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PROJECT FINAL REPORT

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Section A: Project overview

1. Project number: 2014F119R
2. Project title: Development of canola cultivar blackleg resistance groups: feasibility evaluation
3. Abbreviations: AAFC: Agriculture and Agri-Food Canada WCCRRC: Western Canada Canola/Rapeseed Recommending Committee
4. Project start date: (2014/04/01)

5. Project completion date: (2017/03/31)	
6. Final report submission date: (2018/01/31)	
7. Research and development team data	
a) Principal Investigator: (Requires personal data sheet (refer to Section 14) only if Principal Investigator has changed since last report.)	
Name	**Institution**
Ralph Lange	InnoTech Alberta
b) Research team members (List all team members. For each new team member, *i.e*., joined since the last report, include a personal data sheet. Additional rows may be added if necessary.)	
Name	**Institution**
Dr. Dilantha Fernando	University of Manitoba
Dr. Henry Klein-Gebbinck	Agriculture and Agri-Food Canada
Dr. Gary Peng	Agriculture and Agri-Food Canada

Section B: Non-technical summary (max 1 page)

Almost all canola varieties registered in Canada have good levels of resistance to blackleg disease. Unfortunately, *Leptosphaeria. Maculans*, the fungus that causes blackleg, can rapidly overcome resistance, resulting in serious yield losses. The Canola Council of Canada recommends against re-use of canola cultivars because this practice speeds the fungus' adaptation to resistance genes. In practice, simply changing varieties may be ineffective, since producers have no way of knowing which resistance genes they are deploying.

Our goal was to determine if canola varieties could be grouped based on their susceptibility to different *L. maculans* populations. Our initial approach was to emulate a system used in Australia, which involves testing the disease resistance of canola varieties by suspending crop residues over canola plants and then from scoring the resulting infections. We collected crop residues from various sites in Alberta and Saskatchewan and exposed a selection of canola cultivars in this way. Initial results suggested that at least two cultivar groups were present, but these groups were difficult to validate because of differing amounts of spores produced by the crop residues. We therefore decided to remove this problem by devising a new method wherein we could control the number of spores applied to the test plants.

To do this we induced spore formation on the crop residues and pooled spores from many fungal fruiting bodies to represent the combinations of *L. maculans* strains present in field. The suspensions were used to simultaneously point inoculate cotyledons and spray and wound inoculate plants at the three-leaf stage. We found that many varieties were highly susceptible to the pooled fungal populations, although some cultivars were more susceptible to specific populations than others were. Some varieties responded differently to the same populations when inoculated as seedlings than when inoculated at the three-leaf stage, which suggests that major resistance genes, also known as seedling resistance, are not the only genes determining cultivar resistance to *L. maculans*. The results of these tests did not correspond with the performance of cultivars in canola fields. These results suggest that quantitative resistance is important, and that grouping cultivars using this method is not feasible.

Because of this, a system based on planned deployment and withdrawal of resistance genes seemed more practical. Therefore, we selected six representative isolates, that represent the most common pathogen gene combinations, using data provided by the Hossein Borhan laboratory at AAFC in Saskatoon. We tested a number of canola cultivars by inoculating them and scoring disease responses. We again observed that a large number of cultivars that were unable to withstand common *L. maculans* populations. The WCCRRC definition (Western Canada Canola and Rapeseed Recommending Committee 2009)of "moderately-resistant" or "resistant" is defined as mean disease severity (MDS) of ≤30% of Westar. Commercial fields tend to have two dominant blackleg pathotypes, but only about 6% of the cultivars we tested could simultaneously resist two of the most common *L. maculans* strains. This implies that blackleg should be killing a large percentage of canola crops. This is obviously not true, probably because of quantitative resistance genes in existing varieties. This is good news for the canola industry, but also means that resistance grouping is not feasible. Given this, labelling of individual genes, as now being implemented by the canola industry, will allow growers to rotate major genes, while still taking advantage of quantitative resistance.

