress can be easily monitored. However, some lentil lines do not have lateral branches. Colonized oat kernels were used to compare all lentil entries in the greenhouse trials. After comparing different levels of inoculum, two colonized oat kernels per plant were chosen as a standard method. Disease pressure was higher in greenhouse than that we observed in the field in 2002. Cultivars Merritt and Pennel were more resistant to white mold than other lentil cultivars. Among the four advanced breeding lines of winter lentils, LC9976079 was the most susceptible.

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Genetic Analysis of White Mold Resistance Using Microarrays

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Funded Plan: Genetic Analysis of White Mold Resistance Using Microarrays

Sclerotinia sclerotiorum is an important pathogen of soybean in the north-central region of the United States producing the disease known as either Sclerotinia Stem Rot or White Mold. Partial resistance to this pathogen has been reported; however, understanding of the molecular basis of the resistance is limited. The recently developed cDNA microarray technology provides a promising tool to aid our search for genes involved in resistance to this disease. The power of this tool lies in its ability to measure the expression of tens of thousands of genes simultaneously at any specific time point. We will use cDNA microarrays (developed in the lab of Dr. Lila Vodkin at the University of Illinois) representing at least 18,000 different genes from soybean to detail the genetic responses of soybean to this pathogen and to search for specific genes governing soybean resistance. We have completed one round of inoculations using the susceptible cultivar Williams 82 and the resistant plant introduction PI194639. We inoculated the freshly cut end of stems of 4-week old plants with agar plugs containing a fresh culture of *S. sclerotinia* or with sterile agar control plugs. The top 1.5 inches of inoculated or mock inoculate stem was collected at 0, 1, 3, 6, 18, 30, and 48 hours post inoculation and immediately frozen in liquid nitrogen and stored at □80°C. Total RNA was isolated from these tissues by Trizol extraction and will be labelled with fluorescent dUTP to determine gene expression profiles using the soybean microarrays. Genes that show strong correlation with resistance will be converted into molecular markers to determine if they are associated with known QTLs. For mapping purposes, one hundred and fifty F4 plants of a resistant x susceptible cross were grown in the greenhouse during the summer of 2002 for seed increase. These recombinant inbred lines were then planted in the greenhouse in the winter 2002 for scoring their resistance/susceptible phenotypes using the cut-stem inoculation method.

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Identify and Introgress Molecular Markers for White Mold Resistance in Dry Bean

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Funded Plan of Work: Inheritance of field resistance to white mold in an ND88-106-04 (navy) x Aztec (pinto) dry bean mapping population

Pinto bean (*Phaseolus vulgaris* L.) is extremely susceptible to white mold disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, and breeding resistance into them is difficult, due in part to the paucity of resistance sources in a related Middle American background. 'Bunsi' navy bean, a well known source of resistance to white mold from the Middle American gene pool, could be useful for improving resistance of pinto bean. The objective of this project was to determine the inheritance of Bunsi-derived resistance in a pinto bean background. Field research was conducted in North Dakota and Washington in 2001 and 2002 to characterize white mold reaction of an $F_{5:8}$ population of 85 recombinant inbred lines (RIL) from the cross 'Aztec'/ND88-106-04. Aztec pinto bean is susceptible to white mold and ND88-106-04 is a navy bean with resistance to white mold derived from Bunsi. The lines were rated for disease severity (scored from 1 = no disease to 9 = completely susceptible) and yield. Traits associated with disease avoidance such as canopy porosity, plant height, lodging, and maturity were also measured. Average disease severity of Aztec and ND88-106-04 across environments was 6.9 and 2.5, respectively, compared to 4.6 for Bunsi and 7.6 for the susceptible pinto cultivar Montrose. Moderately high heritability (H_{ns} = 59%) and normal distribution of disease score among the RILs were observed, indicating that resistance was influenced by environment and likely conditioned by more than one gene. Disease avoidance contributed to the resistance expressed in the field. For the North Dakota environments, decreased disease severity was correlated ($P< 0.01$) with taller plant canopies (-22%), later maturity (-25%), and reduced lodging (34%). For the Washington environments decreased disease severity contributed to increased yield (-19%) and seed weight (-29%), and was similarly influenced by increased canopy height (-24%), later maturity (-43%), and reduced lodging (29%). Physiological resistance as measured by the greenhouse straw test was not expressed in Bunsi or ND88-106-04, therefore straw test scores for the RILs did not correlate with field reaction. Cultivars with stems that remain green at harvest maturity tend to express greater resistance to white mold, perhaps because the stem tissue is still physiologically active. The green-stem trait, present in Bunsi and ND88-106-04, was expressed among the RILs and was correlated with less disease severity (-30%). Molecular marker analysis of the disease reaction data collected for this mapping population should enable identification of quantitative trait loci (QTL) that specifically condition disease avoidance or physiological resistance, and lead to marker-assisted selection of the QTL with consistent expression across environments.

