Government of Saskatchewan
Ministry of Agriculture

ADF Project Final Report Format

1. Project title and ADF file number.

"FHB screening of CDC barley breeding selections, 2016-2020"
ADF#: 20150126

2. Name of the Principal Investigator and contact information.

Aaron Beattie
Crop Development Centre
University of Saskatchewan
51 Campus Drive
Saskatoon, SK S7N 5A8
Phone: 306-966-2102
Fax: 306-966-5015
Email: aaron.beattie@usask.ca

3. Name of the collaborators and contact information.

Dr. Ana Badea
Brandon Research and Development Centre
Agriculture and Agri-Food Canada
2701 Grand Valley Road
PO Box 1000A, RR3
Brandon, MB R7A 5Y3
Phone: 204-578-6573
Fax: 204-578-6524
Email: ana.badea@canada.ca

Mr. James Tucker
Brandon Research and Development Centre
Agriculture and Agri-Food Canada
2701 Grand Valley Road
PO Box 1000A, RR3
Brandon, MB R7A 5Y3
Phone: 204-578-6592
Fax: 204-578-6528
Email: james.tucker@canada.ca

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.*

Barley is a significant Canadian crop that provided approximately $2.3 billion in revenue to producers, maltsters and exporters in 2019. To build on Canada's position as a supplier of premium quality barley and malt requires developing barley varieties with improved resistance to Fusarium head blight (FHB), or more specifically, resistance to deoxynivalenol (DON) accumulation. The economic impact of FHB across the Canadian cereal industry has been estimated to be somewhere between $50-300 million annually. The purpose of this project was to support activities that would assist in the breeding of barley varieties with improved resistance to DON accumulation, including: 1) evaluation of CDC breeding lines for low DON accumulation, 2) development of tools and methods to conduct DON screening at the CDC, 3) improvement of predictive NIR calibrations of barley grain DON content for use as a screening tool in breeding programs and, 4) evaluation of a genetic population for the purpose of identifying QTL linked to low DON accumulation that could be used as a screening tool in breeding programs.

Approximately 2,250 breeding lines were evaluated for DON content each year with 9-26% of the feed/forage lines and 13-41% of the malting lines showing DON accumulation below that of the low DON check line CDC Mindon. A total of 39 breeding lines that were evaluated under this project were advanced to the western Canadian cooperative registration tests with 80% of them receiving an FHB rating that met or exceeded the intermediate resistance rating required for registration. A total of 5,415 breeding lines were evaluated for DON content at the CDC using the Gallery analyzer and Diagnostix EZ-Tox™ DON kit. DON values obtained from the CDC showed a correlation of r=0.93 to those obtained from a commercial lab. A correlation of 0.57 was observed between the CDC NIR prediction for DON and DON values obtained from the Gallery analyzer using 1,042 lines. A 169-member genetic mapping population was genotyped and evaluated for DON, FHB score, heading date and height. Seven genomic regions were identified as influencing DON content and importantly none of these regions were associated with heading date or height.

A number of significant accomplishments were made through this project including, 1) two lines evaluated under this project, CDC Valdres (HB17340) hulless food barley and CDC Renegade (FB209) forage barley, were registered for commercial production, with one feed barley (TR19175) likely to be registered in 2021, 2) the CDC is now able to evaluate all its breeding lines for DON and make selection decisions based on this information prior spring seeding. This represents a significant achievement in our ability to breed for improved DON tolerance, 3) the NIR prediction for DON content was improved during this project (r=0.57) when compared to the previous 5-year project (r=0.38) and, 4) the seven genomic regions associated with DON content each show a low effect on DON, but collectively they will have value when incorporated as fixed variables into genomic selection models that can be used by breeders to select barley lines with low DON content.

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

Barley is a significant Canadian crop that has been seeded since 2016 on an average of 6.8 million acres and 2.8 million acres across Canada and Saskatchewan, respectively (Statistics Canada, 2020a). In both 2019 and 2020 there were substantial increases in barley acreage with 7.4 million acres seeded across Canada and 3.1 million acres seeded in Saskatchewan (Statistics Canada, 2020a). Acreage devoted to two-row barley varieties continues to heavily dominate in relation to six-row varieties and currently accounts for 95% of barley acres across western Canada (Canadian Grain Commission, 2019). Of these acres, about 55% are devoted to malting barley varieties and 45% to feed varieties (Canadian Grain Commission, 2019). In 2019 Canada produced just over 10 million tonnes of barley, of which 2.3 million tonnes of malting barley was purchased from growers (this represents an estimated farm-gate value of $850 million) with about 300,000 tonnes (with a value of $400 million) used by the domestic malting industry (Statistics Canada, 2020b; Beer Canada, 2020). The malt used domestically by brewing companies generates $5.7 billion in government revenues via taxation and contributes $13.6 billion to the Canadian GDP (Beer Canada, 2020). An additional 530,000 tonnes of malt were exported with a value of $410 million (Statistics Canada, 2020b). In 2019-20 Canada exported 2.2 million tonnes of barley (1.3 million tonnes of malt barley and 850,000 tonnes of feed barley) which contributed another $700 million dollars to the Canadian economy (Statistics Canada, 2020b). Strong exports to China over the past two years have reached 1.5 million tonnes annually and exports may reach 2 million tonnes in 2020-21 due to ongoing trade disputes between China and Australia (P. Watts, CBMTB, personal communication).

To build on Canada's position as a supplier of premium quality barley and malt requires developing barley varieties with improved traits. One such important trait is Fusarium head blight (FHB), or more specifically, resistance to deoxynivalenol (DON) accumulation. Fusarium head blight, most commonly incited in western Canada by *Fusarium graminearum* Schwabe, remains the most important disease in this crop. Within the barley breeding community, breeding for FHB resistance has focused predominantly on resistance to DON accumulation in the grain. This is due to the fact that there is inconsistent, and often poor, correlation between visual FHB symptoms and the corresponding concentration of DON in the grain (Berger et al. 2014; He et al. 2015). The primary economic consequence of FHB infection of barley is via the presence of DON in the grain which significantly impacts quality. DON can render barley grain unacceptable for malting and brewing (above 0.5 ppm), swine and dairy cattle feed (above 1.0 ppm) or beef cattle feed (above 5.0 ppm). With respect to malting and brewing, DON has been shown to negatively impact barley germination, leading to uneven growth and an inability to control the final malt product (Flannigan 1999). DON (specifically DON-3-glucoside) concentrations have been shown to increase during the malting process (Habler et al. 2016), thereby exacerbating the downstream processing problems associated with its presence. These problems include the tendency to increase wort soluble protein, free amino nitrogen and color (Schwarz et al. 2006) and to cause beer gushing (Virkajärvi et al. 2017). For malting barley producers, downgrading their barley crop from malting to feed grade can mean a financial loss of up to $105,000 on 1,000 acres (discount of $1.50/bushel x 70

bushels/acre = $105/acre). Collectively, the economic impact of FHB across the Canadian cereal industry has been estimated to be somewhere between $50-300 million annually since the 1990s (Alberta Fusarium Action Committee, 2015).

The purpose of this project is to partially support several activities, including: 1) the evaluation of new CDC barley germplasm (with unknown levels of FHB resistance) and advanced CDC breeding lines (with FHB resistant parentage) for FHB resistance/low DON accumulation within the collaborative FHB nurseries already established across Canada, 2) develop and conduct DON screening at the CDC using commercially available equipment and assays, 3) to improve predictive NIR calibrations of barley grain DON content for use as an inexpensive screening tool in the CDC barley breeding program and, 4) to evaluate a set of barley lines showing a range of tolerance to FHB and DON accumulation for the purpose of identifying QTL linked to FHB tolerance that could be used as an early generation screening tool in the CDC barley breeding program prior to entering lines into the collaborative FHB nurseries.

Collectively, these activities will help the CDC to continue breeding improved DON resistant varieties that will help alleviate the negative financial costs to growers associated with high DON infection on barley, and support the production of high-quality barley for both the domestic and export markets that western Canada has become known for.

