

Identification of herbicide-tolerant canola breeding lines with resistance to blackleg and evaluation of fungicide treatments to manage blackleg

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Introduction

Blackleg [caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not.] is a serious fungal disease affecting canola (*Brassica napus* L.) in North Dakota. The fungus attacks leaves, stems and pods, and causes stem cankers, girdling and lodging. Seedlings may be killed shortly after emergence, resulting in symptoms that may be mistaken for damping-off. Infections occurring early in the season cause the most severe yield loss.

Blackleg has increased in economic importance over the past 15 years. In the early 1990s most commercial cultivars planted in North Dakota were susceptible to it and as a consequence significant yield losses occurred (Lamey, 1995). In 1991 strains of this pathogen were classified in four pathogenicity groups (PG) based on their reaction to four *B. napus* differentials (Mengistu et al., 1991). PG-1 is an avirulent strain commonly found in canola fields throughout western Canada and North Dakota. PG-2 strains were considered responsible for most of the economic losses at that time. From 1992 to 1995, the incidence of blackleg varied across years from 3 to 28% across all fields surveyed. Since 1996, genes that conferred resistance against strains of PG-2 have been incorporated into the majority of the commercial cultivars grown in North Dakota while susceptible cultivars have been discontinued or grown in a smaller number of fields. More recently, however, strains of PG-3 and PG-4 were isolated from a field in North Dakota (Bradley et al., 2005). Strains of these groups are capable of attacking cultivars considered resistant to PG-2 (del Rio, unpublished data). Frequency and distribution of these strains were limited to a couple of counties early on, although more recently their geographic distribution seems to be expanding (Mazurek et al., not published). Since these strains were not detected until recently, none of the commercially available materials planted in our state has been bred for resistance against them. Since NDSU canola breeding lines may be released soon, it is imperative to characterize them for their reaction to strains of PG 2, 3 and 4. In the meantime, however, it is necessary that fungicide alternatives be evaluated so growers can have options to protect their crops until resistance is incorporated into commercial genotypes.

Current work

Evaluation of existing breeding lines: Blackleg nurseries established in Langdon and Prosper evaluated 37 NDSU canola breeding lines that already have a gene for herbicide-tolerance in 2008; another 50 lines were evaluated in Langdon and Fargo in 2009. The Langdon nurseries were supplemented with blackleg-infected stems collected from commercial fields, while the Prosper and Fargo nurseries were inoculated with a mixture of three PG2 isolates when plants were at the three leaf stage. Floods and warm and dry conditions resulted in the elimination of the nurseries at Prosper and Fargo in 2008 and 2009, respectively. In 2008, at the Langdon trial, lines 0330455, 0427667, 30525, and 0330662 had on average less than 8% incidence with a combined severity of less than 2 in a scale of 0-5 compared to the commercial controls DKL 38-

25 and Invigor 5550 who had an average incidence of 34% and a combined severity of 3.2. In 2009, lines 9041, 9229, 9082, and 9092 had an average incidence of 23% with a mean severity of 1.8 in a scale of 0-5 compared to the commercial controls DKL 30-42 and DKL 52-41 who had an average incidence of 33% and similar severity. In this trial, Westar the universal susceptible had an average blackleg incidence of 54% with a severity of 2.8 which were significantly higher than the breeding lines and commercial controls. Trials conducted in 2008 in Manitoba, Canada, indicated that lines BS-113, BS-122, BS-128, and BS-130, among 44 other lines, had the lowest levels of blackleg with an average of 18% incidence and a mean severity of 1.4 in a 0-5 scale, while other lines, like BS-132, and BS-123 had 100% incidence and a mean severity index of 4.0. In 2009, trials at the same location in Manitoba revealed that lines 9073 and 9228, among 48 other lines, had the lowest levels of blackleg with an average of 7% incidence and a mean severity of 0.1 in a 0-5 scale. Other lines, like 9096 and, 9091, 9051, and 9030 had 13% incidence and a mean severity of 0.1. The commercial control DKL 30-42 and Westar had 53% and 67% incidence and a mean severity of 0.9 and 3.0, respectively. These results are an indication that promising levels of resistance exist among NDSU breeding lines. Thus, continued efforts to screening these materials should be made to secure that only materials with the highest levels of resistance are advanced in the program. At the Langdon sites, strains of PG2 were prevalent, whereas at the Manitoba site the strains prevalent were from PG2, PG3 and PGT.

