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ADF Project Final Report Format

1. Project title and ADF file number.

#20140228
"Breeding for Resistance to Leaf Blotch Pathogens in Saskatchewan Oat"

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4. Abstract/ Summary: *This must include project objectives, results, and conclusions for use in publications and in the Ministry database. Maximum of 300 words in lay language.*

Oat leaf blotch diseases are caused by a complex of *Pyrenophora avenae, Cochliobolus sativus* and *Stagonospora* (*Septoria*) *avenae.* Concern exists as to the potential harm they may pose to oat yield and physical grain quality (i.e. test weight) which can negatively impact production and the potential to meet minimum standards of milling quality. Growing resistant varieties is an effective method of control, but very little is known about sources and effectiveness of resistant oat germplasm. This project was initiated to address these deficiencies through four objectives: 1) to understand the prevalence of these pathogens in commercial oat fields and develop artificial inoculation techniques suitable for evaluating oat germplasm resistance, 2) to study the virulence variability in these pathogens, 3) to identify resistant oat germplasm and understand the genetic inheritance of resistance and, 4) to map resistance quantitative trait loci (QTL) and identify associated genetic markers.

Over 4 field seasons (2014-2017) *P. avena* was identified in 59% of 160 fields surveyed, *Cochliobolus sativus* was present in 23% of

surveyed fields while *S. avenae* was only identified in 3% of fields. Methods to culture these pathogens on artificial media and to develop an effective inoculation procedure to screen oat germplasm were successful. Critical factors included inoculation of 14 day old oat seedlings, maintenance of 100% relative humidity and total darkness following inoculation for a period of 24 hours, and scoring disease reaction seven days after inoculation. A set of 15 *P. avenae* isolates and 17 *C. sativus* isolates were screened across a panel of nine diverse oat lines which revealed a wide range in pathogenicity for isolates of *P. avenae* and *C. sativus*. Several oat lines with effective resistance to both pathogens were identified, the most resistant being 96-21Cn19 (Aberystwyth University, UK), ND061868 (University of North Dakota) and Ave117.02 (INIA, Chile). In general, lines that showed resistance to one pathogen were also more resistant to the other pathogen.

Four bi-parental recombinant inbred line (RIL) populations were used to study the genetic inheritance of resistance against three *P. avenae* isolates. One or two gene models explained the inheritance of resistance in four cases with one population unable to be fit to any model. Three bi-parental recombinant inbred line (RIL) populations were used to study the genetic inheritance of resistance against one *C. sativus* isolate. A three gene model explained the inheritance of resistance in two of the populations with one population unable to be fit to any model. QTL mapping using the OT3011 x Iowa N2052 population revealed a single strong QTL (explaining 67% of the variation) on chromosome 5C that was effective against the two *P. avenae* isolates. A weaker QTL was also located on chromosome 5C, but a different position, in the AC Ass/S42 x CDC Dancer population. A 150 member association mapping panel, composed of elite breeding lines and varieties adapted to Western Canada, was also investigated for loci linked to *P. avenae* resistance. Four loci, identified using different association methods, linked to resistance were located across four chromosomes, including chromosome 5C.

A number of significant accomplishments and findings were made through this project including, 1) *P. avenae* is the most relevant leaf blotch pathogen in terms of prevalence, 2) isolates of *P. avenae* and *C. sativus* display a range of pathogenicity across oat germplasm, 3) oat germplam resistant to these pathogens exist and inheritance of resistance tends to be controlled by 1-3 genes and 4) QTL linked to *P. avenae* resistance were identified and will assist in incorporation of resistance into future oat varieties.

5. Introduction: *Brief project background and rationale.*

Leaf blotch diseases of oat are caused by a complex of *Pyrenophora avenae, Cochliobolus sativus* and *Stagonospora* (*Septoria*) *avenae.* The complex has become more prevalent in recent years, but very little is known about the impact of these diseases on production in Saskatchewan. Given the apparent susceptibility of some oat germplasm to these pathogens, concern exists as to the potential harm they may pose to oat yield and physical grain quality (i.e. test weight), which can negatively impact production and the potential to meet minimum standards of milling quality which would impact price premiums. Disease management practices involve cultural control, foliar fungicide application and growing resistant or tolerant cultivars. Fungicide applications are not an option for organic farmers and may have adverse environmental effects. Growing resistant varieties in combination with adequate crop rotation intervals is the most effective, economical and eco-friendly method of control.

Oat leaf blotch surveys conducted in Saskatchewan over the past decade indicate that both *Pyrenophora* and *Stagonospora* are the most prevalent pathogens which occur in more or less equal frequency. However, no systematic study has been conducted or reported in Canada to; 1) develop artificial inoculation techniques, 2) study the virulence variability in these pathogens, 3) identify resistance in oat germplasm and understand its inheritance, 4) map resistance gene(s) or quantitative trait loci (QTL) and identify genetic markers associated with resistance.

Such information is important for breeding oat varieties resistant to leaf blotch. This project was initiated to address these deficiencies in order to aid in incorporating resistance genes into future oat varieties to sustain oat yield and quality and improve producer returns. Saskatchewan accounts for approximately 50% of the 4 million tonnes of oat produced annually in Canada over the past 5 years (excluding 2010). Almost half of this production is exported to the US, destined for the high quality food market. Exports of oat and oat products were valued at almost $500 million to Western Canadian farmers and processors such as Richardson Milling (Martinsville, SK) and Grain Millers Inc. (Yorkton, SK). Saskatchewan oat production has doubled since the late 1980s and currently the province holds a strong position in supplying the North American oat market. This situation is a result of the steady decline in US oat acres over the same period, and our proximity to the main US oat distribution hub in Minneapolis, MN (removal of the Western Grain Transportation Act made transportation of oat from Alberta and Ontario/Quebec financially unfeasible).

To ensure Saskatchewan's continued role in supplying high quality oat products to the US market (and new markets abroad), investment in oat research and breeding is key. Research and breeding focused at improving oat resistance to these leaf blotch pathogens will increase the desirability of Saskatchewan oat varieties to producers and millers through reduced yield losses and maintenance of good grain test weight.

6. Methodology: *Include approaches, experimental design, methodology, materials, sites, etc.*

Objective 1A. Isolation of leaf blotch pathogens from commercial oat fields

Leaf spotting diseases of oat were surveyed across Saskatchewan in early-August, when the crops were at the milk to soft dough growth stages. Thirty-three fields were surveyed in 2014, 28 in 2015, 43 in 2016, and 64 in 2017. Disease severity was assessed on two to four plants at each of five points approximately 15 m apart and 30 m from the field edge. Oat plants were rated in the field based on disease severity on the upper (flag and penultimate leaves) and lower canopies as follows: 0 (no visible symptoms); trace (<1% leaf area affected); very slight (1-5%); slight (6-15%); moderate (16-40%); and severe (41-100%). Approximately 25 leaves were collected from each field, dried and stored in paper envelopes. Pathogens were identified in the laboratory by cutting and surface sterilizing 10 pieces of infected leaf tissue from 10 different leaves. The leaf cuttings were placed on water agar plates with 10mg/mL ampicillin and 5mg/mL kanamycin for four days to promote sporulation of the pathogen. The identities of the causal agents of the leaf spots were determined by spore size and shape. The identified pathogens were transferred to V8 Juice Agar (V8A) plates for further growth and sporulation. Single spore technique was used to obtain pure cultures of *P. avenae, C. sativus, and S. avenae*. The pure cultures were stored in cryopreservation fluid at -65° C.

Objective 1B. Development of an indoor screening technique

Inoculum production

Isolates were sub-cultured on V8A plates and incubated at room temperature (21±1°C) with a 12-h photoperiod for 10-14 days. To increase inoculum, the culture on the V8A plates was cut into 8 x 8 mm pieces and aseptically transferred to fresh V8A plates and incubated as above. After 10-14 days, conidia was harvested by adding 10 ml of sterile distilled water to the petri plate, scraping the culture with a glass rod and filtering through two layers of cheesecloth. Conidia per milliliter were counted with a hemocytometer and adjusted to the desired concentration.

Oat genotypes

Thirty-two oat lines (Table 1) were selected for screening at the seedling stage with leaf blotch pathogens in controlled environment chambers at the U of S Phytotron. The set of oat lines included potentially resistant and susceptible lines from western Canadian oat breeding programs, including the parents of segregating populations. Seeds were surface sterilized in 0.12% formalin for 1 hr and then rinsed in running water for 10 min and dried over-night. Preliminary experiments were conducted using nine of the oat lines.

Inoculations

Eight seeds per oat line were sown in a bunch with three lines per 15 cm pot containing Sunrise® LG3 Mix and raised in a growth chamber at 24/18°C with a 16/8 h photoperiod. Three replications of each line were planted in a randomized complete block design. Plants were inoculated 14 d after seeding (three leaf stage) with 10K, 20K, 30K, 40K and 50K spores/mL for *P. avenae*, 100K, 170K, 250K and 500K spores/mL for *S. avenae* and 30K, 40K, 50K, 75K, 100K and 150K spores/mL for *C. sativus* (using 10-14 days old fungal culture) to identify the optimal spore concentration for disease development. Using the *P. avenae* pathogen, additional experiments were conducted to understand the effect of inoculation date by inoculating 14 d, 21 d and 28 d after seeding. The inoculum was sprayed on plants using Badger Basic Spray Gun Set Model 250™ (Badger Air-Brush Company, IL, USA) mounted on D500 Air Compressor (Paasche Airbrush Company, IL, USA). Sufficient inoculum was applied until the plants were uniformly wet, approximately 5 mL per pot. Using the *P. avenae* pathogen, inoculated plants were incubated at 21°C in darkness for 24h, 48h and 72h at 100% RH to understand the effect of RH on disease development. Relative humidity was maintained using two Bionarie Ultrasonic Humidifiers (Sunbeam Corporation, Brampton, ON). Inoculated plants were then grown at 21/19°C with a 16/8 h photoperiod at 75% RH.

Assessment of infection response

Using the *P. avenae* pathogen, scoring was performed seven, eight, nine and ten days after inoculation to understand when maximal

disease symptoms were expressed. The 1-9 scale developed by Tekauz (1985) for *P. teres* f. *maculata* (spot form net blotch in barley) was used to rate disease infection. Generally the most common lesion type was scored from the top leaves. Lines were classified as resistant (1.0-3.0), intermediate (3.1-5.0) and susceptible (>5.0) based on their infection response (IR). For each isolate, the test was conducted once and repeated only if symptoms were not fully expressed on susceptible checks.

Objective 2. Assessment of pathogenic variability

Nine oat lines (the first nine listed in Table 1) were screened with 15 isolates of *Pyrenophora avenae* and 17 isolates of *Cochliobolus sativus* using the standardized inoculation method (described below) to assess virulence variability among different isolates.