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

Objective 1. Identify CDC barley breeding lines with improved FHB/DON tolerance

A total of 2,250 CDC breeding lines were planted each year in the FHB nursery at the AAFC-Brandon Research and Development Centre (BRDC). The nursery was planted within the first two weeks of May in 0.9 m long rows, spaced 0.3 m apart at a rate of 30-40 seeds per row. Sets of resistant (CDC Mindon), moderate (AC Metcalfe) and susceptible (CDC Bold) checks were planted every 50 rows. The nursery was fertilized and sprayed to control weeds and insects. A propaconazole fungicide was applied before the elongation phase and before the first *F. graminearum* inoculation to control *Cochliobolus sativus* (spot blotch) which produces head symptoms similar to FHB. About 500 kg of inoculum was prepared in the lab in Brandon from corn seed infested with 4 isolates of *F. graminearum* (2 chemotypes each of 15ADON & 3ADON) when the earliest lines were at flag leaf stage. Three applications were applied by hand at a rate of 5 g per entry at weekly intervals following the initial application. Entries were hand-harvested when mature, individually threshed and a 20 g sample of grain was ground using a Perten 3610 laboratory mill and sent to the CDC for DON analysis. A total of 150 CDC breeding lines were also planted at the AAFC-Morden Research and Development Centre (MRDC), the AAFC-Ottawa Research and Development Centre (ORDC) and the AAFC-Charlottetown Research and Development Centre (CRDC) FHB nurseries each year. Entries were hand-harvested when mature, individually threshed and a sample of grain was ground and sent to either the CDC or AAFC-ORDC for DON analysis.

Objective 2. DON evaluation at the CDC

A 1 g sub-sample from the ground grain sent to the CDC was evaluated at the CDC for DON content using the Thermo Scientific™ Gallery™ analyzer and the Diagnostix EZ-Tox™ DON kit following the manufacturer's instructions. In addition, a 1 g sample of the association mapping (AM) population described below were sent for DON analysis at the University of Guelph-Laboratory Services Division (Guelph, ON) commercial lab, beginning in late November and concluding by early January. In this manner, all 2,250 samples grown in the AAFC-BRDC collaborative nursery was tested for DON content. A selection of 50 samples (cross lab set) encompassing a wide diversity in reaction to FHB (and thus DON accumulation) was also evaluated for DON by both the Gallery analyzer and the University of Guelph-Laboratory Services Division in order to assess infection in the nursery and to gage how well the Gallery analyzer performed.

Objective 3. NIR calibration development for prediction of grain DON content (discontinued in 2018)

The 20 g sample of ground grain sent to the CDC was scanned using the Foss NIRSystems DU 2500 to assist in the development of the DON NIR prediction. Optical wavelength intensities of the sample matrix were captured within the spectral range of 400 – 2500nm, collected at 2 nm segments. To develop the prediction, WinISI calibration software was used to analyze the optical data to identify the most unique samples representative of the entire set of samples analyzed. Stepwise regression then selected the highest coefficient of correlation between the optical signal at all wavelengths and the reference DON data previously measured. New optical

scans with reference data are added to the calibration set each year with the goal of the software identifying additional unique spectra that will help fill in potential gaps in the predictive capacity of the current calibration equation.

Objective 4. Association mapping for FHB/DON tolerance

Association Mapping (AM) Population

An expanded AM population consisting of 169 advanced breeding lines, varieties and exotic germplasm was created. Selection of the new lines were based on prior phenotypic data obtained by Mr. James Tucker (AAFC-BRDC) with the intention of maximizing the phenotypic (i.e. DON accumulation) variability of the population. In addition to the 92 member AM population used in the previous project (FHB screening of CDC barley breeding selections, 2011-2016), which consisted of two-row malt barley lines that were tested in the western Canadian cooperative two-row barley registration trials (2RCoop) spanning the years 1994-2006, the new additions to the population include 2RCoop lines (both malt and feed) tested from 2006-2014, older 2RCoop (malt and feed) lines showing very high or low DON accumulation, breeding lines from the AAFC-BRDC, CDC and North Dakota State University (NDSU) breeding programs and exotic landraces from China. A summary of the new AM population is provided in Table 1 and the detailed list of entries is provided in the Supplementary Data File (Appendix A). A total of 43 current and previously registered cultivars were present in the population including well known cultivars 'AAC Synergy', 'CDC Austenson', 'Oreana', 'Champion', 'AC Metcalfe,' 'CDC Kendall,' 'CDC Copeland,' 'Harrington,' 'CDC Meredith,' 'CDC Landis,' 'CDC Reserve,' 'Merit 16,' and 'Bentley.' This population was grown at the AAFC-BRDC FHB nursery each year as a completely randomized design with 3 replications. DON content (ppm), FHB rating (0-5; based on percentage severity), heading date (days) and height (cm) were recorded on these lines.

Table 1. Breeding program origin of the two-row hulled malt and feed entries (and varieties) which compose the association mapping population used in the current study.

Breeding Program	No. of Lines	Varieties
AAFC-Lethbridge	6	
AAFC-BRDC	29	AAC Synergy, AC Bountiful, AC Metcalfe, Calder, Newdale, Norman
AU	7	Garnet
BARI	15	Conrad, Merit, Merit 16, Merit 57
Cargill	3	
Coors	4	Moravian 19, Moravian 22, Moravian 27, Moravian 28
CDC	63	CDC Aurora Nijo, CDC Austenson, CDC Bold, CDC Copeland, CDC Fraser, CDC Goodale, CDC Kendall, CDC Landis, CDC Meredith, CDC Mindon, CDC Reserve, CDC Select, CDC Unity, Harrington, Manley
FCDC	21	Bentley, Busby, Niobe, Seebe
NDSU	6	Bowman, Conlon
AAFC-ORDC	1	Island
OSU	1	
WestBred/Highland	7	Alexis, Boulder, Brahma, Champion, Oreana, Xena
Landrace	6	

AAFC: Agriculture and Agri-Food Canada; AU: Agricore United (Calgary, AB); BARI: Busch Agricultural Resources Inc. (Ft. Collins, CO); BRDC: Brandon Research and Development Centre (Brandon, MB); CDC: Crop Development Centre, University of Saskatchewan (Saskatoon, SK); FCDC: Field Crop Development Centre (Lacombe, AB); NDSU: North Dakota State University (Fargo, ND); ORDC: Ottawa Research and Development Centre (Ottawa, ON); OSU: Oregon State University (Corvallis, OR).

Genotyping

Genotyping of the AM population was done with the 50K Barley Infinium iSelect SNP Assay at AAFC-MRDC. Allele calling and quality (Gentrain) score was evaluated within the GenomeStudio software package (Illumina Inc.) and data exported to TASSEL (v. 5.0) in order to filter the data set and produce a subset of high-quality markers. The filtering process involved removing markers with a minor allele frequency below 5%, missing data in more than 20% of the lines and markers scored as heterozygous in the lines. This resulted in a set of 23,948 SNP markers available for analysis.

Analysis of variance on the trait data was done using Proc Mixed (SAS Institute Inc, Cary N.C.) with genotype (fixed factor), year (random factor) and blocks (nested within years) as main effects and a genotype by year interaction effect. Least squares (LS) mean values were calculated for each genotype for all traits across years using this model. LS mean values were used for descriptive statistics and generated in Proc Univariate (SAS).

Association mapping of each trait was performed on LS means. Genome wide association analysis was conducted using GAPIT3 software (Wang and Zhang 2018) with the Mixed Linear Model (MLM) procedure (Zhang et al. 2010). To account for population structure, the P + K model described by Price et al. (2006) that accounts for genome-wide relatedness (P) and familial relatedness (kinship; K) was used. The P matrix was generated by principal component analysis (PCA) using three components, while the K matrix was generated from kinship coefficients in GAPIT3. Significant markers ($p<0.001$) were analyzed for SNP effects (Bayer et al. 2017) and the SNP-associated barley genes were investigated within the V1 Morex reference genome via the Barlex website (https://apex.ipk-gatersleben.de/). Genes were investigated for annotation, associated protein information and Gene Ontology (GO) terms.

