Government of Saskatchewan
Ministry of Agriculture

ADF Project Progress Report Format

1. **Project title, ADF file number and reporting period.**

   Development of markers linked to oat crown rust resistance to help breed improved varieties for Saskatchewan producers.
   20180264
   January 1, 2020 – June 30, 2021

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**

   Jennifer Mitchell Fetch (Retired in 2020)
   Agriculture and Agri-Food Canada
   2701 Grand Valley Road
   Brandon Research and Development Centre
   Brandon, MB R7A 5Y3
   Phone: 204-578-6601
   Fax: 204-578-3524
   Email: jennifer.mitchellfetch@agr.gc.ca

   Jim Menzies
   Agriculture and Agri-Food Canada
   101 Route 100
   Morden Research and Development Centre
   Morden, MB R6M 1Y5
   Phone: 204-822-7522
   Fax: 204-822-7507
   Email: jim.menzies@canada.ca

   Curt McCartney
   Department of Plant Science
   Agriculture Building
   University of Manitoba (Fort Garry campus)
   66 Dafoe Road
   Winnipeg, MB R3T 2N2 Canada
   Telephone:
   Fax: 204-474-7528
   Email: curt.mccartney@umanitoba.ca

Randy Kutcher
Crop Development Centre
University of Saskatchewan
51 Campus Drive
Saskatoon, SK S7N 5A8
Telephone: 306-966-4951
Fax: 306-966-5015
Email: randy.kutcher@usask.ca

Kathy Esvelt Klos
USDA-ARS
1691 S 2700 W
Aberdeen, ID 83210
Telephone: 208-397-4162 ext. 123
Fax: 208-397-4165
Email: Kathy.Klos@ars.usda.gov

4. **Abstract (Not more than 250 words).** *Describe in lay language the progress towards the project objectives over the last reporting period. Include any key findings and any interim conclusions. Include any deviations from the original methodology.*

Good progress on the objectives for project 20180264 were made in 2020-21, despite a significant slow down for 6 months due to COVID-19. Phenotyping of populations segregating for *Pc46*, *Pc62*, *Pc67* and one adult plant resistance gene were accomplished, and QTL mapping was completed for *Pc40*, *Pc46* and *Pc62*. A new population for mapping of *Pc67* was created, a population to be used to map *Pc101* has been advanced to the F4 generation, and one adult plant resistance mapping population has been planted for a third year of phenotyping in 2021. The *Pc67* gene does not appear to be an effective resistance gene based on the inability to identify an avirulent crown rust isolate. Thus, the gene may not be of value to oat breeders. Delays over the past year due to COVID-19 may impact our ability to deliver results by the current end-date of the project. We will not ask for an extension at the moment. Instead, we will see how much we can catch-up over the next year and we may ask for an extension at the time of the next progress report in July 2022.

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

Oat is considered a healthy cereal due to a number of nutritional compounds found within the grain, including β-glucan. β-glucan is a soluble fiber that has been shown to lower plasma cholesterol and reduce the risk of heart disease (Queenan et al. 2007; Liatisa et al. 2009). This has resulted in health claims being established in both Canada (Health Canada 2010) and the United States (U.S. FDA 1997). Oat grain also contains a number of antioxidant compounds, including the polyphenolic avenanthramides which have anti-inflammatory effects that may protect against coronary heart disease (Meydani 2009). Oat contains 12-20% protein which is rich in globulins and contains more lysine and threonine than other cereals, thus providing a better balance of essential amino acids (Klose and Arendt 2012). Finally, oat is able to be consumed by most people suffering from celiac disease and is thus considered to be gluten-free (Peraaho et al. 2004).

As a result of these desirable attributes, oat remains a significant Canadian crop that has been seeded on an average of 3.1 million acres over the past 5 years, with Saskatchewan accounting for 50% of these acres (Statistics Canada, 2018). Over the past 5 years, 50% of the 3.2 million metric tonnes (MT) of oat produced annually in Canada has been exported to the U.S., primarily for the food market. In addition to the export of raw oat, oat is critical to the domestic milling industry. Total annual domestic milling in Canada sits close to 750,000 MT, with Saskatchewan

being home to Richardson Milling (Martensville), Grain Millers (Yorkton), Ardent Mills (Saskatoon), and Avena Foods (Regina), or about half of all milling production. Forecasts indicate that domestic milling will grow to just over 900,000 MT within the next 6 years (R. Strychar, Ag Commodity Research). Combined sales of oat and milled oat products were valued at almost $750 million last year. In addition, farm-gate sales of oat represent an estimated value of $450 million to growers.