Inoculum Production

Isolates of *Pyrenophora avenae* and *Cochliobolus sativus* were sub-cultured on V8A plates and incubated at room temperature (21±1°C) with a 12-h photoperiod for 10-14 days. To increase inoculum, cultures on V8A plates were cut into 8 x 8 mm pieces and aseptically transferred to fresh V8A plates and incubated as above. After 10-14 days, conidia was harvested by adding 10 ml of sterile distilled water to the petri plate, scraping the culture with a glass rod and filtering through two layers of cheesecloth. Conidia per milliliter were counted with a hemocytometer and adjusted to the desired concentration.

Inoculation Method

Eight seeds per oat line were sown in a bunch with three lines per 15 cm pot containing Sunrise® LG3 Mix. Plants were grown in a growth chamber at 24/18°C with a 16/8 h photoperiod. Three replications of each line were planted in a randomized complete block design. Plants were inoculated 14 days after seeding (three leaf stage) with 30K spores/ml for *P. avenae* and 50K spores/ml for *C. sativus* (from 10-14 day old fungal culture). The inoculum was sprayed on plants using a Badger Basic Spray Gun Set Model 250™ (Badger Air-Brush Company, IL, USA) mounted on a D500 Air Compressor (Paasche Airbrush Company, IL, USA). Sufficient inoculum was applied until the plants were uniformly wet, approximately 3 mL per pot. Inoculated plants were incubated at 21°C in darkness for 24 h at 100% RH. Relative humidity was maintained using two Bionarie Ultrasonic Humidifiers (Sunbeam Corporation, Brampton, ON). Inoculated plants were then grown at 21/18°C with a 16/8 h photoperiod at 75% RH.

Assessment of Infection Response

Scoring was performed seven days after inoculation on a 1-9 scale (Tekauz 1985), the same scale used for *P. teres* f. *maculata* (spot form net blotch in barley). Generally the most common lesion type was scored using the top leaves. Lines were classified as resistant (1.0-3.0), intermediate (3.1-5.0) and susceptible (>5.0) based on their infection response (IR). For each isolate, the experiment was repeated only if symptoms were not fully expressed on the susceptible checks.

Objective 3. Identification of resistant oat lines

Thirty-two oat lines (Table 1) were selected to evaluate infection response to leaf blotch pathogens. The set of oat lines included potentially resistant and susceptible lines from oat breeding programs located around the world, and included parents of segregating populations. Seeds were surface sterilized in 0.12% formalin for 1 hr and then rinsed in running water for 10 min and dried over-night. The 32 lines were screened with three isolates of *P. avenae* and six isolates of *C. sativus* using the standardized inoculation method.

Table 1. Description of the 32 oat lines used to evaluate infection response to *P. avenae* and *C. sativus* isolates.

Oat Line	Breeding Program	Country
CDC Dancer	Crop Development Centre	Canada
AC Morgan	AAFC-LRDC	Canada
CDC Morrison	Crop Development Centre	Canada
BetaGene	U. of Wisconsin	United Sates
Triactor	Lantmännen SW Seeds	Sweden
CDC Weaver	Crop Development Centre	Canada
CDC Sol-Fi	Crop Development Centre	Canada
HiFi	North Dakota State University	United States
Aslak	Boreal Plant Breeding Ltd.	Finland
Matilda	Lantmännen SW Seeds	Sweden
Aigorudo	UFRGS	Brazil
Iowa N2052	Iowa State University	United Sates
OT3011	Crop Development Centre	Canada
96-21Cn19	Aberystwyth University	United Kingdom
SA99572	Crop Development Centre	Canada
OT3028	Crop Development Centre	Canada
Provena B	USDA-ARS Aberdeen	United Sates
CDC Boyer	Crop Development Centre	Canada
Ave 117.02	INIA	Chile
OT3033	Crop Development Centre	Canada
Bw 1103	INTA	Argentina
OT3031	Crop Development Centre	Canada
CDC Seabiscuit	Crop Development Centre	Canada
MN06212	U. of Minnesota	United Sates
CDC Minstrel	Crop Development Centre	Canada
OT3050	Crop Development Centre	Canada
Robust	Purdue University	United Sates
ND061868	North Dakota State University	United Sates
Vista	U. of Wisconsin	United Sates
AC Ass/S42	AAFC-BRDC	Canada
CDC ProFi	Crop Development Centre	Canada
SA060539	Crop Development Centre	Canada

Objective 4. Inheritance of Resistance and Genetic Mapping

Inheritance studies and Bi-parental QTL mapping

Populations

OT3028 x Robust: Ninety-two $F_{4:5}$ recombinant inbred lines (RILs) were developed from the cross OT3028 x Robust. The population was screened with *P. avenae* isolate PA101. OT3028 is an advanced oat line from Crop Development Centre (CDC), University of

Saskatchewan and resistant to PA101. Robust is an oat variety from Purdue University, USA and susceptible to PA101.

Ave117.02 x OT3033: One hundred and fourteen $F_{4:5}$ RILs were developed from this cross. The population was screened with *P. avenae* isolate PA102. Ave 117.02 is an oat line developed by INIA (Chile) and showed resistance to isolate PA102. OT3033 is an advanced oat line from CDC, University of Saskatchewan and susceptible to PA102.

OT3011 x Iowa N2052: One hundred and fifty $F_{4:5}$ RILs were developed from this cross. All lines were screened with *P. avenae* isolates PA102 and PA114. OT3011 is an advanced oat line from the CDC, University of Saskatchewan resistant to PA102 and PA114, whereas Iowa N2052 was susceptible to these isolates and developed by Iowa State University.

AC Ass/S42 x CDC Dancer: One hundred and fifty $F_{4:5}$ RILs were developed from this cross. All lines were screened with *P. avenae* isolate PA114 and *C. sativus* isolate CS316 in separate experiments. AC Ass/S42 was developed at AAFC-BRDC and is resistant to both isolates while CDC Dancer was developed at the Crop Development Centre and is susceptible to both isolates.

ND061868 x CDC Morrison: One hundred and fifty $F_{4:5}$ RILs were developed from this population. All lines were screened with *C. sativus* isolate CS316. ND061868 was developed at North Dakota State University and is resistant to this isolate, while CDC Morrison was developed at the Crop development Centre and is susceptible.

SA060539 x OT3028: One hundred $F_{4:5}$ RILs were developed from this cross. All lines were screened with *C. sativus* isolate CS316. SA060539 is known to be resistant to this isolate while OT3028 is susceptible. Both lines were developed at the Crop Development Centre.

In experiments involving *P. avenae* CDC Dancer was used as the susceptible check and ND061868was used as a resistant check. Iowa N2052 was used as the susceptible check in experiments where CDC Dancer was a parent of the bi-parental cross. 96-21Cn19, an oat line from Aberystwyth University (UK), was used as the resistant check in experiments where ND061868 was a parent of the bi-parental cross.

Populations were grown and inoculated as described in the standardized inoculation method (Objective 2). For each isolate used, three replications of each line were screened in a randomized incomplete block design due to space limitations in the phytotron. A replication was repeated if symptoms were not fully expressed on the susceptible check.

Statistical Analysis

Independence and normal distribution of the residuals for all class variables were evaluated by the UNIVARIATE procedure in Statistical Analysis Software (SAS, v. 9.4., SAS Institute Inc., Cary, NC). To investigate the effect of line and isolate on infection response, analyses of variance (ANOVA) were conducted, using the PROC MIXED procedure in SAS (v. 9.4) with lines and isolates as fixed effects, and replications and interaction of lines or isolates with replications as random effects. Separation of infection responses between lines or isolates was conducted using Fisher's Least Significant Difference (LSD) at a nominal alpha level of 0.05 (Littell et al. 2006).

Entry (line)-mean heritability (h^2) was calculated for the lines in individual populations for disease severity. The phenotypic variance was $\sigma^2_{\text{lines}} + \sigma^2_{\text{residual}}$/replications. For heritability analysis, all factors were considered random. To evaluate the genetic inheritance of resistance, lines were categorized as resistant or susceptible lines with lines having an IRs of 1 to ≤3.5 classified as resistant, and lines with an IRs of > 3.5 to 9 classified as susceptible. The Chi-square test was used to evaluate segregation ratios against those expected for one-gene (1:1), two-gene (1:3), three-gene (1:7) or four-gene (1:15) inheritance models.

Phenotyping

One hundred and fifty RILs from both the AC Ass/S42 x CDC Dancer and OT3011 x Iowa N2052 populations were surface sterilized in 0.12% formalin for 1 hour and then rinsed with running water for 10 minutes and dried overnight. The 150 lines were grown, inoculated and scored using the standardized inoculation method (Objective 2) with *P. avenae* isolates PA114, PA102 and *C. sativus* isolate CS316. Leaf blotch infection rating for each line was taken as the mean of 3 replications.

Genotyping

Genomic DNA of the 150 lines was extracted from five day old coleoptiles collected from multiple seeds using a modified Micro CTAB DNA Extraction Procedure for Grasses which excluded the phenol washes (modified from Procunier et al., 1990, BNG 20:74-75). Both populations were genotyped with SNP markers using the Oat 6K iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA) at Agriculture and Agri-Food Canada (Morden, MB; lab of Dr. Curt McCartney). SNP genotyping data from the iSELECT platform was visually screened using Illumina GenomeStudio software (Illumina, Inc., San Diego, CA) to ensure correctness of the automated calls. Markers that were monomorphic, had greater than 15% failed or null reactions, or showed highly skewed segregation ($p<0.01$) were removed to create a high quality set of genotyping data.

Linkage Map Creation and QTL Mapping

A genetic map was created using the high quality genotyping data for both populations. Linkage groups were determined using JoinMap 4.0 (Kyazma B.V., Wageningen, Netherlands). Default calculation settings were used when building the groupings tree (initial groups created with independent LOD scores from 2.0 to 10.0 and loci were removed if there was a goodness-of-fit jump greater than 5.00). Marker order within the initial linkage groups was established using the Maximum Likelihood algorithm as described in Van Ooijen (2011) under its default settings.

Linkage groups and marker order data were input into MapQTL 5.0 (Kyazma B.V., Wageningen, Netherlands). QTL mapping parameters were maintained at their default values and phenotypic data were analyzed separately by leaf blotch isolate. A permutation test was run to determine the significant LOD threshold for each pathogen and population. The LOD values were taken at approximately the 95% confidence level for genome-wide significance, as suggested by van Ooijen (1999); when the exact relative cumulative count 0.9500 at the genome-wide level was not present for a particular pathogen, the next highest value was used. Interval mapping was conducted to identify QTLs linked leaf blotch symptom severity.