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

Objectives	**Progress**
1) Identify CDC barley breeding lines with improved FHB/DON tolerance	From 2016-2019 approximately 2,250 breeding lines, checks and genetic populations were evaluated at the four collaborative FHB nurseries (AAAFC-BRDC, AAFC-MRDC, AAFC-ORDC, AAFC-CRDC). In 2020 only 1,573 entries were evaluated due to COVID-19. From 2016-2018, years in which there was excellent FHB disease development in the nurseries, 9-26% of the feed/forage breeding lines and 13-41% of the malting breeding lines showed DON accumulation at or below the low DON check line CDC Mindon. From 2016-2019 a total of 39 breeding lines were advanced to the western Canadian two-row, hulless or forage cooperative registration tests with 19 (49%) receiving an intermediate resistance rating and 12 (31%) receiving a moderate resistance rating from the disease evaluation committee. The FHB rating established by the disease committee for registration is intermediate resistance or better. Two of these lines, CDC Valdres (HB17340) hulless food barley and CDC Renegade (FB209) forage barley, were registered for commercial production, with one feed barley (TR19175) likely to be registered in 2021.
2) DON evaluation at the CDC	The Gallery Analyzer, located at the CDC, allowed DON values to be obtained on breeding lines and checks grown in the AAFC-BRDC collaborative FHB nursery. The Gallery was used to evaluate 1,042 samples in 2016, 840 in 2017, 1,743 in 2018, 1,790 in 2019 and 1,066 in 2020, with the remainder of samples from the nursery evaluated at a commercial lab. The correlation between DON values generated using the Gallery Analyzer and the commercial lab improved each year and an overall value of r=0.93 was obtained.

3) NIR calibration development for prediction of grain DON content	A correlation of 0.69 was observed between the CDC NIR prediction using the Foss NIRS DU2500 and DON values obtained from the Gallery analyzer based on 1,042 lines and checks grown in the 2016 AAFC-BRDC collaborative FHB nursery. A correlation of 0.49 was observed based on 691 lines and checks grown in the 2017 AAFC-BRDC collaborative FHB nursery. Thus, an overall correlation of 0.57 was observed across both years.
4) Association mapping for FHB/DON tolerance	Heading date, plant height, FHB score and DON content was measured on a 169-member association mapping population that was planted, inoculated and harvested from the AAFC-BRDC FHB nursery from 2016-2019 (3 replications each year). Twenty-four lines showed average DON content equal to or lower than CDC Mindon (low DON check), with several lines showing acceptable height and heading date to be potential parents to improve FHB tolerance in future varieties. Seven genomic regions were identified as influencing DON content via association mapping. None of the regions identified were found to be associated with heading date or height. As such, the markers can be used without concern that they may adversely impact maturity or height. The individual influence of each region on DON content was quite low, but collectively they will have value when incorporated as fixed variables into genomic selection models.

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.*

OBJECTIVE 1

Over the 2016-19 time period of this project all objectives were successfully met. The CDC sent between 2,250-2,297 lines annually to the AAFC-BRDC collaborative FHB nursery to be evaluated for DON content (Table 2). Among the breeding entries sent, the malt class dominated, followed by the feed and forage classes and finally the hulless class. This reflected the breeding priorities of the CDC barley program. An additional 140-145 advanced generation breeding lines were also evaluated in the three off-station collaborative FHB nurseries at AAFC-MRDC, AAFC-ORDC and AAFC-CRDC which provided additional DON data for these lines prior to their potential selection into the western cooperative registration tests. Only in 2020, due to COVID-19 restrictions imposed on AAFC staff, was the objective of evaluating 2,250 lines in the collaborative nurseries not met (Table 2). However, a significant amount of work was accomplished despite the restrictions and the most important aspects of the project retained.

Table 2. Sample number for the various barley types evaluated at the four collaborative FHB nurseries from 2016-2020.

Type	2016	2017	2018	2019	2020*
Checks	142	142	138	141	93
Feed/Forage	96	177	272	247	183
Hulless	195	66	76	95	61
Malt	1,173	1,214	1,257	1,307	729
AM Population	507	507	507	507	507
Special FHB Set	137	144	0	0	0
Total	2,250	2,250	2,250	2,297	1,573

*reduced nursery size due to COVID-19.

By examining the DON content and expected ranking of the three FHB nursery check lines, CDC Bold (high DON accumulator), AC Metcalfe (mid-range DON accumulator) and CDC Mindon (low DON accumulator), it was clear that good data was received from most of the nurseries in most years (Table 3). Very good data was received from the main AAFC-BRDC nursery from 2016-18, with dry growing conditions in 2019 leading to low infection and DON levels. AAFC-MRDC also produced good data in 2016 (although CDC Mindon DON was higher than expected) and 2017, moderately good data in 2018, but the dry growing conditions in 2019 also led to low infection and DON levels. AAFC-ORDC provided good data in 2019, moderately good data in 2016 and 2017, and poor data (i.e. low DON levels) in 2018 due to drought conditions. Finally, the AAFC-CRDC provided good data in 2016 (again, CDC Mindon DON was higher than expected) and 2017, low infection and DON levels in 2018 due to dry growing conditions and no data in 2019 due to a fire in their seed storage facility which destroyed harvested samples.

Table 3. DON content (ppm) for the three check lines across the four collaborative FHB nurseries from 2016-2019.

Check	Brandon, MB	Morden, MB	Ottawa, ON	Charlottetown, PEI
2016				
CDC Bold	29.9	40.6	9.2	43.4
AC Metcalfe	19.4	27.5	5.2	17.2
CDC Mindon	17.6	31.9	2.6	25.3
2017				
CDC Bold	64.1	49.5	11.5	55.8
AC Metcalfe	23.1	23.6	8.7	40.0
CDC Mindon	19.0	15.3	6.0	31.2
2018				
CDC Bold	35.0	8.2	7.1*	2.6
AC Metcalfe	16.8	5.8	9.3*	1.6
CDC Mindon	7.4	3.7	5.2*	1.1
2019				
CDC Bold	12.4	3.1	35.4	-**
AC Metcalfe	4.2	1.6	31.3	-**
CDC Mindon	4.1	1.2	16.2	-**

*the 2018 nursery experienced poor germination due to drought. Data from this nursery was thus compromised due to lack of uniformity.
**no data in 2019 due to a fire in the seed storage facility which destroyed harvested samples.

Among the various barley classes (i.e. malt, feed, hulless) evaluated for DON, the hulless barley lines had the lowest average DON content (Table 4). This is not unexpected since the hull is a major site of DON accumulation. The malt, feed and forage lines showed similar DON accumulation (Table 4). To assess the proportion of hulled breeding lines that would be considered to have low DON accumulation, the percentage of lines showing equal or lower DON content than the low DON check, CDC Mindon, was calculated. From 2016-2018, years in which there was excellent FHB disease development in the AAFC-BRDC nursery, 9-26% of the feed/forage breeding lines and 13-41% of the malting breeding lines showed DON accumulation at or below CDC Mindon (Table 4). This was an encouraging finding as CDC Mindon would be rated as having moderate resistance to FHB. The year-to-year variation in the proportion of lines rated as low DON lines varied as a result of the proportion of crosses (made 4-6 years earlier) devoted to improving FHB specifically or devoted to elite-by-elite parents (which typically have good FHB tolerance).

Table 4. DON content (ppm) for the different classes of CDC breeding lines, and the CDC Mindon low DON check, grown in the AAFC-BRDC collaborative FHB nursery from 2016-2019.