The largest on-going threat to oat production in Canada (and worldwide) is the fungal pathogen *Puccinia coronata* Corda f. sp. *avenae* Eriks which causes oat crown rust. In Canada, yield losses averaged 5.1% from 2001 to 2005 (Chong et al. 2008) with the highest loss reported at 11.2% (McCallum et al. 2007). Similarly, yield losses of 50% and 35% were reported in Minnesota and South Dakota in 2014 (USDA-ARS 2014). In addition, crown rust can weaken straw causing plants to lodge (Endo and Boewe 1958). While cultural control methods, such as crop rotation and early seeding, and fungicide application can reduce crown rust severity, incorporating genetic resistance is an important component to an integrated management strategy. Finally, the significant acreage devoted to organic oat production, second only to wheat at 150,000 acres (Guerra 2017), relies heavily on genetic resistance.

Nearly 100 crown rust resistance genes, the vast majority being seedling resistance genes, have been reported in oat (Gnanesh et al. 2014). Despite the effectiveness of these genes to combat crown rust, there has been a history of these genes being defeated with 10 years of widespread deployment in cultivars due to the development of new virulent races of the pathogen (McCallum et al. 2007, Nazareno et al. 2017). As such, gene pyramiding and use of adult plant resistance (APR) are considered viable methods to deal with this issue. For example, the APR present in the Minnesota line MN841801 has been effective since the 1970s (Leonard 2002).

In order to effectively and efficiently implement these approaches, it is necessary to genetically map the location of both seedling and APR genes within the oat genome and develop markers to these genes. Crown rust resistance associated with seedling genes, such as *Pc45* (Gnanesh et al. 2015), *Pc91* (McCartney et al. 2011) and the recently reported, but unnamed, gene derived from *A. strigosa* (Rines et al. 2018), as well as, APR genes from MN841801 (Lin et al. 2014), are examples of genes with mapped locations within the oat genome. The CDC oat breeding program is currently using markers linked to these genes in order to incorporate and select for crown rust resistance. This is not only cost and time efficient, but it also allows identification of individual genes within an oat line containing several resistance genes which normally would not be possible using crown rust nurseries (as the line would simply appear resistant) or because crown rust isolates with the appropriate virulence combinations may not exist to differentiate individual genes.

With the decreasing cost associated with genotype-by-sequencing (GBS) markers, the availability of a 6K Infinium HD SNP genotyping platform and a consensus oat map integrating both marker types (Bekele et al. 2018), it is now possible to not only develop markers linked to oat crown rust genes, but also understand their chromosome locations and more importantly, the allelic relationships among the vast number of reported genes. Such information will allow oat breeders to understand which combinations of resistance genes can be pyramided together and test the effectiveness of such pyramids. Although the presence of some genes has been known for decades, their current distribution within oat germplasm and effectiveness is unclear. For example, although the *Pc45* gene was reported in 1971 (Fleischmann et al. 1971) it remains a useful gene within regions of Western Canada and Ontario (J. Menzies, personal comm.). It is likely that other such useful genes exist within the current pool of reported genes.

To build on Saskatchewan's (and Canada's) position as a supplier of premium quality oat to current US markets (and developing markets in Mexico and China) requires developing varieties with improved crown rust resistance. This will provide value to growers, through improved yield and reduced input costs (i.e. reduced fungicide use) which will help oat remain a viable crop within a grower's rotation, and to millers/food processors, through higher select ability (i.e. good plumpness and test weight).

6. Objectives and the *progress towards meeting each objective*

Objectives (*Please list the original objectives and/or revised objectives if Ministry-approved revisions have been made to original objective. A justification is needed for any deviation from original objectives)*	**Progress (e.g. completed/in progress)**
a) Evaluate crown rust reaction in bi-parental oat populations.	Crown rust reaction was evaluated in 1 population segregating for *Pc46* resistance, 3 populations segregating for *Pc62*, and 1 populations segregating for APR derived from 'CDC Dancer'. Crown rust isolates were tested on parents of the *Pc67* x 'AC Morgan' and *Pc67* x 'Bingo' population to identify isolates which can differentiate the presence or absence of the *Pc67* gene.
b) QTL mapping of crown rust resistance.	QTL mapping was completed for *Pc40, Pc46* and *Pc62*.
c) Development of high throughput marker assays.	High throughput KASP markers associated with *Pc40, Pc46* and *Pc62* are being created and will be evaluated in the associated confirmation populations that have been phenotyped already.

7. Methodology: *Specify project activities undertaken during this reporting period. Include approaches, experimental design, tests, materials, sites, etc. Please note that any significant changes from the original work plan will require written approval from the Ministry.*

Genetic mapping populations

Numerous bi-parental populations segregating for seedling and adult plant resistance (APR) to crown rust were assessed for reaction to crown rust and/or genotyped during the reporting period (Table 1).

Table 1. Details on the oat genetic populations phenotyped, genotyped and/or QTL mapped for crown rust resistance during the current reporting period.