Genome-wide association mapping in Western Canadian elite lines

Population and Phenotyping

An association mapping panel consisting of 150 lines and varieties that were tested over a 1-2 year period in the Western Cooperative Oat Registration Trial between 2002 and 2015 was assembled. These lines represent elite oat breeding germplasm adapted to Western Canada that were bred at seven different breeding programs. Oat lines were surface sterilized in 0.12% formalin for 1 hour and then rinsed with running water for 10 minutes and dried overnight. The 150 lines were grown, inoculated and scored using the standardized inoculation method (Objective 2) with *P. avenae* isolate PA114. Leaf blotch infection rating for each line was taken as the mean of 3 replications.

Genotyping

Genomic DNA of the 150 lines was extracted from five day old coleoptiles collected from multiple seeds using the Qiagen DNeasy Plant Mini Kit (Qiagen Cat. No. 69104, Hilden, Germany). Genotyping was done using the Oat 6K iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA) at the Cereal Crops Research, United States Department of Agriculture-Agricultural Research Service (Fargo, ND). Markers that were monomorphic, had greater than 15% failed or null reactions, or showed highly skewed segregation ($p<0.01$) were removed to create a high quality set of genotyping data.

Kinship and population structure analysis

To account for potential population stratification within the oat line familial several measures of familial relatedness (kinship) and structure were calculated using the set of high quality SNP markers and incorporated into the mixed linear model. A kinship matrix (K) was calculated by estimating kinship coefficients using the method of Loiselle et al (1995) and implemented in SPAGeDi v. 1.5.2 (Hardy and Vekemans, 2002). STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2003, 2007) was used to generate a population structure matrix (Q) using the method of Yu *et al.* (2006). Correlated allele frequencies were used in the linkage ancestry model to

test different group numbers from one to 10. Each test was run 10 times with a burn-in length of 10,000 iterations followed by 100,000 Markov chain Monte Carlo (MCMC) iterations. Results were analyzed by Structure Harvester (Earl and vonHoldt 2012) based on the method of Evanno et al. (2005) to identify the appropriate number of sub-populations, in this case 2.

Association mapping

Association mapping was conducted in R in two ways. Firstly by using the genomic association and prediction integrated tool (GAPIT) package with the compression mixed linear model $y = X\beta + Zu + \varepsilon$, where y is the vector of observed phenotypes; β is an unknown vector containing fixed effects, including the genetic marker, covariates matrix, and the intercept; u is an unknown vector of random additive genetic effects from multiple background QTL for individuals/lines; X and Z are the known design matrices; and e is the unobserved vector of residuals (Zhang et al., 2010). Secondly, by using fixed and random model Circulating Probability Unification (FarmCPU) package (Liu et. al., 2016). Sixteen different association mapping analyses were completed using a variety of calculated K and Q parameters, and a variety of phenotypic data sets. These are outlined below in Table 2.

Table 2. Software, stratification control methods and phenotypic data sets used for association mapping of oat leaf blotch resistance loci in the Western Canadian elite oat line population.

Software Package	Population Stratification Control	Phenotypic Data Set
GAPIT	Self K	150 lines with original scoring
FarmCPU	SPAGeDi K and STRUCTURE Q	150 lines with binary (R/S) scoring
		118 line subset with original scoring
		118 lines with binary (R/S) scoring

Self-K refers to a kinship matrix calculated by the R package and used in GAPIT and FarmCPU. SPAGeDi K and STRUCTURE Q refer to a kinship matrix and population structure matrix generated by the programs SPAGeDi and STRUCTURE, respectively, and used in GAPIT or FarmCPU. 150 Lines indicates that all phenotyped lines were analyzed while 118 lines refers to a subset of lines selected to maximize phenotypic differences in the data set (i.e. removed lines with intermediate infection ratings from 3.0-4.5). Original scoring indicates that the average infection response of each line (as described above) was used in the analysis, while binary (R/S) scoring indicates that lines were classified as being either resistant (R) or susceptible (S) and their phenotypic infection response data was converted to a 1 or 0 score, respectively. A line was considered susceptible if its infection response score was 3.5 or above.

Transformation of the complete phenotypic data set (i.e. 150 lines) were attempted (e.g. squared, cubed, square root, logarithmic, and natural logarithmic) to improve normality of the data, but none yielded a better normal distribution. The default p-threshold in both GAPIT and FarmCPU packages was calculated as 0.01/number of markers used in the association analysis (Bonferroni method), in this case 0.01/2,586=3.87E-06. A relaxed p-threshold of 0.005 was also used to identify significant markers that were not declared using the more stringent Bonferroni correction.

Upon completion of GWAS, trait associated p-values were displayed in Manhattan Plots. Squared correlation coefficients were calculated in GAPIT only and used to describe the variance explained by individual markers. Quantile-Quantile (Q-Q) plots were generated for each run based on the expected vs observed p-values, with markers significantly associated with a trait not following the theoretical linear relationship between the two.

7. Research accomplishments: *(Describe progress towards meeting objectives. Please use revised objectives if Ministry-approved revisions have been made to original objectives.)*

Objectives	Progress
1A) Isolation of leaf blotch pathogens from commercial	Growing conditions in Saskatchewan were wet to very wet in the spring and early summer of 2014. Warm temperatures in July and August, combined with high humidity contributed to disease development. Leaf spots were observed in the canopies of all 33 fields surveyed in 2014. Disease severity in all fields varied from trace to slight in the upper canopy. In the lower canopy, severity ranged from trace to light in 25 fields and moderate in eight fields. Three leaf spotting pathogens were identified from laboratory observation of oat leaf tissue. The most

oat fields

common was *P. avenae*, followed by *C. sativus* and *S. avenae* (Table 3).

Using single spore technique, we obtained pure cultures of *P. avenae* (2 isolates), *C. sativus* (2 isolates) and *S. avenae* (1 isolate) which were stored at -65° C for future research.

Table 3. Prevalence and incidence (isolation frequency) of leaf blotch pathogens of oat in Saskatchewan in 2014.

Pathogen	Prevalence (% crops)	Incidence (% isolations)*
Pyrenophora avenae	91	70
Cochliobolus sativus	61	23
Stagnospora avenae	18	6

* indicative of the relative amount of foliar damage observed

In 2015, growing conditions were relatively dry in the spring and early summer leading to low disease development. Leaf spots were observed in the canopies of all 28 fields surveyed however severity varied from trace to slight in 25 fields and moderate in three fields. *Pyrenophora avenae* and *C. sativus* were identified from the plated oat leaf tissues and their prevalence and incidence was lower as compared to that of 2014 (Table 4). No *S. avenae* was observed in 2015 leaf samples.

We were able to culture 16 isolates of *P. avenae* and 15 isolates of *C. sativus* using the single spore technique. These isolates were stored at -65° C.

Table 4. Prevalence and incidence (isolation frequency) of leaf blotch pathogens of oat in Saskatchewan in 2015.

Pathogen	Prevalence (% crops)	Incidence (% isolations)*
Pyrenophora avenae	65	21
Cochliobolus sativus	37	7

* indicative of the relative amount of foliar damage observed

Leaf spots were observed in the canopies of all 43 crops sampled in 2016, however disease severity was assessed for only 25 of the 43 crops. Leaf spot severity in the upper canopy varied from trace to slight in 22 crops and moderate in three crops, while severity in the lower canopy was rated as moderate in 14 crops, severe in two and slight in the remaining nine.

Only two leaf-spot pathogens were identified from the plated oat leaf tissues (Table 5), *P. avenae* and C. *sativus*, with *P. avenae* being more common. *S. avenae* was not observed in any of the samples, as was the case in 2015. The prevalence and incidence (Table 5) of *P. avenae* and *C. sativus* was lower when compared to that of 2015 when *P. avenae* and *C. sativus* were present in 65% and 37% of crops sampled, respectively (as reported in our 2015 ADF progress report).

We were able to culture 20 isolates of *P. avenae* and 17 isolates of *C. sativus* using the single spore technique. These isolates were stored in cryopreservation fluid at -65° C.

Table 5. Oat leaf blotch disease prevalence and incidence in 43 Saskatchewan oat crops surveyed in 2016.

Pathogen	Prevalence (% crops)	Incidence (% isolations)*
Pyrenophora avenae	33	8
Cochliobolus sativus	9	1

*Number of leaf sections from which pathogens were isolated per total number of leaf sections sampled. Indicative of the relative amount of foliar damage observed.

Leaf spots were observed in the foliar canopies of all 64 crops surveyed in 2017, however, disease severity varied from trace to slight in 40 fields and moderate in eight fields (severity data available for 48 of 64 field samples only).

Of the three leaf-spot pathogens identified from the plated oat leaf tissues (Table 6), *P. avenae* was found to be the most prevalent, followed by *C. sativus* and finally *S. avenae* which was observed in only one field. This ranking of pathogen prevalence follows observations made in both 2016 and 2015.

We were able to culture 5 isolates each of *P. avenae* and *C. sativus,* and one isolate of *S. avenae* isolates of using the single spore technique. These isolates were stored in cryopreservation fluid at -65° C.

Table 6. Oat leaf blotch disease prevalence and incidence in 64 Saskatchewan oat crops surveyed in 2017.

Pathogen	Prevalence (% crops)*	Incidence (% isolations)**
Pyrenophora avenae	59	22.5
Cochliobolus sativus	12	2.3
Stagnospora avenae	2	0.2

*Percentage of fields surveyed from which specified pathogen was identified.
**Number of leaf sections from which pathogens were isolated per total number of leaf sections sampled. Indicative of the relative amount of foliar damage observed.

1B) Development of an indoor screening technique

Effect of inoculum concentration on disease development

Experiments were conducted using different inoculum concentrations of leaf blotch pathogens to identify a spore concentration for each pathogen which produced a wide range of disease scores across the oat lines. For *P. avenae*, three inoculum concentrations (10K, 20K, and 30K spores/mL) were used to inoculate nine oat lines 14 days after seeding. Inoculated plants were incubated at 21°C in darkness for 24 h at 100% relative humidity (RH). The preliminary experiment showed that the 30 K spore concentration was the best (data not shown). Another experiment was conducted where 30K, 40K and 50K spores/mL concentrations were used. Infection response (IR) increased with the increase in spore concentration (Table 7), however, the IR was too high at 40K and 50K as all the tested lines showed a susceptible reaction except CDC Sol-Fi. This suggested that a spore concentration of 30K was the best for *P. avenae* (isolate PA101) which allowed differentiation of oat lines.

Table 7. Reaction of 9 oat lines to different concentrations of *Pyrenophora avenae* isolate PA101 in the Phytotron.