Development of new breeding populations: The canola breeding program at NDSU started in 2006 with 325 canola breeding lines donated by Monsanto Corporation. Between 2006 and 2007 these lines were evaluated at multiple locations for agronomic characteristics including high oil and seed yield as well as adaptation to North Dakota growing season. During the same period, greenhouse and field trials identified four lines as having high levels of resistance against *Sclerotinia sclerotiorum*, the other important disease affecting canola production in North Dakota. These lines were crossed with 14 high-oil and high-seed yield lines in the winter of 2006. Two generations of inbreeding were achieved during 2006-07 and 35 F₂ populations were planted, evaluated, and advanced to F_{2:3} level of inbreeding in the 2007 summer nursery. Seed from each selected F₂ plant was sent to a winter nursery location in Santiago, Chile and grown in individual rows. Three to five plants per row were self-pollinated and advanced to the F_{3:4} generation, and planted in individual rows in the summer of 2008. The remainder of each row was bulk-harvested, and the seed was used to conduct early generation testing (EGT) in 2008 at three locations in North Dakota: Prosper, Minot, and Langdon. The lines were evaluated for seed yield, seed oil content and fatty acid composition, days to flowering, relative maturity, and stand ability traits. Fifty very promising lines were identified on the basis of high seed yield and high oil content with other desirable agronomic characteristics. These lines were advanced to generation F_{5:6} in individual rows in the winter nursery during 2008-09. This project intends to evaluate the F_{6:7} generation of these lines in disease nursery settings to identify the best materials in terms of resistance against blackleg.

Identification of chemical alternatives for disease management: Results of only two field trials have been reported on evaluation of chemical alternatives for management of blackleg in North Dakota. In both, Bradley et al. (2005 and 2006), increased yields were reported when fungicides were applied to canola foliage at the 2-4 leaf stage, although no differences in disease control were observed between protected and non-protected plots. Currently, only two fungicides from the same chemical family, azoxystrobin (Quadris) and pyraclostrobin (Headline) are registered

for use against blackleg in North Dakota (McMullen and Markell, 2009). The lack of statistical differences in disease control reported in these trials and the fact that the only fungicides registered to manage blackleg share similar mode of action warrant the review of other fungicide alternatives.

Objective

This project has two objectives:

- 1) Identify superior genotypes within the NDSU canola breeding program with resistance to blackleg strains prevalent in North Dakota.
- 2) Identify fungicide alternatives to efficiently protect canola plants against blackleg.

Rationale and significance

Blackleg epidemics have been increasing steadily during the past few years in North Dakota. Coinciding with this upward trend blackleg strains of previously undetected pathogenicity groups were collected from fields in two North Dakota counties. It is very likely that the prevalence of these new strains, which are capable of overwhelming the resistance available in commercial cultivars, will increase with time. In order to abort potentially destructive blackleg epidemics it is necessary that all breeding materials be screened for their reaction to this disease. Since genetic resistance is the most effective way to manage a disease, releasing materials that are resistant to this disease will contribute to a more sustainable and environmentally cleaner canola production system. However, until these new materials are made available to growers, it is necessary that fungicide alternatives be evaluated so growers can protect their crops.