Population (R x S)	**Generation**	**Pc Gene**	**Work Accomplished**
Pc40 x 'AC Morgan'	F5	*Pc40*	QTL mapped
Pc46 x 'AC Morgan'	F6	*Pc46*	Phenotyped and QTL Mapped
Pc62 x 'AC Morgan'	F5	*Pc62*	Phenotyped and QTL mapped
Pc62 x 'Kasztan'	F2	*Pc62*	Phenotyped
Pc62 x 'Kasztan'	F2:3	*Pc62*	Phenotyped
Pc67 x 'AC Morgan'	F6:7	*Pc67*	Parental screening with Pca isolates
Pc67 x 'Bingo'	F2	*Pc67*	Parental screening with Pca isolates
Pc101 x Bingo'	F4	*Pc101*	Advanced to F4 generation
'CDC Dancer' x 'AC Morgan'	F4:7	APR	Phenotyped

Greenhouse phenotyping of seedling resistance

Seedlings from each segregating population were grown in controlled environment chambers in root trainers potted with Sunshine Mix#3, comprised of 70-80% Canadian Spaghum peat moss, vermiculite and dolomite limestone (Sun Gro Horticulture Canada Ltd., Seba Beach, AB). Seedlings were watered as needed and grown at a 22-23^{o}C daytime temperature, 18-20^{o}C night temperature and an 18-hour day length. Seedlings at the one leaf stage were inoculated by spraying urediniospores (4 mg/450 µL) suspended in Bayol® on the leaves. Approximately 10 seedlings from each RIL in the F5/F6 RIL populations, and 20 F2:3 families from each of 200 F2 plants in each F2:3 population were

inoculated. The inoculated plants were incubated in a humidity chamber (maintained at 100% relative humidity) overnight at 15-17°C and then returned to the previous growing conditions. Crown rust infection types (ITs) were scored 12 days after inoculation on a 0 to 4 scale (Murphy 1935): 0 (immune), ; (fleck), 1 and 2, resistant; 3 and 4, susceptible. After rust scoring, infected leaves from F2:3 plants were trimmed and the plants were raised to the fourth leaf-stage for DNA collection.

Field phenotyping of adult plant resistance

Lines segregating for APR were grown in a three-replicate lattice design. Lines were tested at AAFC-Morden Research and Development Centre (AAFC-MRDC, Morden MB) and sown as 1 m rows with a row spacing of 0.34 m. Lines were tested at the University of Saskatchewan (Saskatoon, SK) and sown as hills with a row spacing of 0.3 m and spaced 0.6 m apart along the seed row. A spreader row of 'AC Morgan' (U of S) or 'Makuru' (AAFC-MRDC) was sown to increase inoculum in the nursery.

Field nurseries were inoculated with a mixture of field isolates collected from across eastern Saskatchewan and Manitoba the prior year. This mixture contained more than 100 races, and was tested to ensure it is virulent on the seedlings of the parents. Two to three inoculations were done within 10 days when plants in the spreader rows reached the four-leaf stage. At each inoculation, 0.3 g of crown rust urediniospores were mixed with 300 ml light mineral oil (Bayol®, Esso Canada, Toronto, ON.) and sprayed onto spreader rows with a Herbiflex hand-held sprayer (Micron Sprayers Ltd., Bromyard, UK). At the U of S nursery water was sprayed onto the spreader rows after the Bayol had evaporated and covered with white plastic sheets to simulate dew formation and ensure germination of the urediniospores and infection of the spreader rows. The Morden field nursery was irrigated using overhead irrigation once a week, starting just before inoculation until the plants are assessed for crown rust reactions, to ensure dew formation leading to crown rust infection and spread. In Saskatoon, irrigation was applied once a week throughout the season and then 3 times per week in the week preceding rating.

Crown rust was rated on flag leaves. Disease severity (DS) was assessed using the modified Cobb scale (Peterson et al. 1948). Pustules were defined by infection type (IT): resistant (R), moderately resistant (MR), moderately susceptible, and susceptible (S) (Stubbs et al. 1986). All ITs were converted to a numerical value as follows: R = 0, RMR = 0.1667, MR = 0.3333, MRMS = 0.5, MS = 0.6667, MSS = 0.8333, and S = 1, such that a coefficient of infection (CI) can be calculated according to the formula: $CI = (DS \times IT) / 100$.

DNA Extraction, Genotyping, QTL Mapping

DNA was extracted from seedlings (coleoptile from RILs or 4 leaf stage leaves from F2:3 lines) using a CTAB extraction method (Procunier et al. 1991; Anderson et al. 1992) or from freeze-dried leaf tissue using the DNeasy Plant DNA extraction kit (Qiagen, Toronto, Canada). Mapping populations were genotyped with the Oat 6K Infinium SNP Assay at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND) on the iSELECT Genotyping BeadChip (Illumina, Inc., San Diego, CA) or using a genotype-by-sequencing method as described by Huang et al. (2014).

Marker phase was determined following the method of Tinker et al. (2014). Markers significantly deviating from the expected 1:1 segregation ratio (P value < 1e-4) were removed (Gardner et al., 2016; Zuo et al., 2019). The remaining set of markers were filtered to retain those that met the criteria of <20% heterozygosity and <20% missing data. Additionally, lines with more than 30% missing data were removed from the data set. JoinMap v.4 software (Van Ooijen, 2006) was used to group and calculate the order and position of markers within each linkage group (LG) through the Maximum Likelihood algorithm. LGs were assigned to consensus map merge groups described in Chaffin et al. (2016).