	DON (ppm)				Low DON Lines
Type	**Mean**	**Std. Dev.**	**Min.**	**Max.**	**(%)***
2016					
Feed/Forage	23.1	8.1	6.0	52.8	26%
Hulless	9.5	5.9	2.3	39.4	-
Malt	19.4	7.2	4.2	47.2	41%
CDC Mindon**	17.5	6.9	7.4	32.4	
2017					
Feed/Forage	26.2	10.2	3.5	56.0	9%
Hulless	8.6	7.1	0.7	28.3	-
Malt	24.5	11.3	2.5	105.3	14%
CDC Mindon**	13.2	10.2	3.5	55.9	
2018					
Feed/Forage	33.7	11.6	3.1	54.7	12%
Hulless	15.3	11.5	2.2	46.4	-
Malt	29.8	10.7	3.0	53.8	13%
CDC Mindon**	16.8	11.8	3.1	54.6	
2019					
Feed/Forage	4.4	3.8	0.3	22.5	53%
Hulless	1.7	1.7	0.0	8.3	-
Malt	6.7	5.6	0.3	39.0	32%
CDC Mindon**	3.6	3.8	0.1	22.5	

*percentage of lines in that class with DON content lower than the low DON check (CDC Mindon). Not calculated for the hulless class since CDC Mindon is a hulled variety (i.e., hulless lines are naturally lower in DON due to the absence of the hull).

**mean values for CDC Mindon will differ from Table 3 as the values in this table are calculated using only CDC Mindon grown among CDC breeding lines, as opposed to all the CDC Mindon checks grown throughout the entire nursery (Table 3).

The ultimate outcome from the collaborative FHB nurseries was the entry of breeding lines into the various western Canadian registration tests (which ultimately result in the release of future varieties for commercial production). From 2016-2019 a total of 39 breeding lines were advanced to the western Canadian two-row, hulless or forage cooperative registration tests, with 19 (49%) receiving an intermediate resistance rating and 12 (31%) receiving a moderate resistance rating from the disease evaluation sub-committee (Table 5). The FHB rating established by the disease sub-committee for registration is intermediate resistance or better. Two of these lines, CDC Valdres (HB17340) hulless food barley and CDC Renegade (FB209) forage barley, were registered for commercial production, with one feed barley (TR19175) likely to be registered in 2021. These encouraging results demonstrate the value of these nurseries and validate the funding devoted to them and this project.

Table 5. FHB ratings of CDC breeding lines entered into the co-operative registration trials from 2016-2019.

Type	Name and Rating*
2016	
Feed/Forage	TR16160 (I), TR16161 (MS), TR16162 (I)
Hulless	HB16335 (I), HB16336 (MR), HB16337 (S), HB16338 (MR), HB16339 (I)
Malt	TR16156 (I), TR16157 (MS), TR16158 (MS), TR16159 (I)
2017	
Feed/Forage	TR17163 (I)
Hulless	HB17340 (I), HB17341 (I), HB17342 (I), HB17343 (MS)
Malt	TR17164 (S), TR17165 (MR), TR17166 (I), TR17167 (I), TR17168 (MR), TR17169 (I)
2018	
Feed/Forage	FB209 (MR)
Hulless	HB18345 (MR), HB18346 (MR), HB18347 (MR), HB18348 (MR)
Malt	TR18170 (I), TR18171 (I), TR18172 (I), TR18173 (I), TR18174 (MS)
2019	
Feed/Forage	TR19175 (MR), FB210 (MR)
Hulless	None
Malt	TR19176 (MS-I), TR19177 (MR), TR19178 (MR), TR19179 (I)

*MR: moderate resistance, I: intermediate resistance, MS: moderate susceptibility

OBJECTIVE 2

The CDC has established an efficient and accurate in-house DON evaluation system based on the Gallery™ analyzer and the Diagnostix EZ-Tox™ DON kit. This allowed the CDC to evaluate 1,042 samples in 2016, 840 in 2017, 1,743 in 2018, 1,790 in 2019 and 1,066 in 2020, with the remainder of samples from the nursery evaluated at a commercial lab. These numbers far exceeded the 200 samples per year indicated in the original proposal. As shown in Table 6 and Fig. 1, the correlation between DON values generated using the Gallery analyzer and the University of Guelph commercial lab improved each year that a comparison was made, and an overall correlation value of r=0.93 was obtained. The most significant aspect of this accomplishment is that since 2017 the CDC has been able to not only test for DON on all breeding lines entered into the collaborative FHB nurseries, but that the data is obtained prior to when selections are made for the following growing season. This means that decisions on which lines to advance can be made with the inclusion of DON data, rather than having no data or having to wait until the following summer. This is a significant improvement in the CDC's ability to breed for improved DON tolerance and is part of the reason for the high proportion of breeding lines that are now meeting the standard of intermediate or moderate resistance to FHB required for registration.

Table 6. Comparison of DON values determined by the CDC and the University of Guelph commercial lab using the 50-sample cross-lab check set grown each year in the AAFC-BRDC collaborative FHB nursery in 2016, 2017 and 2019.

Year	Correlation Value
2016	0.88
2017	0.90
2019	0.98
Overall	0.93

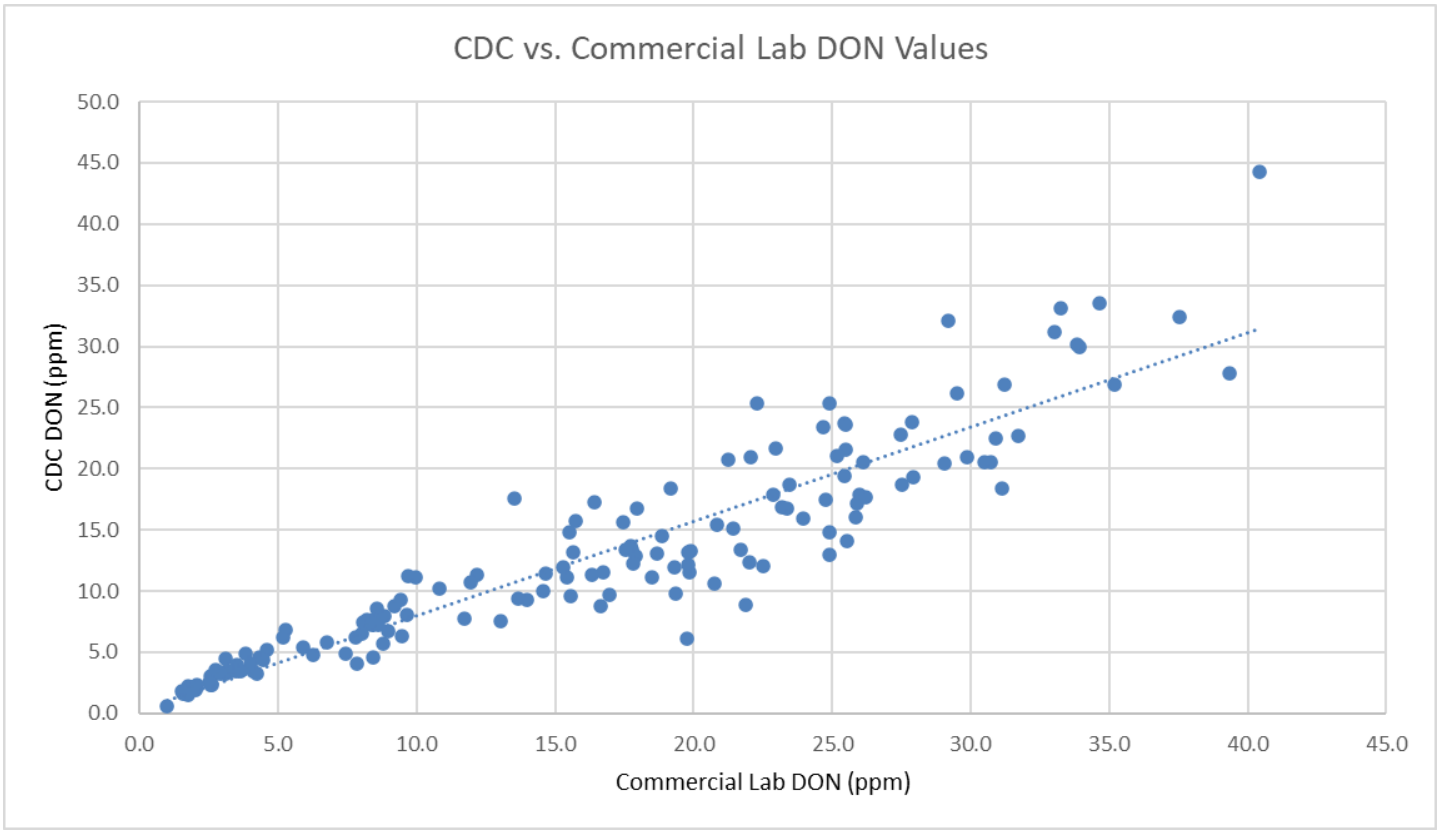

Figure 1. Overall correlation (r=0.93) between the CDC Gallery Analyzer and the University of Guelph commercial lab for DON content within the 50-sample cross-lab check set grown in the AAFC-BRDC FHB nursery in 2016, 2017 and 2019.

