Breeding for Resistance to Leaf Blotch Pathogens in Saskatchewan Oat

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Summary of results

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

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,

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