	Inoculum Concentration (spores/mL)		
Oat Line	30K	40K	50K
CDC Dancer	7.3	8.0	8.7
AC Morgan	6.3	7.0	7.7
CDC Morrison	4.3	5.7	7.3
BetaGene	7.0	7.0	7.7
Triactor	5.0	5.7	8.0
CDC Weaver	3.0	6.3	5.7
CDC Sol-Fi	3.7	4.3	4.3
HiFi	6.7	7.7	7.3
Aslak	5.0	7.0	7.7
Average	5.4	6.5	7.1

Similarly, an experiment was conducted using different concentrations (100K, 170K, 250K and 500K spores/mL) of *S. avenae* isolate SA201. All other conditions were the same as described above. A concentration of 500K provided the highest infection response (Table 8), however, the majority of the oat varieties/lines showed an intermediate

reaction except CDC Morrison which was susceptible at this concentration.

Table 8. Reaction of oat lines to different concentrations of *Stagonospora avenae* isolate SA201 in the Phytotron.

	Inoculum Concentration(spores/mL)			
Oat Line	100K	170K	250K	500K
CDC Dancer	4.3	5.0	4.3	5.0
AC Morgan	3.7	4.0	3.7	4.3
CDC Morrison	4.0	3.7	4.3	5.7
BetaGene	3.3	2.7	3.3	3.0
Triactor	3.0	3.7	3.7	4.3
CDC Weaver	2.3	2.7	3.0	4.3
CDC Sol-Fi	2.0	3.0	4.3	4.3
HiFi	3.3	3.0	5.7	5.0
Aslak	2.3	1.7	2.7	4.3
Average	3.1	3.3	3.9	4.5

An experiment using different inoculum concentrations (30K, 40K, 50K and 75K spores/mL) of *C. sativus* isolate CS301 indicated that none of oat varieties/lines showed a susceptible reaction with spore concentration of 30K and 40K (Table 9). A higher IR was observed with a spore concentration of 50K and 75K, but none of the lines showed an IR higher than 5.

Table 9. Reaction of oat lines to different concentrations of *Cochliobolus sativus* isolate CS301 in the Phytotron.

	Inoculum Concentration (spores/mL)			
Oat Line	30K	40K	50K	75K
CDC Dancer	3.0	2.7	5.0	4.3
AC Morgan	3.0	3.0	5.0	3.7
CDC Morrison	3.0	2.7	4.3	5.0
BetaGene	3.0	2.7	3.3	3.7
Triactor	2.7	2.7	5.0	4.3
CDC Weaver	3.0	2.7	3.7	3.7
CDC Sol-Fi	2.0	2.7	3.0	3.0
HiFi	3.0	3.0	5.0	5.0
Aslak	1.7	2.3	2.0	3.3
Average	2.7	2.7	4.0	4.0

Similar experiments were performed with *C. sativus* isolate CS302. In the first experiment, four different concentrations (30K, 40K, 50K and 75K spores/mL) were used and the IRs were low (Table 10). The experiment was repeated using higher concentrations (50K, 75K, 100K and 150K spores/mL) of CS302. The disease development was better showing higher IRs in many oat varieties/lines (Table 11). The majority of the varieties/lines showed a susceptible reaction at a 75K or higher spore concentration. At 150K spore concentrations, all the varieties/lines showed susceptible reaction except Aslak which showed an intermediate reaction. The results from the second round of higher CS302 concentration screenings suggest that 50K or 75K concentration of CS302 will be appropriate to screen oat lines and populations. The selected 32 varieties/lines were therefore screened using 50K concentration of *C. sativus* isolates.

Table 10. Reaction of oat lines to different concentrations of *Cochliobolus sativus* isolate CS302 in the Phytotron.

	Inoculum Concentration (spores/mL)			
Oat Line	30K	40K	50K	75K
CDC Dancer	2.7	3.7	4.3	4.3
AC Morgan	2.7	3.0	3.0	3.0
CDC Morrison	3.0	2.7	2.7	3.7
BetaGene	2.3	3.3	4.3	3.0
Triactor	3.0	3.7	3.7	4.3
CDC Weaver	2.3	3.0	2.7	3.7
CDC Sol-Fi	3.0	2.7	3.0	3.7
HiFi	3.0	2.7	2.7	3.0
Aslak	2.0	2.3	2.7	3.0
Average	2.7	3.0	3.2	3.5

Table 11. Reaction of oat lines to higher concentrations of *Cochliobolus sativus* isolate CS302 in the Phytotron.

	Inoculum Concentration (spores/mL)			
Oat Line	50K	75K	100K	150K
CDC Dancer	5.7	7.0	7.0	7.3
AC Morgan	5.0	6.3	6.3	6.3
CDC Morrison	3.0	4.3	5.0	6.3
BetaGene	3.7	5.7	5.0	5.7
Triactor	5.7	7.0	7.0	7.0
CDC Weaver	7.0	7.3	7.7	7.0
CDC Sol-Fi	3.7	5.7	5.0	5.7
HiFi	5.0	5.7	7.0	6.7
Aslak	3.7	3.7	3.7	4.3
Average	4.7	5.9	6.0	6.3

Only one isolate of *S. avenae* (SA201) was available (from the 2014 field survey conducted prior to this project) for use as no leaf samples from the 2015 and 2016 surveys yielded spores of this pathogen. As reported in the 2015 ADF progress report, using this isolate we were unable to obtain differentiation in infection response between the oat lines tested. Because of this and the fact that this pathogen has not been observed in the last two field surveys, it was decided to no longer pursue further work on this pathogen. This is in keeping with the original research plan in which one primary leaf blotch pathogen was to be identified (based on highest incidence in field surveys) for further resistance work. Optimal spore concentration was finalized to vary between the two pathogens, with 30K spores/ml for *P. avenae* and 50K spores/ml for *C. sativus*.

Effect of humidity on disease development

Nine oat lines were inoculated with *P. avenae* isolate PA101 using a spore concentrations of 30K spores/mL. Inoculations were performed 14 days after seeding. Inoculated plants were incubated at 21°C in darkness at 100% RH for 24h, 48h and 72h to determine the effect of different durations of humidity. The results indicate that 100% RH for the first 24 h was crucial (Table 12) and increasing the time interval to 48 h or 72 h didn't increase the disease

development as IR were very similar at the three time durations. In future, inoculated plants will be incubated for 24 h at 100% RH.

Table 12. Reaction of oat lines to *Pyrenophora avenae* isolate PA101 using different durations of 100% relative humidity in the Phytotron.

	Humidity Interval		
Oat Line	24 h	48 h	72 h
CDC Dancer	6.3	5.7	6.0
AC Morgan	4.3	3.0	3.7
CDC Morrison	2.7	2.7	2.7
BetaGene	3.7	3.7	3.7
Triactor	4.3	2.7	3.5
CDC Weaver	3.7	3.7	3.7
CDC SO1-Fi	3.7	3.7	3.7
HiFi	6.3	3.7	5.0
Aslak	5.0	3.7	4.3
Average	4.4	3.6	4.0

Effect of other factors on disease development

Scoring was performed 7, 8, 9 and 10 days after inoculations. However, there was no significant increase in symptoms after 7 days. It was decided to score 7 days after inoculation.

Experiments were also conducted to determine the effect of different inoculation dates (14 d, 21 d and 28 d) after seeding. There was no significant difference in the disease reaction when inoculated at different growth stages suggesting that inoculations after 14 days of seeding will be more efficient as we can screen the oat populations faster.

2) Evaluation of pathogenic variability

Evaluation of infection response in nine oat lines with 15 isolates of *P. avenae* revealed that isolate PA111 was the most virulent (average IR across the lines was 6.6) with eight of nine lines displaying a susceptible reaction and only CDC Morrison displaying a resistant reaction (Table 1 in Supplemental file). In contrast, isolate PA102 was the least virulent (average IR across the lines was 2.9) with eight of nine lines showing a resistant reaction and only CDC Dancer having an intermediate reaction (Table 1 in Supplemental file). There were significant effects of line, isolate and line x isolate interaction ($P<0.0001$ in all cases), indicating specificity in the host-pathogen interaction. CDC Dancer was the most susceptible oat line (average IR across the 15 isolates of 6.5), whereas CDC Morrison was the most resistant oat line (average IR across the 15 isolates of 3.5) (Table 1 in Supplemental file).

Most *P. avenae* isolates, except PA106, PA107 and PA113, were good spore producers and would be suitable for further virulence studies (if desired). PA106, PA107 and PA113 were discarded due to the difficulty in working with them.

Among the 17 *C. sativus* isolates tested, CS302 was the most virulent (average IR across the lines was 6.5) with all nine oat lines showing a susceptible reaction (Table 2 in Supplemental file). In contrast, isolate CS309 was the least virulent (average IR across the lines was 2.0) with all nine oat lines showing a resistant reaction (Table 2 in Supplemental file). As with *P. avenae*, there were significant effects of line, isolate and line x isolate interaction ($P<0.0001$ in all cases) again indicating specificity in the host-pathogen interaction. Overall, CDC Dancer was the most susceptible line (average IR across the 17 isolates of 5.0), whereas CDC Sol-Fi, Triactor, Aslak and BetaGene were the most resistant lines (average IR across the 17 isolates of 3.2, 3.3, 3.3 and 3.5, respectively) (Table 2 in