Section C: Project details

1. Background (max 1 page)

Blackleg disease of canola, which is caused by the fungal pathogen *Leptosphaeria maculans*, is responsible for severe crop losses in Canada and worldwide. For example, before the widespread adoption of resistant cultivars in the 1990's, blackleg caused losses of over $500 million to Saskatchewan canola growers annually. Near-total crop losses were experienced in fields in Canada prior to the adoption of resistant cultivars in the mid 1990's, but losses have fallen off significantly since then.

Leptosphaeria maculans can recombine avirulence (avr) genes to overcome cultivar resistance. As a result, the frequency of severely affected canola fields has increased in Alberta since 2011, when heavily blackleg-involved fields were re-encountered for the first time since effective blackleg resistant cultivars became available in the 1990s. Unfortunately, examination of disease survey data from Alberta and elsewhere in western Canada indicates an increasing trend in blackleg since 2006. Worse, blackleg severity equal to that experienced in the worst fields prior to the 1990s has been sporadically observed in Alberta in cultivars rated "Resistant" to blackleg . This indicates that the pathogen is adapting to resistant cultivars, and that widespread, severe losses to blackleg disease are once again a possibility if the problem remains unchecked.

To counter the risk of pathogenic adaptation, the Canola Council of Canada and others recommend against re-use of canola cultivars in individual fields, particularly in fields under rotational intervals of less than one canola crop in four years. Changing cultivars should slow pathogenic adaptation because different combinations of resistance genes ("resistance packages") would be exposed to pathogen populationsthat were adapted to previously planted cultivars, not the new cultivar. Unfortunately, this may be ineffective in practice. Similar resistance packages may inadvertently be presented to the pathogen, as the resistance packages utilized by breeders are either proprietary or unknown. Moreover, the

mix of pathotypes in any field is a moving target (avr gene frequencies change over time). This can make canola producers reluctant to stop planting cultivars known to have good agronomic performance for unknown cultivars in exchange for uncertain protection against blackleg.

2. Objectives and deliverables (max 1 page)

Our goal was to place canola cultivars into resistance groups, based on the type of blackleg resistance genes they carry. Producers can then use this information to select cultivars that will perform against blackleg in their crop rotations. This strategy has been successful in Australia.

Specific objectives of this feasibility study were:

1. Generate inoculum for controlled-environment and field testing using canola crop residues from varieties experiencing resistance breakdown. Generate the inoculum to match that in several environments to reflect the genetic diversity of the pathogen.

2. Using this inoculum, develop methods for controlled-environment evaluation of cultivar performance planted in residues of specific canola varieties.

3. Place cultivars into preliminary resistance groups: Cultivars will be susceptible to pathogen populations generated from residues from the same group, and resistant to residues from different groups.

Per our original proposal, we intended to establish field plots in Beaverlodge (Henry Klein-Gebbinck), Saskatchewan (Gary Peng) and Manitoba (Dilantha Fernando). However, difficulties in obtaining test cultivars in the first year of the project prevented this, because the sites would have had to be established in year 1 to generate residue for subsequent testing. So, to compare field and greenhouse results, we collected residues from cultivars which could be identified, quantified disease severity at those sites and subsequently tested as many of the same cultivars in greenhouse tests as possible. This means that all project activities and expenditure of funds were conducted by InnoTech Alberta.

4. Research design and methodology (max 4 pages)

Canola residues and isolation of *Leptosphaeria maculans* isolates and populations. We collected crop residues from commercial production fields at the locations indicated in Table 1. Only fields with appreciable levels of blackleg disease were selected. All samples were obtained from commercial fields or demonstration strip trials. In-field evaluations of disease severity were conducted immediately post-swathing. Plants were randomly selected along a W-shaped pattern in each field. Disease severity of the infected plants was determined using a standard 0 - 5 severity scale for quantifying vascular discolouration of stem cross-sections (Western Canada Canola and Rapeseed Recommending Committee 2009). Symptomatic stems were retained after evaluation and dried at room temperature (ca. 20° C) on a bench top in open paper bags.