OBJECTIVE 3

A correlation of 0.69 was observed between the CDC NIR prediction using the Foss NIRS DU2500 and DON values obtained from the Gallery analyzer using 1,042 lines and checks grown in the 2016 AAFC-BRDC collaborative FHB nursery. A correlation of 0.49 was observed based on 691 lines and checks grown in the 2017 AAFC-BRDC collaborative FHB nursery. Thus, an overall correlation of 0.57 was observed across both years (Fig. 2).

This was an improvement over the prior 5-year period (2011-2015) in which the correlation between measured and NIR predicted DON was 0.38. This improvement was in part due to the purchase of the Foss DU2500, which has a finer wavelength resolution than the older Foss 6500 and would allow for a larger number of wavelengths to be utilized and potentially incorporated into the calibration. Also, we were able to incorporate additional unique data points, many within the lower range of the DON concentration spectrum. This improvement in precision performance was encouraging, however, due to the ability to measure DON on all breeding material grown in the collaborative FHB nurseries, this activity was discontinued (after informing the funders).

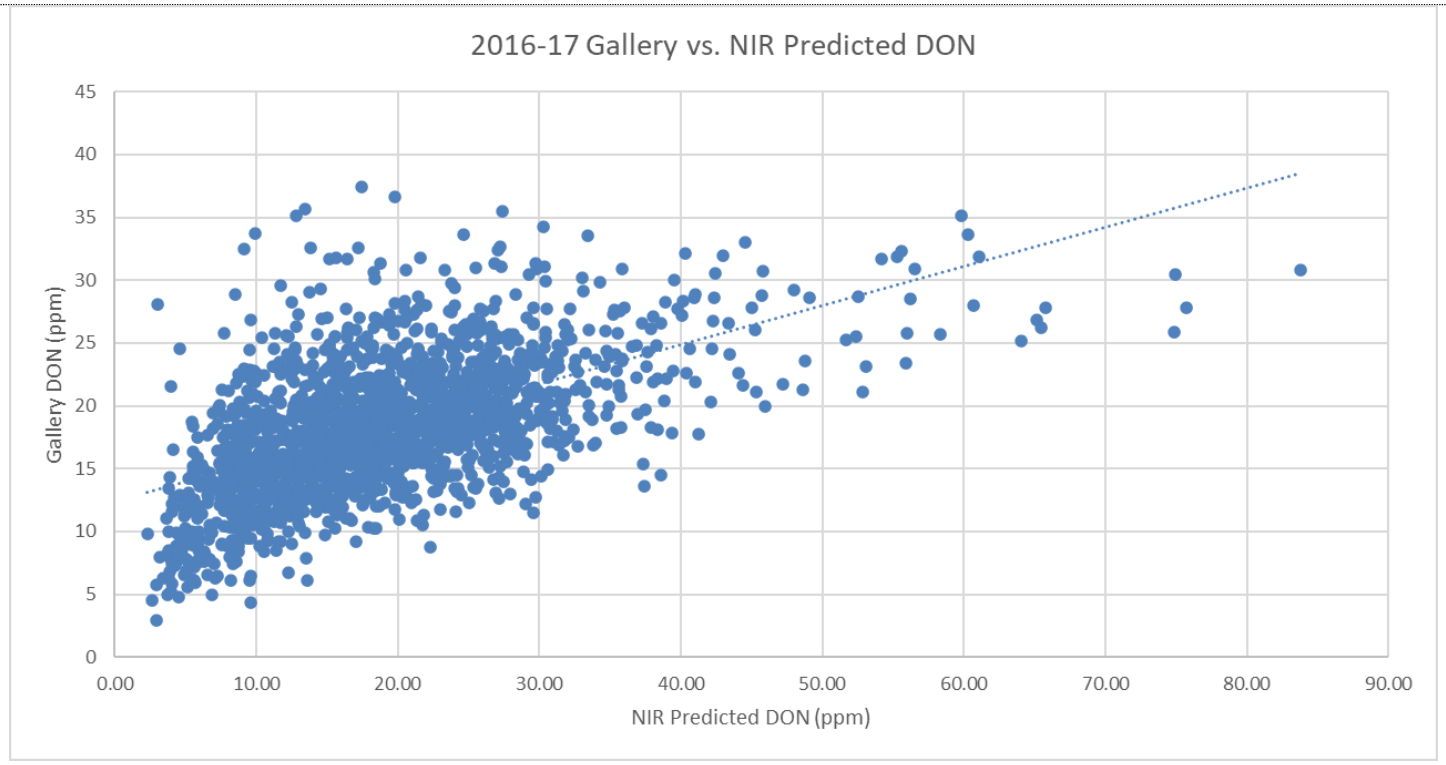

Figure 2. Overall correlation (r=0.57) between the CDC Gallery Analyzer and the CDC NIR prediction for DON content based on 1,783 samples grown in the AAFC-BRDC FHB nursery in 2016 and 2017.

OBJECTIVE 4

Three replicates of an association mapping (AM) population, consisting of 169 members, were grown and evaluated for heading date, height, FHB rating and DON content from 2016-2019 in the AAFC-BRDC FHB nursery. These lines represent elite two-row malting barley germplasm from twelve different breeding programs, 43 past and current varieties, and several landraces (Table 1 and Appendix A).

An analysis of variance indicated that lines (genotypes), years and a line by year interaction were significant sources of variability for heading date, height, FHB rating and DON content (Appendix B). Due to the significant effect of year, summary statistics for the four traits are summarized in Table 7 for each of the four years spanning 2016-19. Average heading date was relatively similar over the four years, while average height was similar in 2017 and 2018, but somewhat taller in 2019. Similarly, average FHB score was similar from 2016-2018, but lower in 2019. Good development of FHB infection was observed from 2016-2018, in which FHB scores were similar and DON content was significant, but poor FHB infection was observed in 2019 due to the dry growing conditions. Significant, but low to moderate correlations were observed between the four traits (Table 8). The expected negative correlations of heading date and height with FHB score and DON were observed, while the positive correlation between FHB score and DON was observed. Figure 3 provides more detailed information pertaining to DON content measured in the AM population from 2016-2019. The significant year-to-year variability in the amount of FHB disease development (and thus DON content) demonstrates the importance of evaluating lines for several years before a clearer understanding of a line's tendency to accumulate DON is able to be obtained.

There were 24 lines in the AM population that displayed average DON content equal to or better than CDC Mindon. Details regarding heading date, height, FHB score and DON content are provided in Appendix C. There are several familiar varieties, like CDC Copeland, Xena and Seebe within the group, along with nine older breeding lines from the CDC, four breeding lines from FCDC, one older breeding line from Lethbridge, four lines from NDSU and three landraces. Four of the lines (TR06676, CI4196, TR01178, TR10692) are 4 days later than the average heading date within the AM population, suggesting that some of their low DON accumulation may be due to

avoidance, or less exposure to the FHB inoculum. Eight lines (SB071676, Katuhya, TR06676, SB03417, CI4196, TR01178, Seebe, H93123023) are 10 cm or taller than the average height of the AM population, again suggesting that avoidance may be part of the explanation for their lower DON accumulation. However, some of these lines (e.g. TR02185, TR01178) do not display either of these characteristics, and do not represent currently used germplasm like CDC Copeland or Xena, and thus would be potential candidates to use a parents to further improve DON accumulation in future varieties.