Approach

Identification of resistant breeding lines:

Identification of genotypes with resistance to PG2: Forty five breeding lines initially developed for high yield and resistance to *S. sclerotiorum* will be evaluated under field conditions in our blackleg nursery plot at Langdon. These elite lines will be planted using a randomized complete blocks design with three replications. Four commercial cultivars and Westar, a universal susceptible check, will be used as controls. Experimental plots (1.5 wide and 5 m long) will be planted at a rate of 140 seeds per m². Plants also will be inoculated, when they reach the third leaf stage, with a mixture of lab-produced inoculum at a concentration of 10⁵ conidia ml⁻¹ to supplement the natural inoculum and secure high disease pressure conditions. Disease intensity will be assessed on ten arbitrarily taken plants per plot when at the 5.4 growth stage of canola (ripening stage); incidence will be measured as the proportion of plants showing disease symptoms, whereas severity will be evaluated using a scale of 0 to 5 where 0 = no penetration or noticeable infection of the stem; 1 = 25% of the stem circumference infected, lesion superficial; 2 = 50% of the stem circumference infected, some penetration; 3 = 75% of the stem circumference infected, significant penetration; 4 = stem completely girdled, but intact at the base, significant penetration; and 5 = stem girdled at the base, constricted, dry and brittle, may be completely severed, plant dead. This scale is widely used by researchers to assess blackleg severity (van de Berg et al., 1993). A disease severity index (DSI) value will be calculated for each line by: (mean disease severity x % incidence)/5. A field trial will evaluate the same materials in a replicated study at Prosper to provide a second environment. Materials at Prosper will be planted in single rows 3 m long. Plants will be inoculated and evaluated as described.

Identification of genotypes with resistance to other PGs: The 45 elite lines that will be evaluated in our blackleg nursery at Langdon also will be evaluated for their reaction to PG 3 and PG 4 strains of blackleg in Fargo. All evaluations in Fargo will be conducted in growth chambers and inoculated plants will be autoclaved before being discarded at the end of the study. Fifteen seedlings of each line will be inoculated in replicated trials at the cotyledon stage with 10 μl of a spore suspension containing 10^7 spores ml^{-1} . Seedlings will be incubated for two weeks and evaluated using a 0-9 scale where 0 is no lesion visible and 9 means dead plant. Plants considered resistant to PG 3 and 4 will be taken to seed production. This same group will be planted at a field nursery in Manitoba, where they will be evaluated for their reaction to PG 3 and PG T. Materials will be planted in single rows and will be evaluated as described above. The information generated in these trials will be combined to identify the best materials.

Other breeding lines: Fifty breeding lines from different populations will be evaluated in non-replicated single row trial at our blackleg nursery in Langdon. This trial will constitute the first blackleg screening of these materials, materials which constitute the second tier in the breeding program. As in previous years, these lines are also being evaluated by the breeder in other ND locations for agronomic traits. The information generated in this trial will help the breeder decide which materials to advance.

Evaluation of fungicide alternatives:

Greenhouse studies: In the first year of this project, GHSE studies will be conducted to determine whether azoxystrobin, pyraclostrobin, prothioconazole, metconazole, and boscalid alone or in combinations have curative effects when applied to canola plants that have already been infected by blackleg. Plants will be inoculated with blackleg spores on the first true leaf. The inoculation will be carried out by depositing a spore suspension ($30 \mu\text{l}$ of a 10^7 spore ml^{-1}) in the center of the leaf. The inoculum will be placed directly on top of a wound created with a needle to facilitate pathogen penetration into the leaf tissue. Immediately after inoculation the plants will be incubated in mist chambers for 12 hours and then moved back to the greenhouse for fungicide applications. Fungicides will be applied at recommended field concentrations at intervals ranging from 12 to 72 hours after incubation. Lesion development will be measured four and eight days after incubation and will be compared to that of controls that were inoculated but not protected with fungicides. To estimate for how long these fungicides can protect plants from infection, plants at the 1 leaf stage will be sprayed with fungicides. Starting the day after fungicide applications, and at three days intervals, the plants will be inoculated with blackleg spores as described. The last inoculation will be made 10 days after fungicide application. Immediately after inoculation plants will be placed in moist chambers for 24 hours to stimulate spore germination and penetration. After that period, plants will be returned to the greenhouse where they will remain for the next 14 days. Lesion development will be monitored as described. Lesion size will be measured for each treatment and treatments will be compared using least significant difference. Data collected from greenhouse trials will be used to identify the best compounds and to prepare replicated field experiments.