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Influence of Crop Rotation on Canola Diseases

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Funded Plan of Work: Sclerotinia Control and Management 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. Seven rotations will be 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 will be treated with fungicide to prevent Sclerotinia stem rot (SSR). Plots will be 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 and 2002.

Blackleg incidence has gradually increased each year. There was no 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. Blackleg severity did not increase with the occurrence of canola in the rotation and yield was not affected by blackleg incidence.

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Interspecific Hybridization, and White Mold Screening of Interspecific and Known Resistant Common and Runner Bean Genotypes

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

Four hundred thirty three recombinant inbred, recurrent inbred-backcross, and congruity inbred-backcross genotypes from 10 interspecific populations between common bean cultivar ICA Pijao and the three species in secondary gene pool, namely, *Phaseolus coccineus*, *P. costaricensis*, and *P. polyanthus* along with susceptible (e.g., Bill Z, UI 114, and UI 537) and resistant (e.g., Ex-Rico 23, G 122, I 9365-25, 92 BG-7, and MO 162) checks were screened under heavy white mold pressure in the field at Hazelton, Idaho and in greenhouse (using the straw test) at Fort Collins, Colorado. A single-row plot 10 ft long without replication, and bordered on either side with a susceptible cultivar was used for field evaluation. An average of 10 plants/genotype were screened in greenhouse. Highly white mold resistant (receiving disease scores of 1 to 3) and susceptible (with disease scores 7 to 9) interspecific genotypes in both field and greenhouse tests were identified in all interspecific populations. Genotypes derived from crosses with *P. costaricensis* (S 33720) seem to be slightly superior for white mold resistance than those derived from crosses with *P. coccineus* (G 35171 and G 35172) and *P. polyanthu*s (G 35877). Lines derived from two or more recurrent inbred-backcrosses did not seem to offer any advantage over lines derived from single crosses and the first backcrosses. Similarly, genotypes from congruity inbred-backcrosses were not superior to recurrent inbred-backcrosses. Sixty to seventy highly resistant interspecific genotypes will be again screened in replicated trials in 2003-2004.

In a separate greenhouse experiment at Fort Collins (CO), 13 white mold resistant *P. coccineus* and eight common bean accessions were screened using the straw test. The mean disease score for *P. coccineus* ranged from 1.9 to 4.4, and for common bean from 3.0 to 3.9. These will be screened in greenhouse using the oxalate test in 2003-2004.

We also initiated new interspecific crosses between two white mold resistant *P. coccineus* accessions, namely PI 433246 and PI 439534, and susceptible pinto bean cultivars Othello and UI 320. PI 433246 is a highly photoperiod sensitive (82 d to flower in a 12-hr light) with indeterminate climbing Type IV growth habit from Guatemala. PI 433246 has scarlet color flowers and a mixture of large seeds of brown, purple, and red colors. PI 439534 is also Type IV with scarlet flowers, but it is photoperiod insensitive (38 d to flower). PI 439534 is from Netherlands and it has large pink spotted seeds. Hopefully, one or two more *P. coccineus* accessions will be used in similar interspecific hybridization in the future. We intend to produce at least 100 F_1 seeds for each single cross, and 250 seeds for each subsequent backcross. Resulting progenies will be allowed to inbreed for, at least, two generations before screening for white mold resistance.

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Management Practices and Sclerotinia on Canola, Dry Pea, Chickpea, Lentil, and Sunflower, 2002

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Funded Plan of Work: Management practices and Sclerotinia on canola, dry pea, lentil, and sunflower.