QTLs were mapped using MapQTL 5 (Van Ooijen, 2004). Interval mapping (IM) was performed using an initial mapping step size of 1 cM. The initial LOD threshold of different LGs were estimated with permutation tests using

10000 permutations. Potential QTL peak markers identified were then chosen as cofactors to perform the multiple-QTL mapping (MQM) to identify further QTLs (Romero et al., 2018; He et al., 2016).

J.A. Anderson, Y. Ogihara, M.E. Sorrells, and S.D. Tanksley, 1992. Development of a chromosomal arm map for wheat based on RFLP markers. Theor. Appl. Genet., 83:1035-1043.

A.S. Chaffin, Y.-F. Huang, S. Smith, W.A. Bekele, E. Babiker, B.N. Gnanesh, B.J. Foresman, S.G. Blanchard, J.J. Jay, R.W. Reid, C.P. Wight, S. Chao, R. Oliver, E. Islamovic, F.L. Kolb, C. McCartney, J.W. Mitchell Fetch, A.D. Beattie, Å. Bjørnstad, J.M. Bonman, T. Langdon, C.J. Howarth, C.R. Brouwer, E.N. Jellen, K. Esvelt Klos, J.A. Poland, T.-F. Hsieh, R. Brown, E. Jackson, J.A. Schluetera, N.A. Tinker, 2016. A consensus map in cultivated hexaploid oat reveals conserved grass synteny with substantial sub-genome rearrangement. The Plant Genome, 9: 21pp

K.A. Gardner, L.M. Wittern, I.J. Mackay, 2016. A highly recombined, high-density, eight-founder wheat MAGIC map reveals extensive segregation distortion and genomic locations of introgression segments. Plant Biotechnol J, 14: 1406-1417.

X. He, M. Lillemo, J. Shi, J. Wu, A. Bjørnstad, T. Belova, S. Dreisigacker, E. Duveiller, P. Singh, 2016. QTL Characterization of Fusarium Head Blight Resistance in CIMMYT Bread Wheat Line Soru#1. PLoS ONE, 11: e0158052

Y.-F. Huang, J.A. Poland, C.P. Wight, E.W. Jackson, N.A. Tinker, 2014. Using genotyping-by-sequencing (GBS) for genomic discovery in cultivated oat. PLoS ONE, 9: e102448.

H.C. Murphy, 1935. Physiologic specialization in *Puccinia coronata* f.sp. *avenae*. U.S. Department of Agriculture Technical Bulletin 433.

C.C.T. Romero, J.P. Vermeulen, A. Vels, A. Himmelbach, M. Mascher, R.E. Niks, 2018. Mapping resistance to powdery mildew in barley reveals a large-effect nonhost resistance QTL. Theor. Appl. Genet., 131: 1031-1045.

R.W. Stubbs, J.M. Prescott, E.E. Saari, H.J. Dubin, 1986. Cereal Disease Methodology Manual. Mexico: CIMMYT Publications.

R.F. Peterson, A.B. Campbell, A.E. Hannah, 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Canadian Journal of Research, 26c:496-500.

J.D. Procunier, X. Jie, K.J. Kasha, 1991. A rapid and reliable DNA extraction method for higher plants. Barley Genet Newsl, 20:74-75.

N.A. Tinker, S. Chao, G.R. Lazo, R.E. Oliver, Y.-F. Huang, J.A. Poland, E.N. Jellen, P.J. Maughan, A. Kilian, E.W. Jackson, 2014. A SNP genotyping array for hexaploid oat. The Plant Genome, 7.

J. Van Ooijen, 2006. JoinMap 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen.

J.-F. Zuo, Y. Niu, P. Cheng, J.-Y. Feng, S.-F. Han, Y.-H. Zhang, G. Shu, Y. Wang, Y.-M. Zhang, 2019. Effect of marker segregation distortion on high density linkage map construction and QTL mapping in soybean (*Glycine max* L.). Heredity, 123: 579–592.

8. **Results and discussion:** *Describe research accomplishments during the reporting period under relevant objectives listed under section 6. The results need to be accompanied with tables, graphs and/or other*

illustrations. 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.

Pc40

As a reminder from the last reporting period, the *Pc40* differential line (Pendek*2/CAV 4997) (Chong et al., 2000) was crossed with three crown rust susceptible cultivars: 'AC Morgan', 'Bingo', and 'Kasztan'. The F5 family of *Pc40* x 'AC Morgan' is comprised of 210 recombinant inbred lines (RILs), the F2 populations derived from *Pc40* x 'Bingo' and *Pc40* x 'Kasztan' consisted of 155 and 156 lines, respectively, and their F2:3 families contained 154 and 150 lines. Pca isolate CR257 (BRBG-97) was used for evaluation of the populations. DNA was extracted from young leaves (one plant/line) of the *Pc40* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). As shown in Table 2, segregation ratios in all 5 populations indicate the presence of a single gene (i.e. *Pc40*) which governs resistance to crown rust (as assessed with Pca isolate CR257).