Field studies: Two types of fungicide trials will be evaluated in field conditions, seed treatment and foliar application. These studies will be conducted in each of the three years this project will last. In the seed treatment trial five fungicides will be applied to seeds prior to planting. In the foliar application study, fungicides will be applied to seedlings at the 2-4 leaf growth stage. These trials will be conducted initially in Langdon in a field that has a history of blackleg, in the second and third years collaborators will be sought to expand trials to at least one or two

additional locations. Experimental plots will be 5 x 20 ft long. Seed treatments will be applied in a randomized complete block design with five replications. The foliar application study will be conducted using a factorial arrangement in a replicated randomized complete block design. At the time of foliar fungicide applications, plastic barriers will be placed at the sides of each plot to reduce fungicide drift. The best compounds will be evaluated in single or double applications, with the first application being made at the 2-4 leaf stage and the second at a time determined by the greenhouse data. To increase disease pressure, canola residues collected in 2009 will be spread in each plot. Disease incidence (proportion of infected plants) and severity (scale of 0-5) will be recorded in 10 plants per plot prior to swathing. The severity scale reflects the amount of lower stem tissue (part of the stem located at the soil line) affected by the disease and goes as 0=no visual symptoms; 1=1 to 10% of stem circumference affected; 2=11 to 25% of stem circumference affected; 3=26-50% of stem circumference affected; 4=51-75% of stem circumference affected; and 5= greater than 75% of stem circumference affected. Yield will be measured at harvest time.

Expected results

The 45 lines to be evaluated in field and growth chamber conditions are very advanced in terms of their degree of homozygosity (these are F_{6,7} lines). We expect the lines showing the highest levels of resistance would be released within a couple of years. The second set of materials (50 lines) are still a few years away from being ready to be released, but screening them at this time will help the breeder decide what materials to use in future population developments. We expect to identify materials within this group that will have promising levels of resistance against blackleg. Information on effective chemical treatments to manage blackleg will be developed from the fungicide studies and the best compounds or mixtures will be identified. This information will be shared with growers during field days.

Pitfalls and limitations

Field trials always have higher risk than trials conducted under controlled conditions of being botched up by bad weather conditions. Sometimes it could be too dry, sometimes it would be too wet; the former happened to our nursery in Fargo in 2009, whereas the latter happened to our nursery in Prosper in 2008 where plants drowned or could not emerge because of too much rain early in the season. The addition of a trial in Manitoba and the conduction of growth chamber screenings will help us produce the data needed to make sound selections among the materials evaluated. The methods selected to screen materials in growth chamber conditions have been used by other researchers as well to characterize reaction of plant materials, thus we do not anticipate major difficulties in conducting the screenings. Weather-related limitations can also occur in our fungicide trials in the field. That is the main reason why we are proposing to conduct this project for three years rather than one or two.

Project timeline

This project will have duration of three years. The evaluation of breeding lines will be carried out every year. Fungicide trials under controlled conditions will be conducted in the first year of the project while field trials will be conducted every year. Field trials, for breeding line and fungicide evaluations, will be installed in the first half of May each year. Foliar fungicide applications will be made when canola plants are at the 2-4 leaf growth stage in late May or early June. Good commercial agronomic practices will be followed to secure growth of the plants.

Disease plants will be evaluated when they approach physiological maturity. The information generated in this project will be used in the future to release breeding lines and to develop new breeding populations with enhanced resistance to blackleg.

Outreach/ Extension activities:

Findings of this project will be shared with the breeder who is a co-PI in this project. Condensed information regarding screening of breeding lines will be presented to growers to keep them abreast of our progress in that area. Results of our fungicide trials will be communicated to growers through field days. Results of this research will also be shared with the scientific community through presentations at professional meetings.

Budget

Student salary and fringe benefits - \$3,393
Research Technician salary and fringe benefits - \$21,420
Domestic Travel - \$2,100
Foreign Travel to Czech Republic - \$500
Materials and Supplies - \$3,500
Publication Costs - \$750
Total - \$31,663