A multi-disciplinary team of scientists is conducting a Crop Sequence Project, which is a multi-phased project to develop guidelines for long-term diversified crop production systems and to provide producers with management flexibility for developing their own cropping systems and managing disease risk. The effect of management practices, crop sequence and biological control, on *Sclerotinia sclerotiorum* disease was evaluated in 2002. 1) Phase II of the Crop Sequence Project includes a crop by crop residue matrix (grown in 2000) to evaluate the impact of previous crops (safflower, canola, crambe, dry pea, dry bean, flax, soybean, sunflower, spring wheat, and barley) and crop residue on Sclerotinia diseases. A uniform spring wheat crop was seeded over the matrix in 2001 and a uniform sunflower crop was seeded in 2002 to evaluate Sclerotinia disease. The number of sunflower plants infected with Sclerotinia basal stalk rot in 2002 was related to the crops grown in 2000. The highest level of Sclerotinia basal stalk rot was associated with plots where crambe was grown. 2) Phase III of the Crop Sequence Project, similar in design to Phase II, was started in 2002. Ten crops (buckwheat, chickpea, corn, lentils, proso millet, grain sorghum, canola, dry pea, sunflower, and wheat) were seeded in strips. Probably, because of the dry conditions in 2002, no Sclerotinia was detected on buckwheat, chickpea, canola, dry pea, or lentil. Sclerotinia basal stalk rot was present on sunflower. The same crops will be evaluated in 2003. 3) The efficiency of *Coniothyrium minitans* (Intercept WG®) in reducing the risk to Sclerotinia disease was evaluated. Treatments included tillage, use of a non-host crop (spring wheat) for one season, followed with a susceptible indicator crop to determine the presence of Sclerotinia disease in 2002. Sclerotinia basal stalk rot tended to be less with the *C. minitans* treatment compared to no treatment, and with the no-till treatment compared to the other tillage treatments.

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Marker-Assisted Backcrossing of Two White Mold Resistant QTL into Susceptible Pinto Bean: I. Early generation assessment.

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

Research was conducted in 2002 to assess the effectiveness of marker-assisted selection for transferring white mold resistance conditioned by quantitative trait loci (QTL) into the susceptible pinto bean market class. The 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. Three marker-assisted backcrosses (BC) were conducted to transfer the QTL from the donor parents to the recurrent pinto bean cultivars 'Winchester' (B7 QTL) and 'Maverick' (B8 QTL). Three populations Pop-1, Pop-2, Pop-3), each consisting of about 45 BC_3F_2 plants, were evaluated for reaction to white mold in the straw test and assayed for presence of the QTL-linked SCAR markers. The marker linked with the B7 QTL explained 15% ($P = 0.02$) of the phenotypic variation for disease resistance among F_2 individuals segregating in Pop-1 as measured by lesion length (mm), and explained 12% ($P = 0.03$) of the variation as measured by disease score (rated from 1 to 9) in Pop-2. The B8 QTL-linked marker explained 19% ($P = 0.002$) of the phenotypic variation for disease score in Pop-3. Further replicated testing in the greenhouse and field of later generation inbred lines derived from these backcrosses are needed to verify the promising results obtained in these early BC_3F_2 generations. Adverse linkage drag effects were noted for the B7 QTL, indicating its use may be restricted to more closely related large-seeded common bean market classes like cranberry and kidney bean.

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Mechanisms of resistance to white mold in Phaseolus coccineus

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Funded Plan of Work: Transfer of total *Sclerotinia* resistance from *Phaseolus coccineus* to *P. vulgaris*

The biochemical basis of white mold resistance was investigated using resistant PI 255956 (25) and PI 535278 (53) and susceptible (Woven Pole) *P. coccineus* lines. Specifically, we tested the contribution of oxalate, an important virulence factor, to white mold decay. *Sclerotinia sclerotiorum* produced more oxalic acid on the susceptible cultivar Woven Pole than the resistant lines 25 and 53. More importantly, these resistant and susceptible lines differed in oxalate sensitivity. A positive correlation between oxalate sensitivity and white mold susceptibility was suggested previously [Kolkman and Kelly (2000) Crop Sci. 40: 281-285]. We reproduced results of Kolkman and Kelly (2000) in that 'Othello' reacted most sensitively to oxalate, whereas 'Huron' the most tolerant cultivar tested, and 'Newport' intermediate. Woven Pole was more sensitive to oxalate than 25 ($P < 0.001$) and 53 ($P < 0.05$), suggesting that differences in white mold susceptibility are at least partially explained by variation in oxalate sensitivity.