Table 2. Disease reaction data for five *Pc40* populations evaluated with the Pca CR257 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc40 x 'AC Morgan'	F5	114	–	96	1:1	0.214
Pc40 x 'Bingo'	F2	120	–	35	3:1	0.575
	F2:3	41	77	36	1:2:1	0.850
Pc40 x 'Kasztan'	F2	116	–	40	3:1	0.853
	F2:3	42	68	41	1:2:1	0.520

During the current reporting period QTL mapping was performed on the *Pc40* x 'AC Morgan' population. After filtering of the 6K SNP dataset a total of 733 high quality markers were used for mapping, of which 656 markers were mapped on the *Pc40* x 'AC Morgan' map, which included 27 linkage groups and a total map length of 1,091 centimorgans (cM) (Table 3). The average density across the entire map was 0.60 markers per cM. The Mrg01, Mrg03, Mrg09, Mrg11, Mrg12 and Mrg20 consensus linkage groups, as defined by Chaffin et al. (2016), were each represented by two linkage groups in the current map (Table 3).

One major QTL, named Pc40 (Mrg17_109.7-110.4 cM), was identified on chromosome Mrg17 (Table 4) and the allele responsible for resistance was contributed by the *Pc40* parent. The Pc40 QTL explained 68.7% of the phenotypic variation within a QTL interval of 1.3 cM (Table 4) and the additive effect of one *Pc40* allele was to reduce the phenotypic score by 1.5 points.

Table 3. Summary information for *Pc40* x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (markers/cM)	Largest Gap (cM)
Mrg01.1	26	13.3	1.95	5.9
Mrg01.2	62	50.5	1.23	8.5
Mrg02	48	61.2	0.78	12.2
Mrg03.1	5	2.3	2.11	0.8
Mrg03.2	17	34.3	0.49	21.8
Mrg04	29	30.9	0.94	6.7
Mrg05	18	11.7	1.54	3.7
Mrg06	29	40.5	0.72	7.4

Mrg08	21	116.1	0.18	24.1
Mrg09.1	19	76.7	0.25	31.3
Mrg09.2	9	48.9	0.18	15.5
Mrg11.1	14	51.5	0.27	24.4
Mrg11.2	12	19.2	0.62	11.6
Mrg12.1	37	81.2	0.46	11.6
Mrg12.2	15	14.2	1.05	6.1
Mrg13	22	18.8	1.17	3.2
Mrg15	10	40.7	0.25	18.4
Mrg17	50	88.1	0.57	17.4
Mrg18	40	84.0	0.48	21.2
Mrg19	7	26.7	0.26	14.3
Mrg20.1	9	23.1	0.39	14.5
Mrg20.2	25	31.7	0.79	17.8
Mrg21	42	40.8	1.03	5.7
Mrg23	34	16.6	2.04	7.0
Mrg24	34	29.9	1.14	4.1
Mrg28	8	4.8	1.66	2.0
Mrg33	14	45.4	0.31	21.6
Total	656	1090.9	0.60	31.3

Table 4. Summary information for the crown rust resistance QTL identified in the *Pc40* x 'AC Morgan' population.

QTL	Chrom.	Consensus Map Location (cM)	*Pc40* x 'AC Morgan' Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc40	Mrg17	109.7-110.4	24.9-26.2	GMI_ES03_c5940_422 GMI_ES01_c15612_54	54.9	68.7	-1.55

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc40* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

Pc46

As a reminder from the last reporting period, the *Pc46* differential line (Pendek*4/CAV 5115) (Chong et al., 2000) was crossed with two crown rust susceptible cultivars: 'AC Morgan' and 'Kasztan'. The F6 family of *Pc46* x 'AC Morgan' is comprised of 202 recombinant inbred lines (RILs), the F2 population derived from *Pc46* x 'Kasztan' consisted of 199 lines, and the F2:3 family contained 189 lines. Pca isolate CR254 (LRBG) was used for evaluation of the populations. DNA was extracted from young leaves (one plant/line) of the *Pc46* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND). As shown in Table 5, the segregation ratio for the *Pc46* x 'Kasztan' F2 population is unclear as to the number of genes. The observed ratio was tested against both a dominant and recessive form of resistance with neither fitting. However, the F2:3 population clearly indicated the presence of a single gene (i.e. *Pc46*) which governs resistance to crown rust (as assessed with Pca isolate CR254).