Canola cultivars. Thirty-nine commercial canola cultivars were used in this study to determine feasibility of the resistance group concept. We also included the Australian cultivars Aurea (*Brassica juncea*), Barra, Darmour, Dunkeld, Garnett, Glacier, Maluka, Oscar, Samourai, and Surpass 400. These cultivars were included for comparative purposes because the major resistance genes present in these cultivar are documented; Australian cultivars were obtained from the Australian Grains Genebank (http://www.seedpartnership.org.au/associates/agg).
All cultivars were in commercial distribution at the time this study was started, with the exception of Westar, which was used in some experiments as a susceptible control, Topas-based canola isolines provided by the Western Canada Canola/Rapeseed Committee, Quantum, Q2 and Excel. We have chosen not to reveal the identities of the cultivars because of intellectual property restrictions and to prevent the possible use of these results to make variety selection prior to implementation of a uniform industry standard for deploying blackleg resistance genes.

Inoculation and cultivar resistance evaluation

To meet project objectives 1 and 2, we developed two methods for generation of inoculum from *L. maculans* field populations: suspension of infected residues to expose target plants to spore showers, based on methods in use in Australia (Marcroft et al. 2012), and the second was a *de novo* procedure using pooled conidiospores populations collected from many individual pycnidia. We also evaluated resistance (and potential cultivar resistance groups) against a set of *L. maculans* isolates.

Preparation of inoculum and inoculation

Spore shower inocula from collected residue

To generate inoculum for method development, we soaked collected residue stems under water for 24 hours, after which the residues were removed from the water and placed onto plastic mesh suspended in plastic tubs. A layer of Grodan expanded clay pellets and 1cm of water were added to the bottom of the container to maintain high relative humidity (Figure 1). Containers were placed under cool white and near-UV fluorescent lights with an 18/6 hour (light/dark) photoperiod at constant room temperature (ca. 21°C) for approximately 60 hours.
To inoculate, potted plants with two expanded true leaves were placed on the clay pellets under the suspended residues for three days. Additional water was added to a 1cm depth, lids were placed on the tubs, and residue was misted hourly to maintain high humidity (Figure 1).
After three days, the lids and residues were removed and the plants placed in a growth chamber set to 21°C for 18 hour photoperiods, and 18°C during dark periods at a constant 90% RH for a further 7 – 10 days. Plants were then transplanted to 10" pots (2/pot) and transferred to a greenhouse bench until severe lesions developed. Disease severity was rated using standard WCCRRC procedures, i.e. classifying severity according to discolouration of stem cross sections, using a 0 – 5 severity scale where 0 = no disease. At least four replicate pots were prepared for each cultivar × residue combination. The entire procedure required approximately four months from the time of seeding to final evaluation.