Because sensitivity to oxalate expresses itself as a wilting phenotype, we became interested in testing whether stomatal complexes are targets of oxalate action. Stomata regulate both the influx of CO_2 to drive photosynthesis and plant water loss through transpiration. Oxalate (100 ¼M) induced stomatal opening in epidermal peels of *Vicia faba*, suggesting a possible link between stomatal opening and disease susceptibility. Moreover, infection of *V. faba* with a GFP-tagged *S. sclerotiorum* strain resulted in stomatal opening as early as 16 hours post inoculation. Besides promoting desiccation stress, guard cells served as entry points for fungal invasion. Hyphae of *S. sclerotiorum* frequently surrounded and penetrated stomatal complexes. Preliminary results indicate that an oxalate-deficient fungal strain is compromised in its ability to induce stomatal opening. Both stomatal aperture is reduced and stomatal opening is delayed after inoculation with this fungal mutant.

We are interested in determining whether the stomatal response oxalate and fungal infection differs between resistant and susceptible *P. coccineus* lines. The relationship between reactive oxygen species, oxalate, and stomatal opening will be investigated.

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Planting System, Fungicide, and Application Timing for Control of White Mold in Field Peas

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Funded Plan of Work: Development of Sclerotinia Management Programs in Field Pea

Two research trials were established in 2002. The goals of the trials were to identify management strategies (planting system, fungicide, and application timing) that will reduce yield loss from white mold infection in field pea. Sites were selected in east central and northeast North Dakota at the Carrington and Langdon Research Extension Centers, respectively. The Carrington location was a conventional-tilled, loam soil with a pH of 7.2 and 2.4% organic matter. Previous crop history was canola that had been infected with sclerotinia. The Langdon site has history of notill system since 1982 with long-term cropping emphasis small grain. The loam soil had a pH of 6.5 and 6.3 % O.M. Previous crop was barley. A core group of fungicides and timings evaluated at both locations included Ronilan at 12 oz/A, Topsin at 16 oz/A, and Quadris at 9.2 fl oz/A at 10 and 40 % bloom stage at both locations. Headline at 8 fl oz/A, and AMS 21619 at 6 fl oz/A and 100% bloom stage were also evaluated at the Carrington location. Conventional and notill planting system were evaluated at Langdon. Environmental conditions affected the disease levels in eastern North Dakota in 2002. Carrington precipitation was 57% of normal early in the growing season. The Langdon site received 6.4 inches precipitation in June but only 0.9 inches in July compared to 30 year normal of 2.9 inches. Only trace levels of sclerotinia developed. Most parameters were not different at either site. The Langdon site had yield interaction among fungicide, tillage, and timing.

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Potential management strategies for Sclerotinia head rot of sunflower from 2002 South Dakota studies

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

Projects studying Sclerotinia diseases on three South Dakota crops, sunflower, soybean, and chickpea were conducted during the 2002 growing season. Sunflower studies examined the use of adjuvants in the Sclerotinia head rot inoculation process, Fungicide efficacy in head rot suppression, and screening for the spectrum of resistance available in commercial sunflower hybrids grown in SD.

Sunflower head rot inoculations were very successful in 2002. Since all hybrids are susceptible, efficiency of the methods used can be measured by the overall incidence of infection. About 85% disease incidence was attained with methods used. The temperatures during sunflower flowering were considerably cooler than a few weeks earlier n the summer. A favorable environment was maintained by mist irrigating for twenty minutes out of every hour over a two-week time period beginning when the first buds opened and through final pollen shed. Heads were inoculated with a 10^4 suspension of ascospores in spring water with 0.01% Tween-20. All heads were inoculated on two dates (August 8 and 14).

Among the adjuvants tested in the method of sunflower head inoculation study, all adjuvants showed significantly more infection that the untreated and spring water checks, but were not unlike one another. Tween 20, Latron CS-7, Agridex crop oil concentrate, and Induce non-ionic surfactant were all equally effective in improving infection success.

Several fungicidal and related treatments were tested in this trial. The treatments were applied directly to the face of the head and challenge inoculated with Sclerotinia ascospores two days later. However, only benomyl significantly reduced incidence, severity, and field severity of Sclerotinia head rot as measured by a reduction in disease relative to the untreated. Tebuconazole, azoxystrobin, iprodione, calcium sulfate, and sulfur were not effective in reducing Sclerotinia head rot.