Table 5. Disease reaction data for three *Pc46* populations evaluated with the Pca CR254 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc46 x 'AC Morgan'	F5	140	–	62	1:1	<0.0001
					3:1	0.07
Pc46 x 'Kasztan'	F2	88	–	111	3:1	<0.0001
					1:3	<0.0001
	F2:3	55	96	38	1:2:1	0.21
Pc46 x 'Ajay'	F2			Not rated yet		
	F2:3			Not rated yet		

During the current reporting period, the *Pc46* x 'AC Morgan' F5 population was evaluated for reaction to the CR254 isolate. As shown in Table 5, the segregation ratio of resistant to susceptible lines best fit a 3:1 ratio, indicative of two independent resistance genes being present in the *Pc46* parent. The *Pc46* x 'Ajay' (susceptible) F2 population was to be phenotyped and DNA extracted by the Klos group in late 2020 or early 2021, but due to COVID-19 this was not completed. This work, along with the F2:3 population from this cross will also be phenotyped in 2021-22.

QTL mapping was performed on the *Pc46* x 'AC Morgan' population. After filtering of the 6K SNP dataset a total of 739 high quality markers were used for mapping, of which 623 markers were mapped on the *Pc46* x 'AC Morgan' map, which included 29 linkage groups and a total map length of 1,073 centimorgans (cM) (Table 6). The average density across the entire map was 0.58 markers per cM. The Mrg01, Mrg03, Mrg08, Mrg09, Mrg11, Mrg12, Mrg20 and Mrg24 consensus linkage groups, as defined by Chaffin et al. (2016), were each represented by two linkage groups in the current map (Table 6).

Despite the indications from the *Pc46* x 'AC Morgan' F5 population that potentially two resistance genes were present in this population, but consistent with the data from the *Pc46* x 'Kasztan' F2:3 population, only one major QTL, named Pc46 (Mrg19_71.3-75.7 cM), was identified on chromosome Mrg19 (Table 7) and the allele responsible for resistance was contributed by the *Pc46* parent. The Pc46 QTL explained 97.2% of the phenotypic variation within a QTL interval of 6.7 cM (Table 7) and the additive effect of one *Pc46* allele was to reduce the phenotypic score by 1.7 points. One could explain the difference between the genetic segregation ratio, indicating 2 genes, and the QTL mapping, indicating 1 gene, by the possibility that there was inadequate genome coverage with the 6K SNP marker data set, such that the second resistance gene was not identified due to the absence of markers in the region harboring the second gene. However, the fact that the one QTL identified explained nearly all the phenotypic variation would more strongly indicate that there was only a single gene responsible for resistance and that the population used for the mapping study was sampled by chance in such a manner as to be skewed toward lines with resistance.

Table 6. Summary information for *Pc46* x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (marker/cM)	Largest Gap (cM)
Mrg01.1	52	47.2	1.10	7.2
Mrg01.2	25	13.2	1.89	6.1
Mrg02	15	100.5	0.15	26.2
Mrg03.1	5	1.5	3.25	1.2
Mrg03.2	17	16.2	1.05	5.9
Mrg04	28	27.2	1.03	10.4
Mrg05	12	18.6	0.64	8.2

Mrg06	19	28.9	0.66	14.6
Mrg08.1	40	50.8	0.79	18.1
Mrg08.2	6	35.2	0.17	17.4
Mrg09.1	16	42.2	0.38	15.6
Mrg09.2	8	7.9	1.00	2.6
Mrg11.1	21	10.5	2.00	7.5
Mrg11.2	8	72.7	0.11	17.4
Mrg12.1	19	18.1	1.05	10.6
Mrg12.2	15	22.8	0.66	14.6
Mrg13	25	31.9	0.78	12.5
Mrg15	8	30.3	0.26	14.2
Mrg17	51	112.6	0.45	25.3
Mrg18	23	87.1	0.26	21.4
Mrg19	27	104.9	0.26	30.5
Mrg20.1	6	17.1	0.35	15.2
Mrg20.2	31	41.2	0.75	14.9
Mrg21	60	50.2	1.19	8.6
Mrg23	30	12.1	2.47	4.6
Mrg24.1	21	31.8	0.66	21.9
Mrg24.2	14	1.0	13.59	0.3
Mrg28	8	5.0	1.57	3.4
Mrg33	13	32.9	0.39	10.0
Total	623	1072.8	0.58	30.5

Table 7. Summary information for the crown rust resistance QTL identified in the *Pc46* x 'AC Morgan' population.

QTL	Chrom.	Consensus Map Location (cM)	*Pc46* x 'AC Morgan' Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc46	Mrg19	71.3-75.7	64.3-71.0	GMI_DS_LB_4867 GMI_GBS_35330	103.4	97.2	-1.71

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc40* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

Pc48 and *Pc59*

The *Pc48* differential line (Pendek*2/ A. sterilis CAV 5401) (Chong et al., 2000) was crossed with two crown rust susceptible cultivars: 'Bingo' and 'Kasztan'. There are 227 F2:3 families from the *Pc48* x 'Bingo' cross and 170 F2:3 families derived from *Pc48* x 'Kasztan'. Pca isolate CR259 (LQCB-91) will be used for evaluation of the populations and the *Pc48* x 'Bingo' population will be used for genotyping.

No work done on *Pc59* yet.