Table 7. Summary statistics for the 169-member association mapping population grown in the AAFC-BRDC collaborative FHB nursery from 2016-2019.

Trait	Year	Mean	Std. Dev.	Minimum	Maximum
Heading Date (days)	2016	55.9	2.3	49.7	60.7
	2017	56.3	3.6	45.7	66.0
	2018	53.4	1.8	48.0	58.7
	2019	56.8	1.8	49.3	61.3
Height (cm)	2016*	-	-	-	-
	2017	82.0	7.0	59.0	100.3
	2018	83.9	7.2	49.7	103.0
	2019	93.8	9.6	67.0	194.0
FHB Score (0-5)	2016	2.3	0.7	1.0	5.0
	2017	2.6	1.1	0.7	5.0
	2018	2.2	0.8	0.3	5.0
	2019	1.3	0.6	0.3	4.3
DON (ppm)	2016	15.6	6.3	4.0	44.4
	2017	20.7	8.2	5.1	52.5
	2018	33.8	13.6	7.1	89.8
	2019	3.0	1.6	0.6	9.7

*no data due to storm damage.

Table 8. Correlation values among the four traits measured on the 169-member association mapping population grown in the AAFC-BRDC collaborative FHB nursery from 2016-2019.

	Heading Date	Height	FHB
Height	0.22*		
FHB Score	-0.17*	-0.46*	
DON	-0.27*	-0.35*	0.48*

*significant at $p<0.05$.

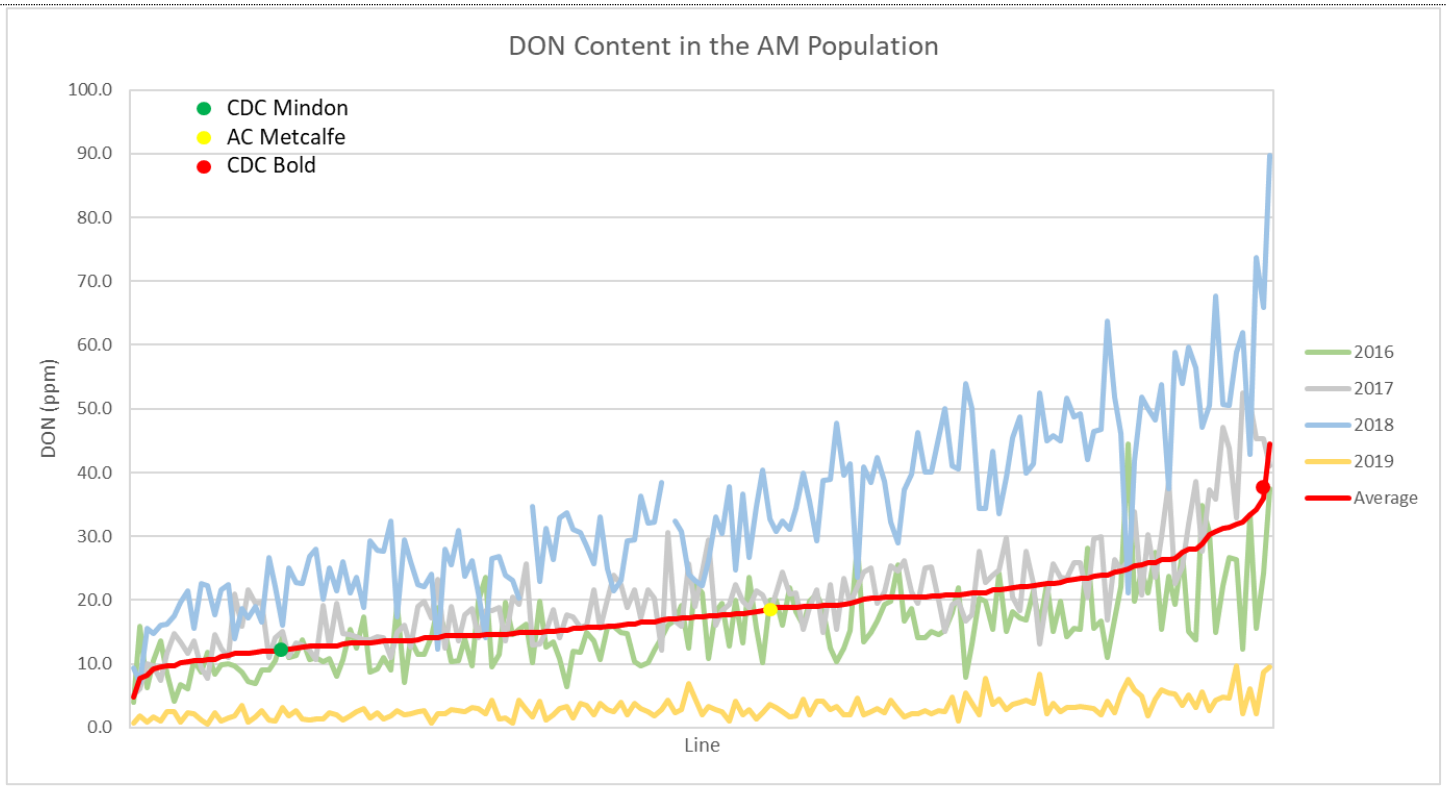

Figure 3. DON content measured in the association mapping population grown in the AAFC-BRDC nursery from 2016-2019. Lines are ordered from lowest to highest average DON content. Average DON content for CDC Mindon (low DON check), AC Metcalfe (mid-DON check) and CDC Bold (high DON check) are indicated for perspective on the range of DON observed in the AM population.

Analysis of the association mapping population using almost 24K SNP markers identified a number of genomic regions associated with DON, FHB score, heading date and height data. A total of 18 markers distributed across 7 genomic regions (Figure 4 and Table 9) were associated with DON. The phenotypic effect of these markers varied from 1.6 to 4.9 ppm. Some regions, like the 103 cM locus on chromosome 4H or the 129 cM locus of chromosome 7H, had numerous markers (i.e. genes) associated with DON, however, it is likely that only one of the genes is responsible for the effect. Appendix D provides information on the genes, and their proposed functions, associated with the markers listed in Table 9. Within the list, there are several interesting candidate genes that could logically be associated with DON. For example, NFXL-1, associated with JHI-Hv50k-2016-512498 on chromosome 7H (129.6 cM), is a transcription factor that has been shown to repress FHB resistance in wheat (Brauer et al. 2019). NFXL-1 is also down-regulated when barley is inoculated with a trichothecene-deficient mutant of Fusarium versus a wild-type strain (Boddu et al. 2007). Similarly, cytochrome P450, associated with JHI-Hv50k-2016-321115 on chromosome 5H (97.3 cM), are a family of proteins directly involved with removing toxins and again were shown to be down-regulated when barley is inoculated with a trichothecene-deficient mutant of Fusarium versus a wild-type strain (Boddu et al. 2007). Identifying these same genes in this project validates the importance of these genes and also provides confidence in the mapping results. The impact of some of these loci on the DON content appear fairy high, for example 4.9 ppm for the cytochrome P450-related marker, and is likely an overestimation of their impact. This is due to the very low minor allele frequency value associated with these markers in which the phenotypic value associated with the minor allele is not estimated with as much accuracy as the major allele, and thus may be artificially inflated or deflated. Although the individual influence of each locus identified in Table 9 is quite low and thus the design and use of marker-assisted selection for these individual loci is not warranted, collectively they have value when incorporated as fixed variables into genomic selection models.

It was also important to note that none of the markers identified in Table 9 were found to be associated with heading date or height. Some barley lines can have low DON as a result of late heading or being tall, however these two characteristics are not generally desired in varieties. As such, the markers identified in Table 9 can be used without concern that they may adversely impact maturity or height.