	Supplemental file).
3) Identification of resistance sources in oat germplasm	The isolates used to screen these 32 lines were selected based on their ability to produce abundant spores and to span the range of virulence observed within the smaller set of nine oat lines used in Objective 2. Among the 32 oat lines evaluated, 10 showed resistance, 11 were intermediate and 11 displayed a susceptible reaction to isolate PA101 (Table 3 in Supplemental file). Oat lines 96-21Cn19, ND061868 and AC Ass/S42 were the most resistant lines (average IR of 1.7, 1.7, and 1.3, respectively), while CDC Dancer and Iowa N2052 (average IR of 7.7 each) were the most susceptible lines. The differential reactions observed within these oat lines suggests that isolate PA101 could be used to screen some bi-parental populations in order to study inheritance of resistance. Twenty-four lines showed a resistant IR, seven displayed an intermediate response and only Iowa N2052 had a susceptible reaction to isolate PA102 (Table 3 in Supplemental file). Seventeen lines showed a resistant infection response, 13 displayed an intermediate response and only Iowa N2052 and CDC Dancer had susceptible reactions to isolate PA114 (Table 3 in Supplemental file). Across all three isolates, 96-21Cn19 was the most resistant line (average IR was 1.2), followed by ND061868 (average IR was 1.6), whereas Iowa N2052 was the most susceptible (average IR was 7.0) followed by CDC Dancer lines (average IR was 6.3) (Table 3 in Supplemental file). PA101 tended to have low spore number production in comparison to PA102 and PA114. As such, the latter two isolates were chosen to screen a larger number of bi-parental oat populations for the purpose of QTL mapping. With respect to screening the 32 oat line set with the *C. sativus* isolates, 31 of the lines showed a resistant infection response and SA060539 had an intermediate reaction to isolate CS301 (Table 4 in Supplemental file). Only seven lines displayed an intermediate response to isolate CS302 while the rest showed susceptible reactions (Table 4 in Supplemental file). There was more variability observed for isolate CS305, with four lines showing resistance, 21 showing intermediate and seven showing susceptibility (Table 4 in Supplemental file). Similarly for isolate CS307, three lines showed resistance, 18 showed intermediate and 11 showed susceptibility (Table 4 in Supplemental file). More resistance was observed against isolate CS312, with 20 lines showing resistance, nine showing intermediate and only three showing susceptible infection responses (Table 4 in Supplemental file). Finally, 17 lines showed resistance, 11 showed intermediate and four showed susceptible infection responses to isolate CS316 (Table 4 in Supplemental file). Overall, ND061868 was the most resistant oat line (average IR of 2.9), followed by Ave 117.02 and 96-21Cn19 (average IR of 3.0 and 3.1, respectively), whereas SA060539 was the most susceptible lines (average IR of 6.1). The differential reactions observed within these oat lines suggested that isolates CS305, CD307, CS312 and CS316 could be used to screen some bi-parental populations in order to study inheritance of resistance, and in fact, several parents of currently existing bi-parental oat populations could be screened with these isolates. However, CS316 consistently produced sufficient spores for inoculation in addition to producing differential reactions in these 32 oat lines, and was therefore selected for further bi-parental oat population screenings.
4) Genetic mapping of resistance in oat	**Inheritance studies** Four bi-parental oat populations were screened at the seedling stage in the growth chamber to study the inheritance of resistance to different *P. avenae* isolates. **OT3028 x Robust**: The resistant check ND061868 displayed an IR=1.3 while the susceptible check CDC Dancer showed an IR=7.5 against isolate PA101 (Table 13). OT3028 showed resistance (IR=3.0) and Robust susceptibility (IR=5.7; Table 13 and Figure 1). The 92 RILs in this population segregated 28:64 (R:S; Table 15, Figure 1), which is not significantly different from a 1:3 ratio (χ^2=1.45; P=0.23) indicating the presence of two resistance genes. Many lines within the population showed transgressive segregation (better than the resistant parent or worse than the susceptible parent) (Table 13 and Figure 1). The high heritability value (0.90) indicated that the phenotypic variation was mainly due to genetic factors.

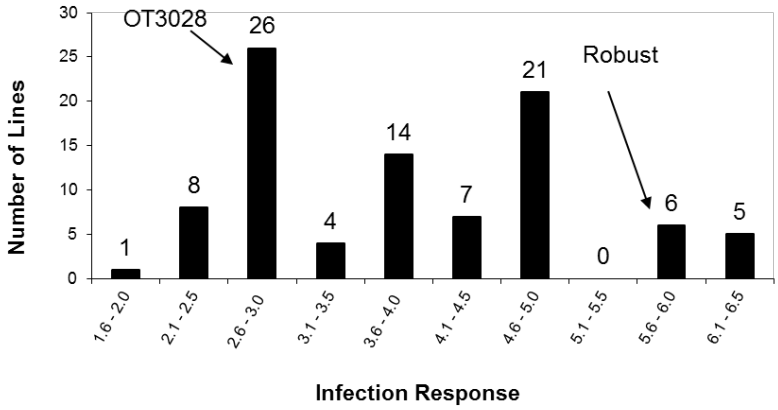

Figure 1. Frequency distribution of infection responses among the 92 recombinant inbred lines of OT3028 x Robust population when screened with *P. avenae* isolate PA101 at the seedling stage in growth chambers.

Ave 117.02 x OT3033: The resistant check ND061868 displayed an IR=1.7 while the susceptible check CDC Dancer showed an IR=6.1 against isolate PA102 (Table 13). Ave 117.02 showed resistance (IR=2.1) and Robust susceptibility (IR=4.4) (Table 13 and Figure 2). The 114 RILs in this population segregated 67:47 (R:S; Table 15, Figure 2), which is not significantly different from a 1:1 ratio (χ^2=3.51; P=0.06) indicating the presence of one resistance gene. Lines within the population also showed transgressive segregation (Table 13 and Figure 2). The heritability value (0.74) remained high, indicating that the phenotypic variation was mainly due to genetic factors.

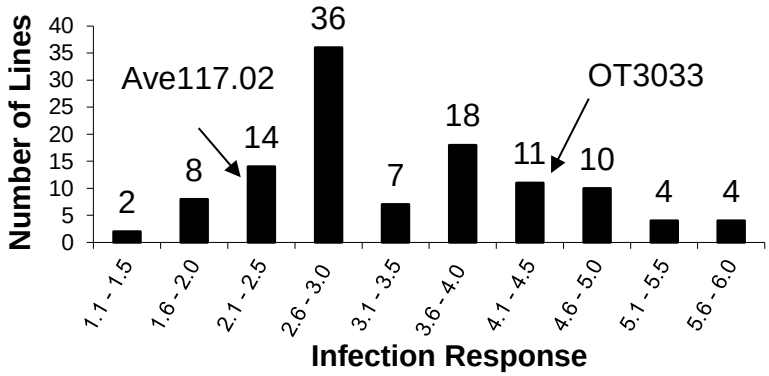

Figure 2. Frequency distribution of infection responses among the 114 recombinant inbred lines of OT3033 x Ave117.02 population when screened with *P. avenae* isolate PA102 at the seedling stage in growth chambers.

OT3011 x Iowa N2052: The resistant check ND061868 displayed an IR=1.7 while the susceptible check CDC Dancer showed an IR=5.1 against isolate PA102 (Table 13). OT3011 showed resistance (IR=3.0) and Iowa N2052 susceptibility (IR=5.8) (Table 13 and Figure 3). The 150 RILs in this population segregated 71:79 (R:S; Table 15, Figure 3), which is not significantly different from a 1:1 ratio (χ^2=0.43; P=0.51) indicating the presence of one resistance gene. Again, lines within the population showed transgressive segregation (Table 13 and Figure 3). The heritability value (0.72) remained high, indicating that the phenotypic variation was mainly due to genetic factors.

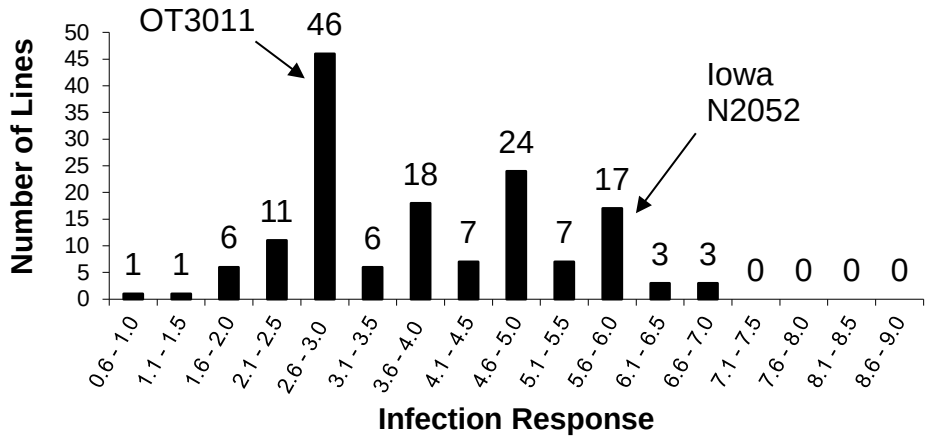

Figure 3. Frequency distribution of infection responses among the 150 recombinant inbred lines of OT3011 x Iowa N2052 population when screened with *P. avenae* isolate PA102 at the seedling stage in growth chambers.

The resistant check ND061868 displayed an IR=1.6 while the susceptible check CDC Dancer showed an IR=6.8 against isolate PA114 (Table 13). OT3011 showed resistance (IR=2.8) and Iowa N2052 susceptibility (IR=6.6) (Table 13 and Figure 4). The 150 RILs in this population segregated 58:92 (R:S; Table 15, Figure 4), which could not be fit to any of the gene models evaluated. Again, lines within the population showed transgressive segregation (Table and Figure 4). The heritability value (0.84) remained high, indicating that the phenotypic variation was mainly due to genetic factors.

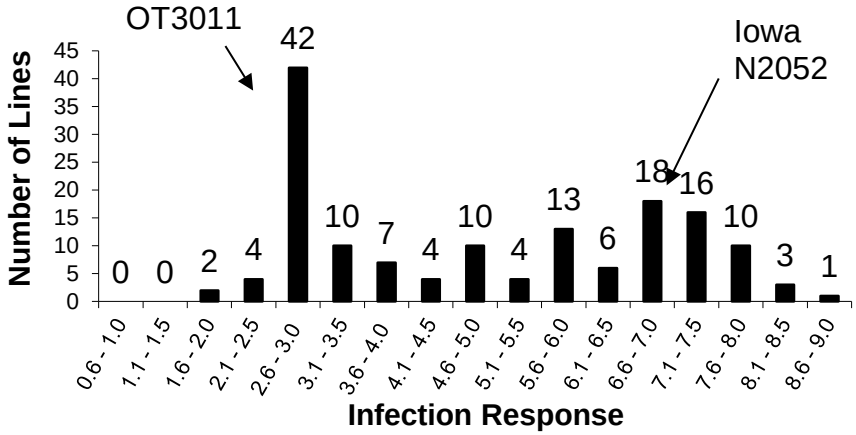

Figure 4. Frequency distribution of infection responses among the 150 recombinant inbred lines of OT3011 x Iowa N2052 population when screened with *P. avenae* isolate PA114 at the seedling stage in growth chambers.

AC Ass/S42 x CDC Dancer: The resistant check ND061868 displayed an IR=1.2 while the susceptible check Iowa N2052 showed an IR=7.3 against isolate PA114 (Table 13). AC Ass/S42 showed resistance (IR=2.9) and CDC Dancer susceptibility (IR=6.8) (Table 13 and Figure 5). The 150 RILs in this population segregated 71:79 (R:S; Table 15, Figure 5), which is not significantly different from a 1:1 ratio (χ^2=0.43; P=0.51) indicating the presence of one resistance gene. Again, lines within the population showed transgressive segregation (Table 13 and Figure 5). The heritability value (0.80) remained high, indicating that the phenotypic variation was mainly due to genetic factors.

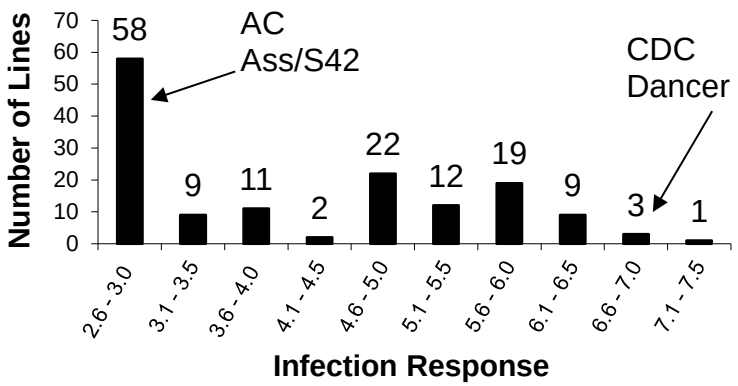

Figure 5. Frequency distribution of infection responses among the 150 recombinant inbred lines of AC Ass/S42 x CDC Dancer population when screened with *P. avenae* isolate PA114 at the seedling stage in growth chambers.