Nine hybrids were submitted from seed companies for Sclerotinia head rot screening. A resistant and susceptible standard were selected to provide a semblance of uniformity between testing sites at Brookings, SD and Carrington, ND where some entries are duplicated. Resistant standards always had the lowest ratings in the study. None of the nine entries exhibited a significantly lower rating than the resistant standard, but several entries did respond to inoculation with a significantly higher rating (more disease) than the susceptible control.

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Progress in genetic analysis of white mold resistance in Phaseolus coccineus

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Funded Plan of Work: Transfer of total *Sclerotinia* resistance from *Phaseolus coccineus* to *P. vulgaris*

Building on our previous work to screen all available *Phaseolus coccineus* accessions from the USDA NPGS plant introduction collection using the straw test, we identified several resistant and several susceptible accessions for use in genetic studies. Two resistant accessions, PI 255956 (25) and PI 535278 (53) and two susceptible accessions, 'Woven Pole' (WP) and PI 153209 (15) were selected for inheritance studies and marker assisted selection. Two susceptible *P. vulgaris* parents ('OR 91G' and 5-593) were also crossed to the resistant *P. coccineus* accessions. In general, 25 > 53 >WP = 15 > OR 91G = 5-593 for straw test reaction.

The 25 parent could not be used successfully as a female with any cross combination and was only successful as a male in crosses to WP and 'OR 91G'. F_1 and F_2 seeds were obtained from these crosses. The 53 parent was successfully crossed to the two susceptible *P. coccineus* accessions and two *P. vulgaris* lines. Dwarf lethals were produced in the F_1 from crosses between OR91G X 53. The cross 5-593 X 53 produced stunted and sterileF_1 plants in the greenhouse, but growth in the field was normal. Inheritance of resistance differed between the two sources of resistance, with 53 X 15 F_1 susceptible whereas WP X 25 segregated in the F_1 in a 3:2 R:S ratio. From 53 X 15, 73 F_2 individuals were tested for resistance, with three individuals showing high levels of resistance after two inoculations. Data fit either a two or three gene model ($X^2_{15:1}$ = 0.57, Prob. = 0.44; $X^2_{63:1}$ = 3.07, Prob. = 0.07).

From a screen of 500 RAPD primers, 370 primers possessed one or more polymorphic bands between the two parents. Polymorphic primers were screened against a panel of 3 very susceptible offspring and 3 resistant offspring plus parents. Seven primer bands ($OPA18_{1300}$, $OPB3_{600}$, $OPC13_{1600}$, $OPD10_{1000}$, $OPS13_{500}$, $OPT4_{1100}$, and $OPT5_{1650}$) exhibited polymorphism associated with resistance.

The WP X 25 F_2 segregated 68:11 S:R, from resistant F_1s, contradicting apparent dominance observed in the F_1. Resistance appears to be controlled by more than one gene. Screening for RAPD markers associated with resistance is underway, with the identification of one band ($OPQ19_{1400}$) to date. The marker is in trans to resistance.

An additional six PI accessions have been identified with resistance levels comparable or better than 25. These are currently being used in crosses to *P. vulgaris* to determine if these have better cross compatibility than 25.

Because of the apparent complexity of inheritance, QTL mapping may be required to understand the genetic architecture of white mold resistance, and to identify markers associated with all factors for resistance in *P. coccineus*.

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

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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 two aspects of disease management: breeding for resistance and monitoring durability of control practices, such as effectiveness of chemical treatments or new crop varieties. The population structure of *Sclerotinia* on cruciferous crops in the US and Canada and on soybean in central Canada is very well elucidated, based on both phylogenetic analysis of single nucleotide polymorphism (SNPs) that shows the historical relationships of genotypes, and identification of genotypes (genetic individuals) by three genetically unlinked types of markers, mycelial compatibility grouping, DNA fingerprinting (RFLPs), and microsatellites. 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 exists 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 124 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-AC/MseI-CT and EcoRI-AC/MseI-CT were selected for further studies. These primer combinations show that pea and lentil isolates from WA characterized to date are clonal confirming expectations from previous work in Canada. 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. RFLP fingerprinting using probe pLK44.20 will be conducted on a subset of isolates to provide for cross-referencing of isolates to the global database in Dr. Kohn's laboratory.