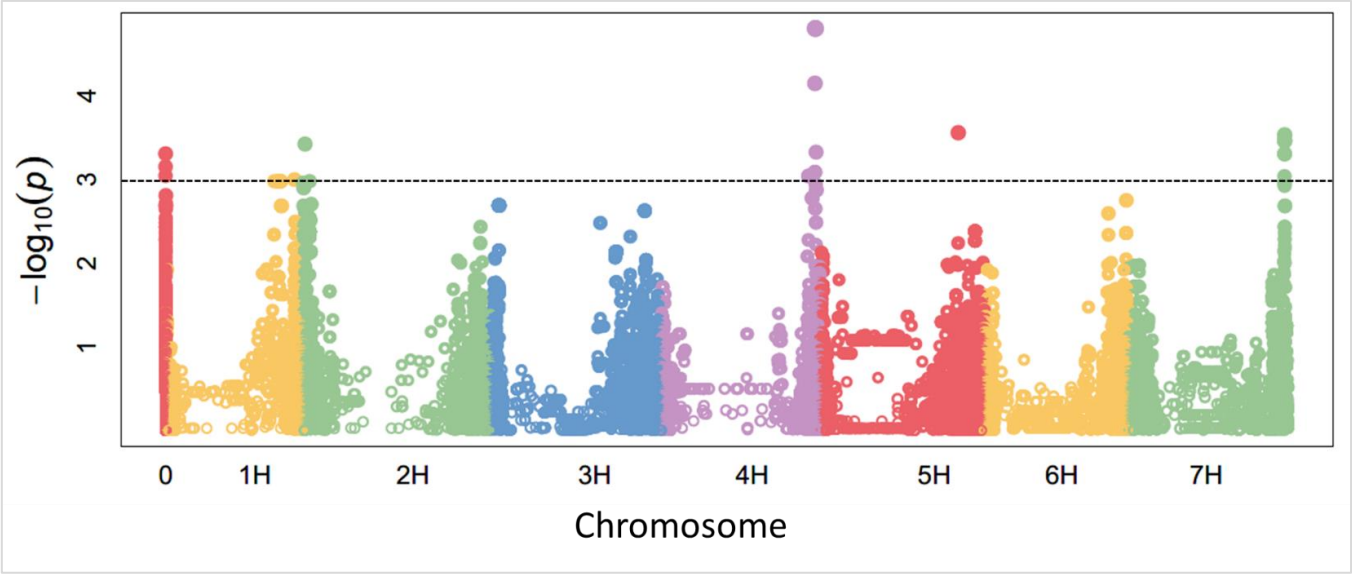

Figure 4. Manhattan plot showing the location of SNP markers within the barley genome that are significantly associated with DON content in the association mapping population based on data collected in the AAFC-BRDC FHB nursery from 2016-2019. Each marker is represented by a circular data point and markers are color-coded for each chromosome. The left most markers of each chromosome represent the top of the chromosome. The black dashed line indicates the minimum threshold to declare a marker significantly associated with the trait.

Table 9. Detailed information for SNP markers found to be significantly associated with DON in the association mapping population. Markers are colored coded to match the chromosome maps in Figure 4. Different shading within each chromosome represents blocks of markers that map to a similar genetic location.

		Chrom. Position			Allele	Minor	
SNP Marker	Chrom.	bp	cM	P value	Effect*	Allele Freq.	Co-Segregating Traits**
BOPA1_8758-564	NA	NA	NA	0.000489	1.8	0.47	Unknown
SCRI_RS_163314	NA	NA	NA	0.000698	-3.5	0.09	Unknown
BOPA2_12_10218	NA	NA	NA	0.000896	3.0	0.10	Unknown
JHI-Hv50k-2016-47293	1H	526,215,272	102.7	0.000994	-1.9	0.28	None
JHI-Hv50k-2016-64093	2H	10,055,848	6.5	0.000375	-3.3	0.09	None
JHI-Hv50k-2016-261184	4H	599,795,020	81.3	0.000900	2.8	0.08	None
JHI-Hv50k-2016-267752	4H	626,077,094	103.3	0.000810	3.0	0.06	None
JHI-Hv50k-2016-267833	4H	626,164,301	103.3	0.000810	-3.0	0.06	None
JHI-Hv50k-2016-267907	4H	626,279,385	103.9	0.000070	-3.2	0.07	None
JHI-Hv50k-2016-268238	4H	626,928,609	103.5	0.000016	4.6	0.05	None
JHI-Hv50k-2016-268379	4H	627,029,342	103.7	0.000016	4.6	0.05	None
JHI-Hv50k-2016-268868	4H	630,337,488	109.9	0.000468	2.9	0.08	None
JHI-Hv50k-2016-321115	5H	563,627,618	97.3	0.000275	4.9	0.03	None
JHI-Hv50k-2016-512498	7H	642,028,967	129.6	0.000343	1.8	0.31	None
JHI-Hv50k-2016-512521	7H	642,061,132	129.6	0.000910	-1.6	0.36	None
JHI-Hv50k-2016-512596	7H	642,107,979	129.6	0.000291	1.8	0.31	None
JHI-Hv50k-2016-512623	7H	642,218,587	129.6	0.000494	-1.7	0.31	None
JHI-Hv50k-2016-512666	7H	642,243,408	129.6	0.000343	-1.8	0.31	None

*difference in grain DON content (ppm) due to presence of allele 1 versus allele 2 at a given marker.
**indicates if FHB score, heading date or height traits were also found to be associated with this genomic region.

Appendices D and E contain additional information regarding markers linked to FHB score, heading data and height. As with DON, several markers identified have previously been associated with these traits. For example, the *FLT1* gene, associated with JHI-Hv50k-2016-459853 on chromosome 7H (33.6 cM), is a transcription factor know to influence flowering time (Faure et al. 2007). Also, the *HvCen* gene, located in the middle of the large linkage block of genes on chromosome 2H (58.7 cM), is an important regulator of flowering time (Bi et al. 2019). Finally, the heading time gene *Ppd-H1* has previously been reported to have an impact on plant height which would explain its association to height in this study (Karsai et al. 1999). Again, identifying these genes in this study validates the importance of these genes and also provides confidence in the mapping results. While the primary importance of identifying markers associated with heading date and height in this study was to understand if they were also associated with DON, these loci may also be used in genomic selection models to help prevent selected lines from getting too tall or late.

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) each year a significant percentage of breeding lines, 9-26% of the feed/forage lines and 13-41% of the malting lines, showed DON accumulation at or below CDC Mindon. This bodes well for the release of future varieties with better FHB resistance since CDC Mindon would be rated as having moderate resistance,

2) two lines evaluated under this project, CDC Valdres (HB17340) hulless food barley and CDC Renegade (FB209) forage barley, were registered for commercial production, with one feed barley (TR19175) likely to be registered in 2021,

3) the CDC is now able to evaluate all its breeding lines for DON and make selection decisions based on this information prior spring seeding. This represents a significant achievement in our ability to breed for improved DON tolerance,

4) the NIR prediction for DON content was improved during this project (r=0.57) when compared to the previous 5-year project (r=0.38),

5) twenty-four lines in the AM population showed average DON content equal to or lower than CDC Mindon (low DON check), with several lines showing acceptable height and heading date to be potential parents to improve FHB tolerance in future varieties,

6) seven genomic regions were identified as influencing DON content via association mapping. None of the regions identified were found to be associated with heading date or height. As such, the markers can be used without concern that they may adversely impact maturity or height. The individual influence of each region on DON content was quite low, but collectively they will have value when incorporated as fixed variables into genomic selection models.

Recommendations:

1) some of the lines in the AM population (e.g. TR02185, TR01178) do not display excessive height or late maturity, and do not represent currently used germplasm like CDC Copeland or Xena, and thus would be potential candidates to use a parents to further improve DON accumulation in future varieties,

2) increase the capacity of the AAFC-BRDC collaborative FHB nursery since the CDC's in-house capacity to evaluate DON using the Gallery analyzer now exceeds the number of entries grown in the nursery,

3) given that markers associated with DON accumulation explain a small percentage of the variation, incorporating such markers into marker-assisted section programs provides little value. In contrast, genomic section (GS) considers the genetic contribution of many markers to a trait collectively (no matter the explained variance they provide) and thus this approach has shown promise to not only select superior lines, but also to select the best combinations of parents to cross, with quantitative traits like FHB resistance. It is suggested that a GS model be created to breed for low DON accumulation. The data collected on the AM population over the past 5

years of this project can be leveraged to create the initial GS model, and the markers found associated to DON can be incorporated into the model as fixed factors. The annual breeding lines entered into the nurseries can then be used for validation, selection and improvement of the GS model.