Inoculum from pooled pycnidiospores

Twenty symptomatic stem pieces (each ca. 8-10cm in length) from each variety and sampling location were surface sterilized with 10% (v/v) commercial bleach (10.8% NaClO) , rinsed twice for two minutes with sterile distilled water, and placed onto water agar amended with a 1:1000 dilution of 5% Rose Bengal in water. Plates were placed under alternating fluorescent and near-UV light and darkness (18 and 6 hours, respectively) for 7-10 days. Conidiospores were harvested with a needle from sporulating pycnidia on each stem piece and suspended in a 1.5mL tube of sterile water amended with 150ppm streptomycin sulfate. A separate tube was prepared for each cultivar from each sample location. The suspended conidiospores were stored at 4°C for up to three days before being used to inoculate plants. Immediately prior to inoculation, conidiospores were loosely pelleted by centrifugation; the supernatant was discarded and the pellet resuspended in sterile distilled water. The density of the conidiospores suspension was then set to 2×10^{7} $\bullet mL^{-1}$ with a haemocytometer. One pool of conidiospores was prepared for each location, and used to inoculate all test cultivars. Separate inoculation experiments were performed on the test cultivars for each sample location, and separate inoculations of seedling and rosette-stage plants were performed. Seedling inoculations were performed by planting canola seeds in soilless growth medium and allowing them to germinate and grow for six days in a greenhouse, by which point they had reached BBCH growth stage 10 (Lancashire et al. 1991). A 10 µL droplet of conidiospore suspension was placed on each of four wounds made in both lobes of each cotyledon with an insect pin, creating four inoculation points per plant. Adult plants (BBCH 13) were wounded on the second and third true leaves with a bundle of 12 insect pins. Adult inoculations were performed by spraying each plant with ca. 50 µL of spore suspension amended with 0.01% Silwet L-77 with an airbrush at 124 kPa. Each experimental unit consisted of four inoculated plants for both the seedling and adult inoculations, and each group of plants was replicated four times and arranged in a completely randomized design.

Inoculation with known avirulence genotypes

Leptosphaeria maculans isolates used as inoculum were selected based on avirulence gene profiles, as determined by the M. Hossein Borhan laboratory of Agriculture and Agri-Food Canada in Saskatoon. Ninety-eight isolates held by InnoTech Alberta, and collected from various locations in western Canada, were submitted to the Borhan laboratory and Isolate phenotypes and genotypes generated using molecular (KASP) markers and/or inoculation of a set of single-Avr Topas *B. napus* lines. This work was conducted under separate funding from this project, and data kindly provided to InnoTech Alberta. Isolates that represented gene combinations commonly found in Alberta and Saskatchewan were selected for subsequent inoculations. Two isolates were chosen with each selected avr profile. In addition, one isolate with an avr gene profile of *AvrLm1-2-4-6-7-11-J1-S, AvrLep1-2-3* was supplied by the Borhan laboratory to provide an isolate with a profile common in Manitoba. Data from isolates with the same avr gene profile were pooled, which generated interaction phenotype data for six avr gene profiles (five pools of two isolates plus the isolate from Manitoba).

Evaluation of resistance to blackleg was conducted using the standard cotyledon inoculation procedure of Bosland and Williams (1986). *Brassica napus / L. maculans* interaction

phenotype was calculated as the mean of up to 80 data points (four wounds × five plants × four replicates)determined according to a 0 (no symptoms) to 5 (unrestricted lesions with pycnidia) severity scale (Bosland and Williams 1986). Data were standardized by expressing disease severity as a percentage of disease severity on Westar susceptible control plants included in each planting tray.

Table 1. *Brassica napus* crop residues used as inoculum sources

Field (Residue identification no.	Crop year collected	Cultivar(s)	Location
1048	2014	1876, 9998	Daysland, AB
1056	2014	9997	Lomond AB
1058	2014	1840, 1849, 1857	Coaldale AB
1059	2014	Undetermined	Coaldale AB
1062	2014	1877	Sedgewick AB
1063	2014	1879	Lougheed AB
1065	2014	1875	Vegreville AB
1067	2013	1877	Meota, SK
1070	2015	1876	Lavoy AB
1071	2015	1840	Lavoy AB
1072	2015	1876	Killam AB
1073	2015	1840	Lavoy AB
AITF	2014	1868	InnoTech Alberta, Vegreville AB

Figure 1. *Brassica napus* plants exposed to blackleg-infected *B. napus* residues. Residues are suspended in the mesh above the plants. Plant pots are on clay pellets, with water in bottom of the plastic container to maintain high humidity.

Screening canola cultivars against *Avir*-gene characterized *L. maculans* isolates

Agglomerative cluster analyses were performed on the percent-Westar standardized severity scores and visualized as heat maps using the Pheatmap package (Kolde 2015) in R version 3.4.3

(R Core Team 2017). Clustering analyses were performed on Euclidian distances or squared Euclidian distances among cultivar and isolate data points, as appropriate, and examined for common trends. Ward's minimum variance criterion calculated on squared Euclidian distances (Murtagh and Legendre 2014) was used to prepare the final heatmap/dendrogram figures.