Three bi-parental oat populations were screened at the seedling stage in the growth chamber to study the inheritance of resistance to different *C. sativus* isolates.

AC Ass/S42 x CDC Dancer: The resistant check ND061868 displayed an IR=1.4 while the susceptible check Iowa N2052 showed an IR=4.6 against isolate CS316 (Table 14). AC Ass/S42 showed resistance (IR=3.2) and CDC Dancer susceptibility (IR=6.1) (Table 14 and Figure 6). The 150 RILs in this population segregated 21:129 (R:S; Table 16, Figure 6), which is not significantly different from a 1:6 ratio (χ^2=3.23; P=0.58) indicating the presence of three resistance genes. Again, lines within the population showed transgressive segregation (Table 14 and Figure 6). The heritability value (0.51) was moderate for this trait, indicating that the phenotypic variation was controlled less by genetic factors.

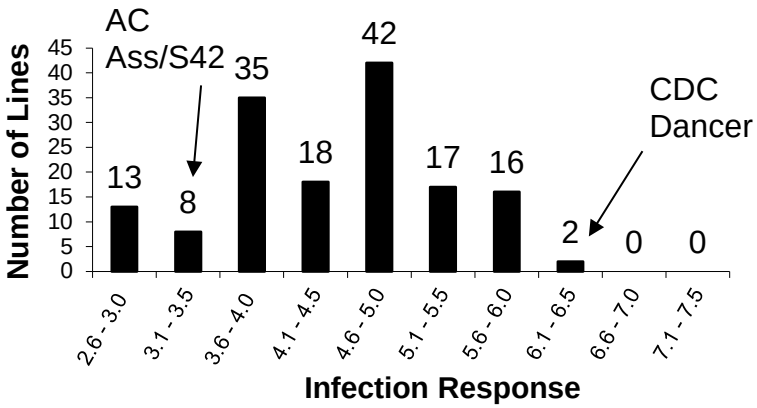

Figure 6. Frequency distribution of infection responses among the 150 recombinant inbred lines of AC Ass/S42 x CDC Dancer population when screened with *C. sativus* isolate CS316 at the seedling stage in growth chambers.

SA060539 x OT3028: The resistant check ND061868 displayed an IR=2.0 while the susceptible check CDC Dancer showed an IR=6.2 against isolate CS316 (Table 14). SA060539 showed resistance (IR=3.2) and OT3028 susceptibility (IR=5.2) (Table 14 and Figure 7). The 100 RILs in this population segregated 14:86 (R:S; Table 16, Figure 7), which could not be fit to any of the gene models evaluated. Again, lines within the population showed transgressive segregation (Table 14 and Figure 7). The heritability value (0.42) was moderate for this trait, indicating that the phenotypic variation was controlled less by genetic factors.

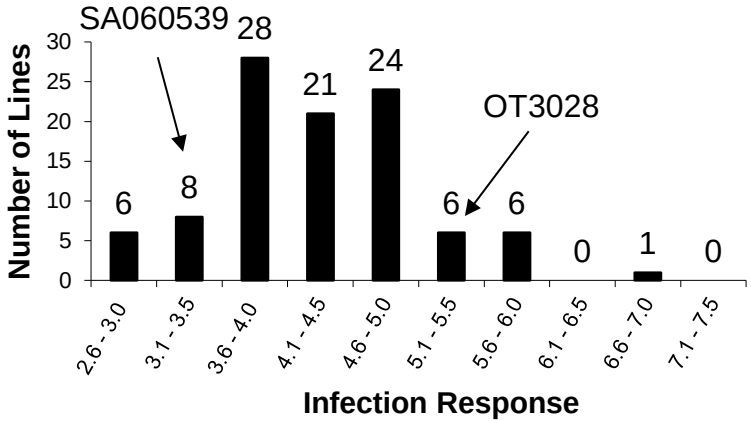

Figure 7. Frequency distribution of infection responses among the 100 recombinant inbred lines of SA060539 x OT3028 population when screened with *C. sativus* isolate CS316 at the seedling stage in growth chambers.

ND061868 x CDC Morrison: The resistant check 96-21Cn19 displayed an IR=1.4 while the susceptible check CDC Dancer showed an IR=6.4 against isolate CS316 (Table 14). ND061868 showed resistance (IR=2.7) and CDC Morrison susceptibility (IR=5.6) (Table 14 and Figure 8). The 150 RILs in this population segregated 21:129 (R:S; Table 16, Figure 8), which is not significantly different from a 1:6 ratio (χ^2=0.10; P=0.76) indicating the presence of three resistance genes. Again, lines within the population showed transgressive segregation (Table 14 and Figure 8). The heritability value (0.42) was moderate for this trait, indicating that the phenotypic variation was controlled less by genetic factors.

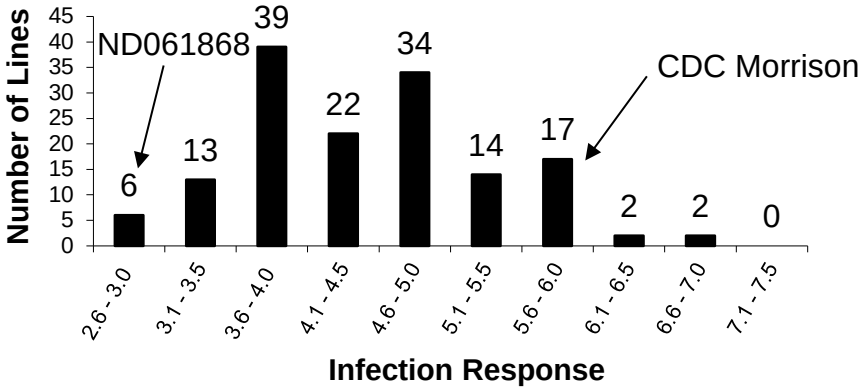

Figure 8. Frequency distribution of infection responses among the 150 recombinant inbred lines of ND061868 x CDC Morrison population when screened with *C. sativus* isolate CS316 at the seedling stage in growth chambers.

Table 13. Infection responses of parents and recombinant inbred lines from four different populations to isolates of *P. avenae* when screened at the seeding stage.

Population (R x S)	Isolate[a]	Resistant Parent	Susceptible Parent	RIL Population		Checks		$LSD_{0.05}$[b]	Heritability
				Mean	Range	ND061868 (R)	CDC Dancer (S)		
OT3028 x Robust	PA101	3.0	5.7	4.7	2.0-9.0	1.3	7.5	1.5	0.90
Ave117.02 x OT3033	PA102	2.1	4.4	3.4	1.3-6.0	1.7	6.1	1.4	0.74
OT3011 x Iowa N2052	PA102	3.0	5.8	3.9	1.0-7.0	1.7	5.1	1.4	0.72
OT3011 x Iowa N2052	PA114	2.8	6.6	5.0	2.0-8.7	1.6	6.8	1.2	0.84
AC Ass/S42 x CDC Dancer	PA114	2.9	6.8	4.2	2.0-7.5	1.2	7.3*	1.2	0.80

[a]*Pyrenophora avenae* isolate.
[b]Least significant differences (P=0.05).
*Iowa N2052 was used as the susceptible check

Table 14. Infection responses of parents and recombinant inbred lines from three different populations to isolates of *C. sativus when* screened at the seeding stage.

Population (R x S)	Isolate[a]	Resistant Parent	Susceptible Parent	RIL Population		Checks		$LSD_{0.05}$[b]	Heritability
				Mean	Range	ND061868 (R)	CDC Dancer (S)		
AC Ass/S42 x CDC Dancer	CS316	3.2	6.1	4.5	3.0-6.3	1.4	4.6*	1.4	0.51
SA060539 x OT3028	CS316	3.2	5.2	4.3	3.0-6.0	2.0	6.2	1.5	0.42
ND061868 x CDC Morrison	CS316	2.7	5.6	4.5	2.6-6.3	1.4**	6.4	1.5	0.42

[a]*Cochliobolus sativus* isolate.
[b]Least significant differences (P=0.05).
*Iowa N2052 was used as the susceptible check
**96-21Cn19 was used as the resistant check.

Table 15. Chi-square analysis in four oat populations to determine inheritance of resistance to *P. avenae.*

Population	Isolate	No. R Lines	No. S Lines	R:S Ratio	X^2 Value	P value	No. Genes*
OT3028 x Robust	PA101	28	64	1:3	1.45	0.23	Two
Ave117.02 x OT3033	PA102	67	47	1:1	3.51	0.06	One
OT3011 x Iowa N2052	PA102	71	79	1:1	0.43	0.51	One
OT3011 x Iowa N2052	PA114	58	92	1:1	7.71	0.01	-
AC Ass/S42 x CDC Dancer	PA114	71	79	1:1	0.43	0.51	One

*****Other gene ratios were evaluated, but only those with the best fit are given here. -: no model could be identified.

Table 16. Chi-square analysis in three oat populations to determine inheritance of resistance to *C. sativus.*

Population	Isolate	No. R Lines	No. S Lines	R:S Ratio	X^2 Value	P value	No. Genes*
AC Ass/S42 x CDC Dancer	CS316	21	129	1:6	3.23	0.58	Three
SA060539 x OT3028	CS316	14	86	1:6	51.23	0.00	-
ND061868 x CDC Morrison	CS316	20	130	1:7	0.10	0.76	Three

*Other gene ratios were evaluated, but only those with the best fit are given here. -: no model could be identified.

Bi-parental QTL mapping

The bi-parental crosses AC Ass/S42 x CDC Dancer and OT3011 x Iowa N2052 were chosen for QTL genetic mapping studies because, 1) their phenotypic segregation ratios to isolates PA114 and PA102, respectively, appeared to fit a single gene model of resistance inheritance, 2) the two populations displayed resistance to different isolates so it would be valuable to know if the same QTL were effective against both isolates, and 3) the AC Ass/S42 x CDC Dancer population also showed resistance against the CS316 isolate.

A total of 4975 high quality markers were used for linkage mapping in the AC Ass/S42 x CDC Dancer and OT3011 x Iowa N2052 populations. 909 markers were mapped to 36 linkage groups for a total map length of 3562.520 cM in the AC Ass/S42 x CDC Dancer population, while 848 markers were mapped to 27 linkage groups for a total map length of 3448.923 cM in the OT3011 x Iowa N2052 population.