4) continued funding and operation of the collaborative FHB nurseries is essential to permit on-going improvement and release of barley varieties with resistance to DON accumulation.

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.*

The primary outputs from this project were the registration of several varieties with lower DON accumulation for commercial production, CDC Valdres (HB17340) hulless food barley and CDC Renegade (FB209) forage barley. In addition to these varieties, lines such as TR02185 and TR01178 will be useful parents to improve FHB tolerance/low DON accumulation in breeding programs.

Developing in-house capacity to evaluate the DON content of CDC breeding lines grown in the collaborative FHB nurseries is a significant improvement to the cost and efficiency of breeding for better FHB tolerance/low DON accumulation. The equipment and methods adopted by the CDC can be incorporated into other breeding programs, for example, the AAFC-BRDC breeding program has recently purchased a Gallery analyzer for this purpose.

Molecular markers linked to DON accumulation were identified in this project. Although using these markers for marker-assisted selection would not be efficient, they can instead be incorporated into genomic selection models which would be a more effective means of improving DON accumulation in breeding programs. Genomic selection could be applied very early in the breeding process (prior to lines entering the FHB nursery) to select only the most promising lines to be screened in the nursery. This would be a more efficient use of the nursery as it would help limit the number of lines with poor DON resistance from taking up valuable space in the nursery.

The release of varieties with better low DON accumulation can mitigate financial losses due to yield reduction and poor physical grain quality. More importantly, lower DON accumulation in varieties reduces the risk of the grain being unsuitable for malt production, swine feed and human consumption. This will help protect the western Canadian barley industry and maintain its current status as a source of quality barley. Genetic resistance to FHB complements other approaches, including crop rotation, use of clean seed and treatment of seed with a Fusarium-controlling product, increasing seeding rates and the use of fungicides. Good genetic resistance may alleviate some of the financial costs associated with fungicide use by reducing the number of applications.

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

CDC Valdres (HB17340) hulless food barley (licensed to Tomtene Seeds, registered March 6, 2020; #8955).

CDC Renegade (FB209) forage barley (licensed to SeCan, registration pending).

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

Industry Presentations:

Beattie AD (2016) Crop Development Centre Two-Row Barley Research Check-Off Project. WGRF Technical Meeting and Field Tour, Brandon, MB. August 3, 2016.

Beattie AD (2017) CDC Malting Barley Breeding Program. Syngenta Malt Masters Grower Meeting, Saskatoon, SK. March 16, 2017.

Beattie AD (2018) Barley Breeding at the Crop Development Centre. Beef Cattle Research Council. Calgary, AB. June 26, 2018.

Beattie AD (2019) Canadian Malting Barley Breeding for the Chinese Market. Canadian Malting Barley Technical Centre, Winnipeg, MB. June 19, 2019.

Conference Presentations:

Tucker J, Badea A, Beattie AD, Hiebert C, Legge B, McCartney C, Fernando D (2018) Genome-wide association study of a diverse two-row barley genomic panel identifies genomic regions associated with resistance to FHB and DON accumulation. 9th Canadian Workshop on Fusarium Head Blight / 4th Canadian Wheat Symposium Joint Conference. Winnipeg, MB. November 19-22, 2018.

13. List any industry contributions or support received.

We gratefully acknowledge the financial support from the Western Grains Research Foundation and the Saskatchewan Barley 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.*

Further follow-up research is required and has been summarized in the ADF Project 20200268 proposal "Phenotyping and Genomic Selection for Improved Barley Deoxynivalenol (DON) Resistance." To summarize, several activities should continue, including:

1) evaluation of DON content in CDC breeding lines grown in the collaborative FHB nurseries located in Brandon, MB, Morden, MB, Ottawa, ON and Charlottetown, PEI. DON data will be used: a) as part of the CDC breeding selection process to improve DON resistance in CDC barley varieties and, b) as part of the genomic selection process outlined below,

2) data previously collected on the AM population grown in the AAFC-BRDC nursery from 2016-2020, along with similar data to be collected annually on a subset (approximately 150-200) of CDC breeding lines grown in the nursery will be used: a) to develop and validate a genomic selection model for DON content, b) to select and then evaluate lines selected using the genomic selection model.

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

Acknowledgements to ADF, WGRF and SBDC for their support of this work were made at the industry presentations given by A. Beattie and the conference presentation given by J. Tucker which are listed in Section 12.

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

Literature Cited:

Alberta Fusarium Action Committee (2015) Alberta *Fusarium graminearum* Management Plan. Alberta Agriculture and Rural Development, Agdex 110/632-3. pp. 23.

Bayer MM, Rapazote-Flores P, Ganal M, Hedley PE, Macaulay M, Plieske J, Ramsay L, Russell J, Shaw PD, Thomas W, Waugh R (2017) Development and Evaluation of a Barley 50k iSelect SNP Array. Frontiers in Plant Science 8: 1792.

Beer Canada (2020) https://industry.beercanada.com/ (Accessed August 20, 2020)

Bi X, van Esse W, Mulki MA, Kirschner G, Zhong J, Simon R, von Korff M (2019) CENTRORADIALIS interacts with *FLOWERING LOCUS T*-like genes to control floret development and grain number. Plant Phys 180: 1013-1030.

Boddu J, Cho S, Muehlbauer G (2007) Transcriptome analysis of trichothecene-induced gene expression in barley. Mol Plant-Microbe Interact 20:1364-75.

Brauer EK, Balcerzak M, Rocheleau H, Leung W, Schernthaner J, Subramaniam R, Ouellet T (2019) Genome editing of a DON-induced transcription factor confers resistance to Fusarium graminearum in wheat. Mol Plant-Microbe Interact 33: DOI: 10.1094/MPMI-11-19-0332-R.

Canadian Grain Commission (2019) Cereal Varieties - 2019 Insured Commercial Acres. Canadian Grain Commission, Statistics and Business Information. (Released September 19, 2019)

Faure S, Higgins J, Turner A, Laurie DA (2007) The FLOWERING LOCUS T-Like gene family in barley (*Hordeum vulgare*). Genetics 176: 599–609.

Flannigan B (1999) The microflora of barley and malt. In: Preist FG, Campbell I (eds) Brewing microbiology, 2nd ed. Aspen Publishers, Maryland, pp 83-126.

Habler K, Hofer K, Geissinger C, Schuler J, Huckelhoven R, Hess M, Gastl M, Rychlik M (2016) Fate of Fusarium toxins during the malting process. J Agric Food Chem 64:1377-1384.

Karsai I, Eszaros KM, Szucs P, Hayes PM, Lang L, Bedo Z (1999) Effects of loci determining photoperiod sensitivity (*Ppd-H1*) and vernalization response (*Sh2*) on agronomic traits in the 'Dicktoo' x 'Morex' barley mapping population. Plant Breed. 118:399-403.

Price, A.L., N.J. Patterson, R.M. Plenge, M.E. Weinblatt, N.A. Shadick, and D. Reich. 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nat. Genet. 38:904-909.

Statistics Canada (2020a) Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units. (Accessed August 12, 2020).

Statistics Canada (2020b) Canadian International Merchandise Trade Database. (Accessed August 20, 2020).

Virkajärvi V, Sarlin T, Laitila A (2017) Fusarium Profiling and Barley Malt Gushing Propensity, J Am Soc of Brew Chem 75:181-192.

Wang J, Zhang Z (2018) GAPIT Version 3: An Interactive Analytical Tool for Genomic Association and Prediction. (preprint).

Zhang Z, Ersoz E, Lai C-Q, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM, Buckler ES (2010) Mixed linear model approach adapted for genome-wide association studies. Nat. Genet. 42: 355-360.