5. Results, discussion and conclusions (max 8 pages)

Spore shower inocula from collected residue

We initially experienced difficulties obtaining seed from breeding companies for this project. For example, some were unwilling to allow use of their most recent products, or were unable to supply products that were being withdrawn from the market. As a result, we were unable to expose some cultivars to the blackleg-affected residues of the same cultivar, but we were able transmit propagules and generate disease symptoms using the spore shower method. We were able to examine a large inventory of cultivars and residues (Figure 2). Cultivars seemed to fall into two groups, seemingly based on susceptibility; one group consisting of Q2, Westar and Aurea. The remaining cultivars exhibited near-uniformly low levels of disease severity. This included cultivars with low disease severity when challenged with residues that should provide ample aggressive inoculum, such as Westar residues, or the same cultivar as the test plants. Judging from the low-severity reaction of Westar to exposure to several residues, we assume that low disease pressure is likely the reason for the low severity scores we observed (Figure 2). When symptoms did develop, the severity and incidence of disease were much lower than those commonly attained using other inoculation methods such as point inoculation or pycnidiospore sprays. One important reason for this is likely the uncontrolled level of *L. maculans* infection of crop residues from different fields. In this study, profuse pseudothecia developed on some, but not all of the canola residue pieces used as inoculum, so we feel that the most important propagules were pycnidia, which are less infective than ascospores. Australian researchers use field-collected crop residues in a ascospore shower technique (Huang et al. 2009) that reliably infects test plants. Our experience from disease surveys and field disease nurseries is that pynidia are much more common than pseudothecia on crop residues in Canada. In Australia, pseudothecia are formed after a single growing season, in contrast to Canada where two crop cycles are needed for ascospore production, meaning pycnidia and pycnidiospores are more likely the primary inoculum on our residues, including the residue pieces we used as inoculum. Therefore, to attempt to overcome the lower level of infectivity of pynidiospores, we modified the Australian inoculation technique to obtain uniform inoculum loads from field populations of *L. maculans.*