Pc62

As a reminder from the last reporting period, The *Pc62* differential line (Fraser*4/CAV 4274) (Chong et al., 2000) was crossed with two crown rust susceptible cultivars: 'AC Morgan' and 'Kasztan'. The F5 family of *Pc62* x 'AC Morgan' is comprised of 198 recombinant inbred lines (RILs), the F2 population derived from *Pc62* x 'Kasztan' consisted of 156 lines. Pca isolate CR254 (LRBG) will be used for evaluation of the populations. DNA was extracted from young leaves (one plant/line) of the *Pc62* x 'AC Morgan' population (and the parents) and was genotyped with the Oat 6K SNP Array at the Biosciences Research Laboratory, USDA-ARS (Fargo, ND).

During the current reporting period, the *Pc62* x 'AC Morgan' F5:6 population was evaluated for reaction to the CR254 isolate. As shown in Table 8, the segregation ratio of resistant to susceptible lines did not fit either a 1:1 or 3:1 ratio, indicative of a one or two independent resistance genes, respectively, being present in the *Pc62* parent. However, the *Pc62* x 'Kasztan' F2 lines and F2:3 families both were consistent with a single gene model for resistance derived from the *Pc62* parent (Table 8).

Table 8. Disease reaction data for three *Pc62* populations evaluated with the Pca CR254 isolate.

Population	Generation	Resistant	Segregating	Susceptible	Ratio	P-Value
Pc62 x 'AC Morgan'	F5:6	125	–	73	1:1	<0.0001
					3:1	<0.0001
Pc62 x 'Kasztan'	F2	108	–	35	3:1	0.29
	F2:3	38	82	35	1:2:1	0.68

QTL mapping was performed on the *Pc62* x 'AC Morgan' population. After filtering of the 6K SNP dataset a total of 692 high quality markers were used for mapping, of which 619 markers were mapped on the *Pc62* x 'AC Morgan' map, which included 24 linkage groups and a total map length of 1,120 centimorgans (cM) (Table 9). The average density across the entire map was 0.55 markers per cM. The Mrg03, Mrg09, Mrg11, Mrg23 and Mrg24 consensus linkage groups, as defined by Chaffin et al. (2016), were each represented by two linkage groups in the current map, there were no markers associated with Mrg 19 consensus linkage group and markers of Mrg01 and Mrg17 were mapped together into one LG (Table 9).

Despite no clear indication from the *Pc62* x 'AC Morgan' F5:6 population regarding the number of resistance genes segregating in the population, but consistent with the data from the *Pc62* x 'Kasztan' populations, one major QTL, named Pc62 (Mrg5_14.4-21.1 cM), was identified on chromosome Mrg5 (Table 10) and the allele responsible for resistance was contributed by the *Pc62* parent. The Pc62 QTL explained 97.9% of the phenotypic variation within a QTL interval of 6.7 cM (Table 10) and the additive effect of one *Pc62* allele was to reduce the phenotypic score by 1.8 points. As with the disparity between the genetic segregation results and the QTL mapping results for *Pc46*, the fact that the one QTL identified explained nearly all the phenotypic variation for *Pc62* would strongly indicate that there was only a single gene responsible for resistance and that the population used for the mapping study was sampled by chance in such a manner as to be skewed toward lines with resistance.

Table 9. Summary information for *Pc62* x 'AC Morgan' population genetic linkage map.

Linkage Group	Mapped Markers	Length (cM)	Marker Density (marker/cM)	Largest Gap (cM)
Mrg01/17	111	106.1	1.05	9.8
Mrg02	10	26.1	0.38	10.3
Mrg03.1	20	50.1	0.40	12.0
Mrg03.2	13	35.6	0.36	9.6
Mrg04	33	13.4	2.46	4.7
Mrg05	33	94.1	0.35	33.4

Mrg06	17	2.7	6.12	1.3
Mrg08	45	93.1	0.48	19.3
Mrg09.1	11	52.4	0.21	17.6
Mrg09.2	9	11.4	0.79	5.1
Mrg11.1	8	7.6	1.04	6.4
Mrg11.2	63	42.9	1.47	4.5
Mrg12	25	67.3	0.37	17.3
Mrg13	11	24.1	0.45	20.9
Mrg15	19	82.1	0.23	19.4
Mrg18	28	56.4	0.50	13.0
Mrg19	-	-	-	-
Mrg20	47	83.9	0.56	19.6
Mrg21	27	56.5	0.48	15.9
Mrg23.1	14	4.9	2.86	2.0
Mrg23.2	18	36.5	0.49	25.8
Mrg24.1	6	10.4	0.57	4.2
Mrg24.2	13	40.0	0.32	21.6
Mrg28	16	58.9	0.27	14.6
Mrg33	22	62.9	0.35	16.0
Total	619	1120.2	0.55	33.4

Table 10. Summary information for the crown rust resistance QTL identified in the *Pc62* x 'AC Morgan' population.