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Screening Canola Germplasm for Sclerotinia Resistance and Molecular Marker Polymorphisms

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Funded Plan of Work: Genetics of Sclerotinia resistance in Canola

The goal of our project is to identify quantitative trait loci (QTL) for Sclerotinia resistance in Brassica napus, verify the effects of QTL alleles in different genetic backgrounds, and apply this information to canola breeding. The preliminary approach we are using is to map quantitative trait loci for resistance segregating in a population of recombinant inbred (RI) lines derived from cross of two Chinese lines: Ning RS-1 (resistant) and H5200 (susceptible). We have compared the two parents for molecular marker polymorphisms using 270 SSR and RFLP markers. In total, about 160 markers show polymorphism.

In order to establish disease-screening methods and to determine the reaction of the parents of the mapping population, we screened nine Brassica accessions for disease reaction to Sclerotinia (one Canadian spring cultivar, one European winter cultivar, five Chinese winter cultivars, including the parents of our current mapping population, and one accession each of B. carinata and B. nigra). Plants were grown in a greenhouse under fluorescent and incandescent lights (12 hr photoperiod) at 26-27°C during the day and 19-20°C at night. Sclerotinia was applied to three-week old seedlings using a petiole inoculation technique (PIT) and a soybean isolate (105HT). The day on which each plant wilted was recorded beginning three days after inoculation. Plants that had not wilted by day 11 were given a score of 12.

We found that Ning RS-1 and H5200, from which the RI population was derived, were not significantly different (both appeared to have good levels of resistance, scores of 11.1 and 10.2, respectively). Other inoculation methods, as well as additional isolates and cultivars, will be used in future experiments to determine if the response of these parental lines to Sclerotinia can be distinguished and how they compare to a wider group of germplasm. We did find large differences between the Canadian spring cultivar Stellar (susceptible, score of 3.6) and some of the Chinese winter cultivars (resistant, scores of 10 - 11.5). We plan to make crosses between these two types to develop a DH population for potential use in mapping resistance genes.

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The Status of Sclerotinia sclerotiorum on Canola in North America

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Data was used from 11 years (1991 and 1993-2002) of published disease surveys in North Dakota (ND) and from 7 years (1996-2002) of published disease surveys in Minnesota (MN) (North Dakota State University Extension Reports). Data also was used from published surveys in the Canadian Prairie Provinces for selected years from 1991-2001 (Canadian Plant Disease Surveys). Sclerotinia stem rot varied from year to year and area-to-area depending on weather conditions. Often it was the most serious disease problem in ND, MN and Manitoba (MB), but in some years blackleg, caused by *Leptosphaeria maculans*, was more serious. In ND and MN, blackleg incidence exceeded that of Sclerotinia only in ND in 1991 and 2002. Survey data from Saskatchewan (SK) and Alberta (AB) often reported on other diseases and Sclerotinia was a minor problem in many years.

The highest incidence (percent girdled stems) recorded in both ND and MB was in 1993, with 18.7% in ND and 29.1% in MB; one district in MB had 41.4% incidence. The incidence in 1994 was 14.8% in ND, 15% in MB and 6% in SK. In 1997, the incidence in MN was 18.8% and that in ND was 14.2%. In 1999, the incidence was 12.5% in ND, 15.0% in MN, 7.6% in MB and 13% in SK. The incidence in 2000 was the second highest in both the ND and the MN surveys with an incidence of 17.0% in ND, 17.8% in MN, 13% in MB, 8% in SK and 7.3% in AB. Incidence in 2002 was only 4.4% in ND and 7.3% in MN, the lowest incidence in 11 years of ND survey and in 7 years of MN survey.

Published data from Canada indicate that a 0.5% loss (Can. J. Plant Pathol. 6:265) to a 0.7% loss (Can. J. Plant Pathol. 6:75-77) occurs for each 1% of incidence. Since the ND and MN disease surveys were done after swathing, with only the lower portions of the plants observed, the higher figure of 0.7% was used to estimate yield losses. Yield losses for ND and MN were estimated as (0.7 X incidence X average yield) for each year and state. Dollar losses were estimated using the average price of canola for each year, or the loan prices for 2000 and 2001. Yield losses were estimated to be over 1,000,000 cwt for ND in 1998 and 1999 and over 2,000,000 cwt in 2000. Yield losses in MN were estimated to be over 200,000 cwt in 1997, 1998 and 2000. Total yield losses in ND and MN from 1997 to 2002 were estimated to be 8,488,885 cwt. Dollar losses for ND and MN were estimated to be over $13,000,000 in 1998, over $11,000,000 in 1999, over $24,000,000 in 2000 and over $20,000,000 in 2001. The total dollar losses in North Dakota and Minnesota for 1991 through 2002 were estimated to exceed $94,000,000.