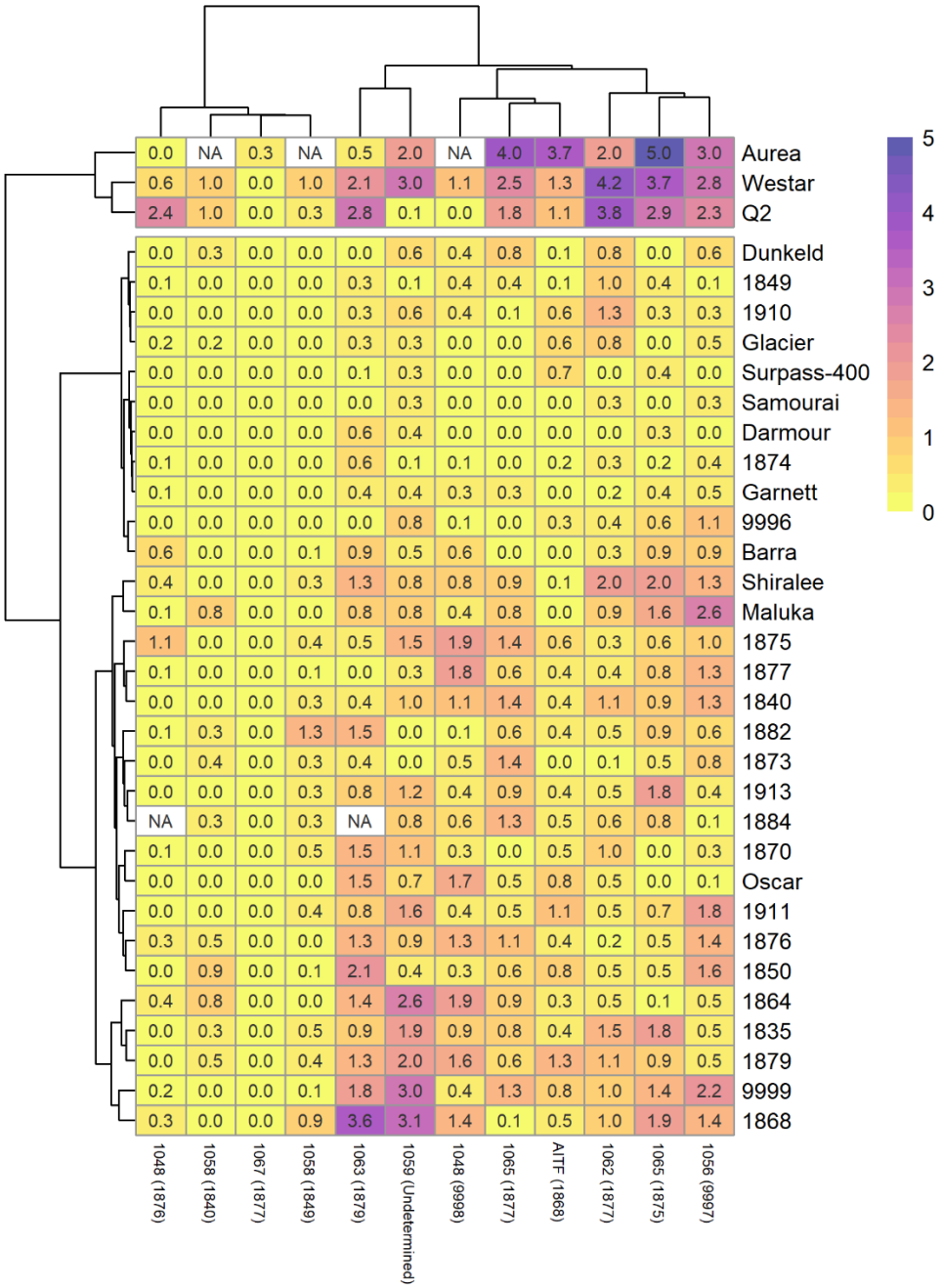

Figure 2 Severity of blackleg disease on cultivars exposed to spore showers from suspended canola residues. Numbers indicate the mean disease severity on a 0-5 scale where 0= no disease and 5 = dead plants. Columns indicate pathogen populations as described in Table 1; column label text in parentheses indicates cultivar producing the residue used as inoculum. Rows indicate cultivars. Asterisks indicate cultivars that were included in greenhouse tests. Dendrograms illustrate hierarchical clustering by Euclidian distance using Ward's criterion. Colours indicate the range of disease severity within each of the three tests. Rows are cut to indicate the two highest-order clusters. NA indicates missing data.

Inocula from pooled pycnidiospores

Our second method of inoculation was designed to remove the effects of uncontrolled differences in inoculum potential among different *L. maculans* populations. We did this by ensuring a uniform number of conidiospores from each pathogen population was applied. When we challenged a set of canola cultivars with *L. maculans* populations at uniform inoculum loads (Figure 3), we found that resistance groups could be discerned, although these groups did not meet the criterion that members of the same group should be susceptible to residues from the same group, and resistant to residues from different groups. For example, we did not observe the expected high level of disease in cultivar 1840 when exposed to populations 1071 and 1073, which included pycnidiospores harvested from 1840 residues. When seedlings were inoculated (leftmost diagram in Figure 3), we found a group with substantially lower disease than Westar (cultivars 1876, 1886, 1840, and 1874), regardless of the *L. maculans* population used as an inoculum source. This group discriminated from the remaining cultivars (and from Westar) at a high level. The remaining cultivars performed variably, depending on the inoculum source, especially the group of 9999, 1848, and 1878, which exhibited markedly higher susceptibility to population 1058 than the other populations.