QTL	Chrom.	Consensus Map Location (cM)	*Pc62* x 'AC Morgan' Map Location (cM)	Flanking Markers	LOD[a]	Variation Explained (%)[b]	Additive[c]
Pc62	Mrg05	14.4-21.1	2.49-9.69	GMI_ES14_c11707_426 GMI_ES17_c11370_658	99.1	97.9	-1.8

[a] indicates significance of the QTL, a LOD score of 3.0 was established to declare a QTL significant.
[b] indicates the percentage of phenotypic variation explained by the QTL.
[c] indicates the direction (in this case negative) and magnitude of the effect of substituting one *Pc40* parental allele for one 'AC Morgan' parental allele on the overall phenotypic score.

Pc67

The *Pc67* differential line (Harder, 1983) was crossed with crown rust susceptible cultivar 'AC Morgan.' The F6 family of *Pc67* x 'AC Morgan' is comprised 200 recombinant inbred lines (RILs) and was grown in the CDC oat breeding program field plots in Saskatoon in the summer of 2020. The population was harvested and is ready for phenotyping. In the winter of 2021, a second population derived from the cross *Pc67* x 'Bingo' was made at the CDC to serve as the marker confirmation population. The Klos group has made a cross between *Pc67* x 'Ajay' (susceptible) and the F2 population has been genotyped. This population could serve as a second marker confirmation population. The CDC screened the parents of these crosses to identify isolates which can differentiate the presence or absence of the *Pc67* gene (Table 11). However, the CDC (as well as the Klos group) have been unable to identify a suitable isolate which is avirulent on the *Pc67* parent while being virulent on 'AC Morgan' and 'Bingo'. A few more isolates will be assessed, but if none can be identified then mapping of this gene will not be possible. Given the apparent

ineffectiveness of this gene to so many isolates it would seem to indicate this gene may not have much value to oat breeders in their attempts to incorporate and stack multiple crown rust resistance genes.

Table 11. Disease reaction of the *Pc67*, 'AC Morgan' and 'Bingo' parents to various crown rust (Pca) isolates.

Isolate	*Pc67*	'AC Morgan'	'Bingo'
CR13	4	4	4
CR77	4	4	4
CR185	4	4	4
CR192	4	4	4
CR223	4	4	4
CR249	3	3	4
CR250	2+, 3, 4	4	4
CR251	4	4	4
CR254	4	4	4
CR259	2+, 3, 4	4	4

Pc101

The *Pc101* differential line was crossed with crown rust susceptible cultivars: 'Bingo' and 'Kasztan'. Currently the *Pc101* x 'Bingo' is being advanced to the F5 generation in the CDC oat breeding program. Over the winter of 2020-21 it was grown in the greenhouse and as of summer 2021, the population is currently at the F4 generation in field plots being grown in Saskatoon. It will be sent to our winter nursery in New Zealand in 2021-22 and be ready for evaluation in summer 2022. A third population derived from the cross *Pc101* x 'AC Morgan' will be created started in fall 2021 as a second marker confirmation population. Pca isolate CR254 (LRBG) will be used for evaluation of the populations and the *Pc101* x 'Bingo' will be used for genotyping.

Adult Plant Resistance (APR)

The 'CDC Dancer' x 'AC Morgan' population was grown and evaluated at the AAFC-Morden (Menzies) field nursery in 2020. The population was unable to be grown at the U of S nursery (Kutcher) field nursery due to COVID-19. Data was received from the AAFC-Morden nursery. This population has been planted in the nurseries again in 2021. The 'CDC Dancer' x 'AC Morgan' has also been genotyped. Analysis of marker-trait relationships will be reported once several field seasons of data have been obtained due to the quantitative nature of APR. The PI 184004 x 'AC Morgan' was not planted in the 2020 or 2021 field nurseries due to a combination of COVID-19 (in 2020) and that Dr. McCartney took a new position at the University of Manitoba. We will have discussions in September 2021 to determine how to proceed with this population.

9. Interim conclusions (If any).

Work was delayed in 2020-21 due to COVID-19. My primary staff member working on this project was unable to access our lab or growth facilities from March 2020-August 2020 and a fire in the growth chamber we were using prevented its use from March-May 2021. Similarly, COVID impacted the ability to have the two APR populations assessed at the U of S field nursery and the ability to have the *Pc46* x 'Ajay' (susceptible) F2 population phenotyped and DNA extracted by the Klos group. These delays may impact our ability to deliver results by the current end-date of the project. We will not ask for an extension at the moment. Instead, we will see how much we can catch-up over the next year and we may ask for an extension at the time of the next progress report in July 2022.

10. **List any technology transfer activities undertaken in relation to this project:** *Include conference presentations, talks, papers published etc.*

    A.D. Beattie, 2021. Canadian Oat Quality. Prairie Oat Growers Association Presentation to Japanese Oat Industry, Webinar, February 24, 2021.

    A.D. Beattie, 2020. CDC Forage Barley and Oat Breeding: How it all Works. Saskatchewan Ministry of Agriculture Regional Services Branch, Saskatoon, SK, December 11, 2020.

11. **Identify any changes expected to industry contributions, in-kind support, collaborations or other resources.**

    None.

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

    None.