One major QTL was significantly associated with resistance to both PA102 and PA114 isolates in the OT3011 x Iowa N2052 population. The peak of this QTL was located on linkage group 1 at approximately 159-162 cM (Table 17). This locus is located on chromosome 5C at position 89.5 cM of the consensus map (Chaffin et al. 2016). Gene names, Gene Ontology information, Protein ID, and product name for genes located within 15 cM of the QTL peak are provided in Supplemental Table 5. Many of the genes have functions related to cell proliferation, cell death, and DNA replication.

Table 17. Resistance QTL identified in the OT3011 x Iowa N2052 population when inoculated with *P. avenae* isolates PA 101 and PA114.

Isolate	Linkage Group	QTL Peak and Width (cM)	QTL Peak Marker	LOD Score	LOD Significance Threshold	Mu_A[a]	Mu_B[b]	Explained Variance (%)[c]
PA102	1	162.1 (110.3-216.6)	ES22_c1052_894	29.6	10.7	4.8	2.7	59.2
PA114	1	159.9 (115.5-176.4)	ES15_lrc9414_222	36.7	19.9	6.5	3.2	66.7

[a]estimated mean disease reaction associated with the homozygous OT3011 allele.
[b]estimated mean disease reaction associated with the homozygous Iowa N2052 allele.
[c]percentage of variance explained by the QTL.

No QTL were identified in the AC Ass/S42 x CDC Dancer population associated with resistance to *C. sativus* isolate CS316. However, a QTL associated with resistance to *P. avenae* isolate PA114 was very close to meeting the LOD significance threshold (Table 18). Interestingly, this QTL also mapped to chromosome 5C on the oat consensus map at position 71.5 cM (Chaffin et al. 2016).

Table 18. Resistance QTL identified in the AC Ass/S42 x CDC Dancer population when inoculated with *P. avenae* isolate PA114.

Isolate	Linkage Group	QTL Peak and Width (cM)	QTL Peak Marker*	LOD Score	LOD Significance Threshold	Mu_A[a]	Mu_B[b]	Explained Variance (%)[c]
PA114	5	71.5 (66.5-78.9)	ES22_c7656_144	3.5	3.8	4.6	3.8	7.4
			ES07_c1546_339			4.5	3.8	7.2

[a]estimated mean disease reaction associated with the homozygous OT3011 allele.
[b]estimated mean disease reaction associated with the homozygous Iowa N2052 allele.
[c]percentage of variance explained by the QTL.
*QTL Peak lies between two markers

Genome-wide association mapping in Western Canadian elite lines

The distribution of disease reactions in the 150 member panel of the association mapping population can be seen in Figure 9. A visual examination of the plot suggests a possible bi-modal distribution of phenotypes with the 3.6-4.0 category lying between the two modes of the distributions. In an attempt to accentuate this bimodal distribution and understand the impact on association mapping results, the 32 lines representing disease reactions that fell within the 3.1-4.5 categories were removed (Figure 10).

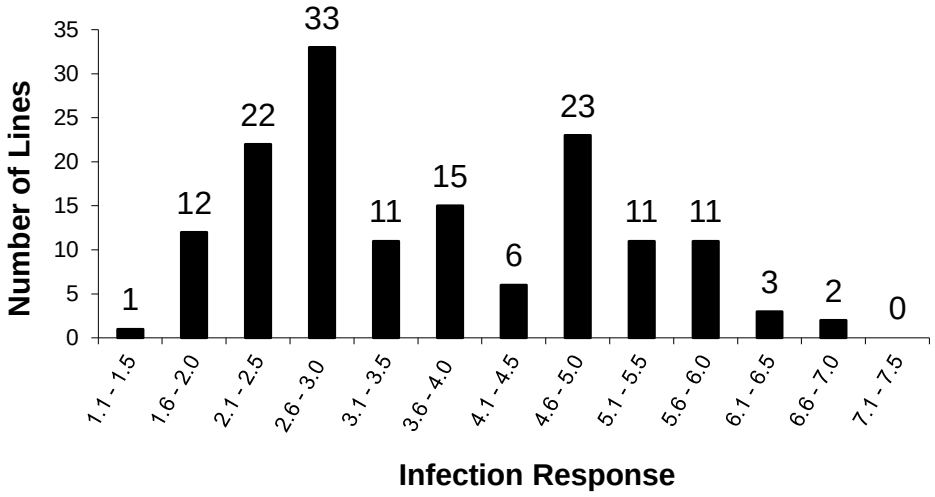

Figure 9. Histogram showing disease reactions of the 150 member association mapping panel in response to *P. avenae* isolate PA114.

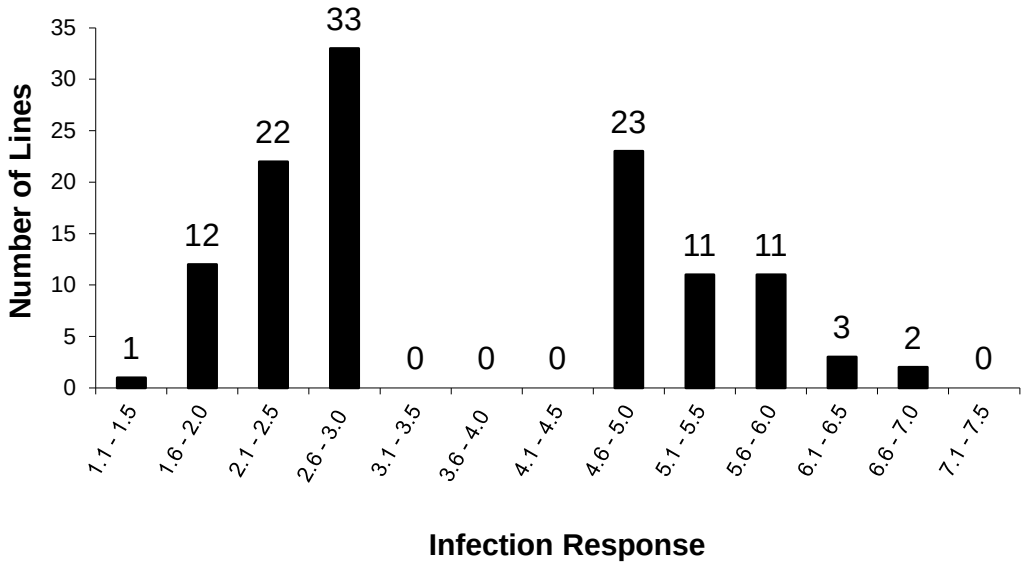

Figure 10. Histogram showing disease reactions of the 118 member association mapping panel in response to *P. avenae* isolate PA114.

After removing markers that were monomorphic, had greater than 15% failed or null reactions, or showed highly skewed segregation ($p<0.01$) a high quality set of 2586 markers were available for association mapping analysis. After evaluation of the data using the 16 different association mapping methods four markers were identified, using a minimum of 3 methods, as being associated with resistance to *P. avenae* isolate PA114 (Table 14, Supplemental Figure 1 and Supplemental Figure 2). In all four cases, it did not matter if the Self-K or K + Q methods of population stratification control were used as both were equally effective at detecting significant markers (Table 14). Similarly, reducing the significance threshold did not increase the number of loci detected, that is, the default p-values detected the same loci (Table 14). As such, future association studies could just use one method of population stratification control in conjunction with the default significance threshold. For three of the four markers (GMI_ES14_c2536_539, GMI_ES22_c1163_38 and GMI_ES03_c10561_484) both software programs detected the same loci. It was only the GMI_ES02_c15089_196 loci that was detected with FarmCPU, and this was also the only marker detected using the reduced population size. Similarly, three of the four markers were detected using the original disease reaction data and the R/S binary scoring method. Only GMI_ES03_c10561_484 was detected using only the R/S binary scoring method. Using multiple methods to declare a significant association provided confidence that these loci are worth further investigation for providing resistance to *P. avenae*. Of these four loci, GMI_ES02_c15089_196 mapped to chromosome 5C at position 23.6 cM (Supplemental Table 6).

Table 14. Genetic markers identified by a minimum of three different methods as being associated with resistance to *P. avenae* isolate PA114 in the association mapping panel population.

Marker ID	Mapping Method[a]	p-value[b]	R^2 value[c]
GMI_ES02_c15089_196	FarmCPU 118 Lines Original Scoring Self-K Default p-threshold	2.73E-08	
	FarmCPU 118 Lines R/S Scoring Self-K lowered p-threshold	4.46E-07	
	Farm CPU 118 Lines R/S Scoring K + Q lowered p-threshold	1.12E-06	
	Farm CPU 118 Lines R/S Scoring Self-K default p-threshold	6.82E-04	
	Farm CPU 118 Lines R/S Scoring K+Q default p-threshold	8.06E-04	
GMI_ES14_c2536_539	FarmCPU 150 Lines Original Scoring Self-K lowered p-threshold	6.17E-07	
	GAPIT 150 Lines R/S Scoring K+Q default p-threshold	1.45E-03	0.08
	FarmCPU 150 Lines R/S Scoring Self-K default p-threshold	6.51E-04	
	FarmCPU 150 Lines R/S Scoring K+Q default p-threshold	7.51E-04	
GMI_ES22_c1163_38	GAPIT 150 Lines Original Scoring Self-K default p-threshold	1.60E-03	0.08
	GAPIT 150 Lines R/S Scoring Self-K default p-threshold	6.32E-04	0.09
	GAPIT 150 Lines R/S Scoring K+Q default p-threshold	2.23E-03	0.07
	FarmCPU 150 Lines R/S Scoring Self-K lowered p-threshold	4.38E-07	
	FarmCPU 150 Lines R/S Scoring K+Q lowered p-threshold	1.18E-07	
	FarmCPU 150 Lines R/S Scoring K+Q default p-threshold	1.26E-03	
GMI_ES03_c10561_484	GAPIT 150 Lines R/S Scoring K+Q default p-threshold	2.25E-03	0.08
	FarmCPU 150 Lines R/S Scoring Self-K default p-threshold	7.78E-04	
	FarmCPU 150 Lines R/S Scoring K+Q default p-threshold	1.27E-03	

[a] Mapping methods have five criteria: GAPIT or FarmCPU software, 150 lines or 118 lines, original scoring method or binary (R/S) scoring, Kinship matrix calculated by R-studio (Self-K) or Kinship and Structure calculations (K + Q) from SPAGeDi and Structure programs respectively, and default p-threshold of 0.01/number of markers used for association mapping (0.01/2,586=3.87E-06) or the lowered p-threshold of 0.005.

[b] Significance value associated with the marker

[c] Variance explained by the marker. Values were only available using the GAPIT software.