We observed similar, but not identical resistance groups when the test cultivars were inoculated in the rosette stage. Group membership shifted between the two tests. Some of this was apparently due to relatively minor changes in disease severity compared to seedling inoculations, such as cultivar 1876 shifting out of the 1840-1874-1886 grouping. However, some variation from the seedling tests was due to large changes in disease resistance, notably cultivar 9999 showing disease severity approximately equal to Westar, in spite of showing a moderate level of resistance in the seedling test, especially to pathogen populations 1070 and 1071. Other cultivars, such as 1877 and 1878 responded differently to particular pathogen populations when inoculated at the seedling vs. the rosette stage. Some of this can be explained by slight variations in overall disease severity (likely due to small environmental variations in the greenhouse) or differing sets of pathogens analyzed between the two sets (we lost disease severity data in the seedling test for population 1065 due to a laptop failure). However, large shifts between seedling and adult plant tests (e.g. the large relative adult plant disease severity on cultivar 1877 compared to seedling symptoms when challenged with population 1058) are likely due to differences in the relative efficacy of major vs. quantitative resistance. Thus, any implementation of resistance group labelling of canola cultivars would have to account for the activities of these two kinds of resistance, e.g. by testing plants at adult and immature growth stages, or by evaluating cultivar performance in diverse environmental conditions and *L. maculans* populations in multiple site-year field trials.

Our results also illustrate differences in avirulence gene distribution among *L. maculans* populations (i.e. pathogen populations differ across sites), as can be seed by the column dendrograms in Figure 3. In the field, cultivars seemed to cluster into groups with high (1878), low (1810 and 1886) and intermediate mean susceptibility (the remaining cultivars), when averaged across sites (right hand column of Figure 3). Some of these groupings are well conserved in relation to the greenhouse tests (e.g. cultivar 1886) but others differed markedly between field and greenhouse tests. In particular, cultivars that appeared to be resistant in greenhouse tests could be among the most severely affected in the field (e.g. cultivar 1840). In

the latter case, greenhouse testing falsely predicted resistance in the field. We also found (when the same cultivars were planted in multiple locations) that cultivars performed non-uniformly, such as the range of responses of cultivar 1876 across four different sites (Figure 4). Such site-to-site differences are probably due in part to environmental factors, but presumably also due to differences in avr gene distribution in the local *L. maculans* populations. This implies that a resistance group scheme would have to account for such variability. Characterizing avr gene profiles could be done practically using new molecular tools, for example the KASP marker system developed under the Growing Forward 2 project "Rapid field diagnostics of the blackleg pathogen races through the identification of pathogen avirulence (Avr) genes and the development of Avr-specific markers" led by M. Hossein Borhan of AAFC in Saskatoon. Would the availability of such diagnostic tools mean canola cultivars can be arranged into resistance groups that are effective against specific *L. maculans* populations? An affirmative answer would remove much of the ambiguity and variability we observed to this point in the project.