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Uniform Fungicide Trials for Canola in North Dakota and Minnesota

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Funded Plan of Work: Sclerotinia Control and Management in Canola

Uniform fungicide trials were initiated in 2001 with funding from a USDA-ARS grant to the University of Minnesota. Rather than have different fungicide trials at each of the four locations, we initiated a uniform trial with core treatments common to all four sites: the NDSU research extension centers in Carrington, ND; Langdon, ND; Minot, ND; and an on-farm site near Red Lake Falls, MN. This trial was inoculated with commercially available ascospores, and plots were misted at all locations except Minot. The misting was done periodically to keep the plants wet and favor infection. Sclerotinia incidence in the untreated plots was 73% at Carrington, 58% at Langdon and 61% at Red Lake Falls. Incidence was minimal at Minot without a misting system.

The 2001 uniform trials included registered and experimental fungicides. Applications were made at: 10-20% bloom, 30-40% bloom, and 50-60% bloom. Benlate (no longer available), Topsin M, BAS 510 (nicobifen), Ronilan and Rovral all provided good control and were most effective when applied at 50-60% bloom. Folicur provided marginal Sclerotinia control.

The 2002 uniform trials included the same fungicides, except for Folicur, which was not tested. Although Minot inoculated with ascospores and misted in 2002, only 12% incidence developed in the untreated plots. Despite the misting system, hot weather between inoculation and petal fall several days later may have reduced the survival of ascospores. Hot weather at inoculation also may have reduced survival of ascospores and subsequent infection at Carrington, where 12% incidence developed in the untreated plots. Langdon had 51% incidence in the untreated plots; temperatures were only slightly lower than at Carrington, and high temperatures persisted for slightly fewer hours. Since the temperatures were marginal at both locations, a slight difference in temperature and duration of high temperatures may have made a significant difference in disease development. Hail prevented harvest at Red Lake Falls. Results at Langdon in 2002 were similar to those in 2001. The 2002 uniform trials were funded primarily by a grant from the USDA-ARS National Canola Research Program for funding of summer labor and supplies at all locations, with funding from the Sclerotinia Initiative for purchase of a well at Minot to enable installation of a misting system.

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Use of Intercept for control of Sclerotinia on dry beans, canola, and sunflower 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

The objectives of this project are to identify the most effective procedure to infest soils with the biofungicide Intercept, to study the relationship between tillage systems, crop rotations and use of intercept in the control of *Sclerotinia* diseases, and to evaluate the feasibility of applying Intercept in tank mixes with herbicides. Two sets of replicated experiments were established at two locations. The first set was designed to study the efficacy of fall and spring applications of three doses of Intercept (0, 1, and 2 lb/A). The second set was designed to study the impact of annual applications of Intercept on plots under two tillage systems (no till and disking) and two crop rotations schemes. All plots for both sets of experiments were supplemented with field sclerotia before the application of intercept. Dry and warm spells of weather during the flowering of all crops resulted in very low incidence of white mold in all locations. *C. minitans*, the active ingredient of Intercept, was detected in soil samples collected from each experimental plot at all locations. The same plots will be planted in the 2003 season.

Harness,Lasso, Python, Valor, Balance, Prowl, and Authority, herbicides used in crops planted in rotation with dry beans, canola, and sunflowers were evaluated in the laboratory for their compatibility with Intercept. These herbicides need to be shallow-incorporated in the ground after application and could be used in tank mixes with Intercept. The herbicides were mixed with water at the recommended concentrations for field applications and then spores of *C. minitans* (Intercept) were suspended in the solution. One and two hours later aliquots were taken from the spore-herbicide mix and spread on the surface of dishes containing water agar. The spores were incubated at room temperature for 48 hours and then the number of colonies produced was counted. Valor, Harness, and Lasso reduced germination significantly compared to the untreated control. The other herbicides did not affect germination. Additional experiments to measure the impact of mixing the herbicides with *C. minitans* spores on their parasitic ability are in progress.

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