8. Discussion: *Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.*

Over the four years (2014-2017) that field surveys were conducted in commercial oat fields to evaluate the prevalence of oat leaf blotch pathogens *P. avenae* was the most often identified, being present in 59% of the 160 fields surveyed. *Cochliobolus sativus* was present in 23% of surveyed fields while *S. avenae* was only identified in 3% of fields. The ranking prevalence of these pathogens was consistent across all four years and differs from prior surveys conducted where S*. avenae* was observed in all years and with greater prevalence than *C. sativus* in most years (2011-2013). While it is hard to draw firm conclusions as to why this difference was observed, the higher average summer temperatures (May-August) from 2015-2017 may suggest temperature, as opposed to precipitation amount (which varied across all years from below to above average) may favour the growth of *C. sativus* over *S. avenae* The observation that *P. avenae* is consistently the most prevalent oat leaf spot pathogen regardless of growing conditions indicates it may be less impacted by these environmental factors.

Over the course of the project we were able to culture and preserve pure isolates of *P. avenae*, *C. sativus* and *S. avenae*. These isolates may be useful, and are available upon request, for future genetic mapping, germplasm screening or pathogen virulence studies.

Methods to culture these pathogens on artificial media and to develop an effective inoculation procedure to screen oat germplasm were successful. Critical factors evaluated to establish an inoculation procedure included spore concentration, exposure duration of inoculated plants to high humidity to promote spore germination, age of plants when inoculated and number of days after inoculation to conduct disease reaction evaluation. Based on the prevalence (and thus assumed importance) of *P. avenae* all of these factors were evaluated for this pathogen. Once optimal disease producing conditions were identified pertaining to humidity duration, age of plants at inoculation and days after inoculation to reach maximal disease expression (for scoring), only spore concentration was assessed for *C. sativus*. Due to the near absence of *S. avenae* in field surveys and the difficulty of producing sufficient spores from the isolated *S. avenae* isolates, no further work was done with this pathogen. Experiments with different spore concentrations of *P. avenae* revealed that 30K spores/mL was optimal for producing differential reactions among oat lines. For *C. sativus*, 75K or 100K spores/mL produced similarly good differential among oat lines. Exposure of inoculated plants to different periods of humidity suggested that incubation at 100% humidity for 24h after inoculation was effective and necessary during the initial stage of infection in order to develop disease symptoms. Further experiments indicated that plants inoculated 2 weeks after seeding developed as much disease as plants inoculated at later stages of development, while 7 days of incubation following inoculation was sufficient to allow maximal disease development and that. Overall, methods to culture these pathogens on artificial media and to develop an effective inoculation procedure to screen oat germplasm were successful

A set of nine oat varieties selected based on their genetic diversity (i.e. they represent varieties from 6 breeding programs and different end-uses) were used to assess the virulence phenotype of 15 *P. avenae* isolates and 17 *C. sativus* isolates. These studies revealed variability in pathogen virulence and infection response in oat lines. There was significant line x isolate interaction with both pathogens ($P < 0.0001$), indicating specificity in the host-pathogen interaction. There was a similar spectrum of virulence between the two pathogens with some isolates producing susceptible reactions against 8 of 9 lines (e.g. PA108 or CS302), whereas others were unable to produce significant disease on all lines (e.g. PA102 or CS309). There was a slightly lower overall infection rating among the *C. sativus* isolates (3.7) versus the *P. avenae* isolates (4.8). Given the small sample size this may simply represent sampling bias or perhaps this may reflect less virulence of this pathogen which may explain its lower prevalence in field surveys. Based on these results, a smaller subset of *P. avenae* and *C. sativus* isolates were selected to screen a set of 32 oat lines in order to identify resistant germplasm for breeding, as well as, identify parents of currently existing bi-parental populations that could be used for QTL mapping.

Evaluation of the 32 lines with three isolates of *P. avenae* and six isolates of *C. sativus* indicated many lines carried resistance, in particular, 96-21Cn19 (Aberystwyth University, UK), ND061868 (University of North Dakota) and Ave117.02 (INIA, Chile) showed a high level of resistance to all isolates of *P. avenae* and most isolates of *C. sativus*. These lines would make good parents within breeding programs to transfer leaf blotch resistance to future oat varieties. On the other end of the spectrum CDC Dancer was the most susceptible line to *P. avenae*, along with Iowa N2052, while CDC Dancer and SA060539 were most susceptible to *C. sativus*. In general, lines that showed better resistance to one pathogen were also more resistant to the other pathogen.

Based on the results from screening the 32 oat lines four bi-parental populations were identified to evaluate genetic inheritance of resistance to three *P. avenae* isolates and three bi-parental populations were identified to study resistance against one *C. sativus* isolate. In six of the eight inheritance studies segregation of resistance fit a one or two gene model for *P. avenae* or a three gene model for *C. sativus*. In two of the studies no model could be fit. Reflecting the number of genes responsible for resistance to each pathogen, the heritability for *P. avenae* resistance was significant higher (0.72-0.90) than for *C. sativus* (0.42-0.51). Populations derived from OT3011 x Iowa N2052 and AC Ass/S42 x CDC Dancer were selected for genotyping and QTL mapping because OT3011 x Iowa N2052 showed resistance to two isolates of *P. avenae* so it was of interest if the same locus was responsible for resistance to both isolates, and because the AC Ass/S42 x CDC Dancer showed resistance to both *P. avenae* and *C. sativus*. QTL mapping revealed a single, strong QTL (explaining 67% of the variation) on chromosome 5C (position: 160 cM) that was effective against both *P. avenae* isolates used to screen the OT3011 x Iowa N2052 population. This finding indicated that perhaps this locus may be suitable to provide resistance against a broad range of *P. avenae* isolates which would make breeding for this trait simpler. However, additional QTL mapping with other isolates would need to be done to confirm this. A weak QTL was also located on chromosome 5C in the AC Ass/S42 x CDC Dancer, but it was located at a different position (71 cM) than the one found in the OT3011 x Iowa N2052 population. Given that AC Ass/S42 and OT3011 are derived from different breeding programs and have no common ancestors going back 5 generations it is perhaps not surprising that different resistance loci would exists in these two lines. The strong QTL identified in the OT3011 x Iowa N2052 population is now being converted to a TaqMan marker to allow high throughput evaluation of oat breeding lines. Such a marker will allow selection for this trait which previously had not been a criteria within the CDC oat breeding program.

In an attempt to identify additional resistance loci from a broader spectrum of oat germplasm, an association mapping study was conducted with 150 elite breeding lines adapted to Western Canada which were bred at seven different breeding programs. Using a variety of association mapping parameters to assess the robustness of our methods and provide confidence in any loci identified, four loci were associated with resistance to *P. avenae* isolate PA114. These loci were present on chromosomes 5C, 8A, 9D and 21D. It was interesting to note the involvement of the 5C chromosome in resistance, but again the locus position at 23 cM differed from the QTL results. Identification of lines carry one or more of these resistance loci could be used in more traditional bi-parental QTL mapping studies to more clearly understand the inheritance and variance explained (i.e. strength of the QTL) by these loci.

A number of significant accomplishments and findings were made through this project including, 1) *P. avenae* is the most relevant leaf blotch pathogen in terms of prevalence, 2) isolates of *P. avenae* and *C. sativus* display a range of pathogenicity across oat germplasm, 3) oat germplam resistant to these pathogens exist and inheritance of resistance tends to be controlled by 1-3 genes and 4) QTL linked to *P. avenae* resistance were identified and will assist in incorporation of resistance into future oat varieties.

9. Conclusions and Recommendations: *Highlight significant conclusions based on the previous sections, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project.*

Conclusions:

1) *P. avenae* is the most prevalent oat leaf pathogen, followed by *C. sativus*. *S. avenae* appears to have limited relevance to oat leaf blotch disease,

2) Isolates of *P. avenae* and *C. sativus* display a range in virulence phenotypes, i.e. different pathotypes likely exist,

3) Effective inoculation of oat with *P. avenae* and *C. sativus* requires:

- Inoculation of 14 day old oat seedlings
- 50K and 30K colonies/mL inoculum concentrations for *C. sativus* and *P. avenae*
- 100% humidity for 24 hours in darkness following inoculation for optimal spore germination
- Assessment of disease development 7 days after inoculation

4) Resistant oat germplasm, such as OT3011, 96-21Cn19, ND061868 and Ave117.02, can serve as parents in breeding programs to incorporate useful resistance to oat leaf blotch,

5) Resistance to *P. avenae* appears to be more heritable and governed by 1-2 genes while resistance to C. sativus is moderately

heritable and governed by 3 genes,

6) A major resistance QTL explaining 67% of the phenotypic variation for two *P. avenae* isolates was identified from OT3011 and is the focus of TaqMan marker development. This marker will aid in the incorporation of leaf blotch resistance in future oat varieties,

7) Four additional resistance loci were identified in an association mapping population. Future work to understand these resistance loci through bi-parental QTL mapping would be useful.

Recommendations

1) develop and use TaqMan marker linked to OT3011 resistance QTL to select leaf blotch resistance in breeding programs,

2) prioritize resistance breeding against *P. avenae* due to its consistent and significant prevalence in commercial fields. Use isolate PA114 to assess future breeding lines for resistance,

3) determine if resistance from other sources (e.g. 96-21Cn19, ND061868, Ave117.02 and selected lines in association mapping population) differs from that in OT3011.

10. Success stories/ practical implications for producers or industry: *Identify new innovations and /or technologies developed through this project; and elaborate on how they might impact the producers /industry.*

This is the first report on methods to inoculate and evaluate oat germplasm for resistance to *P. avenae* and *C. sativus* which will allow future research and breeding for resistance to these pathogens to take place. Resistant oat germplasm was identified to these pathogens and a major resistance QTL effective against multiple isolates of *P. avenae* was identified. Molecular markers linked to this QTL are being developed to allow genotyping of resistant oat breeding lines.

Collectively these successes will allow oat breeding programs to start breeding for oat leaf blotch resistance, a previously neglected disease of oat, which should lead to improved varieties better able to protect their yield potential from losses caused by leaf blotch pathogens.

11. Patents/ IP generated/ commercialized products: *List any products developed from this research.*

None.

12. List technology transfer activities: *Include presentations to conferences, producer groups or articles published in science journals or other magazines.*

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13. List any industry contributions or support received.

We gratefully acknowledge the financial support from the Western Grains Research Foundation and the Saskatchewan Oat Development Commission for this project.

14. Is there a need to conduct follow up research? *Detail any further research, development and/or communication needs arising from this project.*

1) Develop TaqMan marker for the chromosome 5C resistance QTL,

2) Screen additional isolates of *P. avenae* (especially more virulent ones like PA108) on the OT3011 x Iowa N2052 population to better understand the effectiveness of this resistance gene,

3) Characterize resistance from other sources (e.g. 96-21Cn19, ND061868, Ave117.02 and selected lines in association mapping population) to understand if they harbour different QTL than that found in OT3011.

15. Acknowledgements. *Include actions taken to acknowledge support by the Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement.*

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16. Appendices: *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited*

A file containing supplemental tables and figures is being sent along with this report.

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