To test this, we challenged a set of Canadian canola cultivars with isolates representing the common pathotypes found in Alberta and elsewhere. In order to ensure that we represented *L. maculans* populations accurately, we selected representative isolate pools based on phenotypic and genotypic analysis using KASP markers performed by the Hossein Borhan laboratory under GF2 funding. We selected a subsample of six *L. maculans* isolates from 98 cultures held in Alberta Innovates collections originally from 33 locations in MB, SK, and AB. These six isolates represented the most common avr gene combinations present in western Canada (Borhan, unpublished data) who found a limited number of avr gene combinations in western Canada. Cultivars can be identified in our results (Figure 5) that are resistant to each pathotype we tested. However, many other cultivars cannot be considered to be resistant. The WCCRRC definition of "moderately-resistant" or "resistant" is defined as mean disease severity (MDS) of ≤30% of Westar (Western Canada Canola and Rapeseed Recommending Committee 2009). The cultivars that meet this criterion nearly all fall into a single cluster, with the only exceptions being cultivars resistant to specific isolates (e.g. cultivars 1845 and 1865 are susceptible to all pathotypes except *A2-3-5-6-9-11-S-L1-L2*). Commercial fields tend to have two dominant pathotypes (Larkan and Borhan, unpublished data) which suggests that cultivars must be simultaneously resistant to two pathotypes to ensure a high level of disease resistance. Data provided to us by the Borhan laboratory suggest that pathotypes *A2-2- 4- 5- 6- 7- 11- S- L1-L2* and *A2-5-6-7-11-S-L1-L2* are most prevalent in Alberta (Borhan, unpublished), Figure 5 suggests that only 2 of 33 (ca. 6% of the cultivars tested) would be resistant to both the pathotypes based on these data. The same frequency of commercial cultivars simultaneously resistant to pathotypes *A1-2-4-5-6-7-11-S-L1-L2-L3* and *A1-4-5-6-7-11-S-L1-L2-L3*, which appear to be most prevalent in Manitoba (Borhan, unpublished) was observed. Even if we arbitrarily widen the definition of resistance to ≤50% of Westar on the grounds that this test exerted very severe disease pressure on the test cultivars, we still observe only 30% and 19% of varieties that are simultaneously resistant to prevalent Alberta and Manitoba pathotypes, respectively.
The low frequency of cultivars resistant to *L. maculans* populations observed in this study, combined with the high prevalence and moderate to high incidence of the pathogen (McLaren et al. 2017; Harding et al. 2017) suggest that severe blackleg epidemics should be very common in western Canada yet this is clearly not the case. Disease severity in most years is very low on

average, with only a small percentage of fields experiencing high mean disease severities. It therefore appears that quantitative resistance plays a significant role in providing practical levels of blackleg resistance in most cultivars.

It may be possible to construct resistance groups based on greenhouse or field-testing using the methods developed in this study, provided site-to-site variability in *L. maculans* populations could be accounted for. This would most feasibly be accomplished by developing representative test populations with which to screen cultivars similar to the approach of Marcroft et al. 2012. Alternatively, site-to-site variability could be accounted for in multi-site, multi-year testing at locations with well-characterized populations. Field-testing would also account for environmental effects, given a sufficient number of sites for each year of testing. Such testing would be similar to the current public co-op tests conducted by the WCCRRC and would likely incur similar costs and risks. Such a scheme would also constitute an additional layer of testing above that already required for cultivar registration. If implemented, a resistance grouping protocol would likely identify only two groups with any degree of confidence, the first group consisting of generally resistant cultivars that would perform well against most, but not all *L. maculans* populations, and a susceptible group that would contain members that are nevertheless resistant to specific pathogen populations. We feel that the resources needed to make such a system work would not justify the outcome. Labelling of specific major genes in cultivars would be simpler and less costly to implement, and generate information that could be applied to specific *L. maculans* field populations. Our results and conclusions were communicated in preliminary form to the Canola Council of Canada's blackleg steering group, and formed part of that group's recommendation to institute gene labelling in preference over cultivar resistance groups.

High levels of blackleg disease do occur sporadically (Harding et al. 2017), and these are severe enough to underscore the need for identification of additional major resistance genes and better information on *L. maculans* population structures over all geospatial and time scales. Disclosure of major resistance genes in commercial cultivars, as proposed by the Canola Council of Canada, when combined with KASP markers or other avr-gene diagnostic techniques, should provide sufficient information, specific to individual fields, to allow specific susceptible host-pathogen interactions (e.g. susceptibility of cultivar 1835 to *A1-2-4-5-6-7-11-S-L1-L2-L3*, Figure 5) to be avoided. This strategy would be strengthened by generating better knowledge of the efficacy and genetics of quantitative resistance, combined with continued emphasis on crop rotation and other blackleg control methods to reduce reliance on genetic resistance.

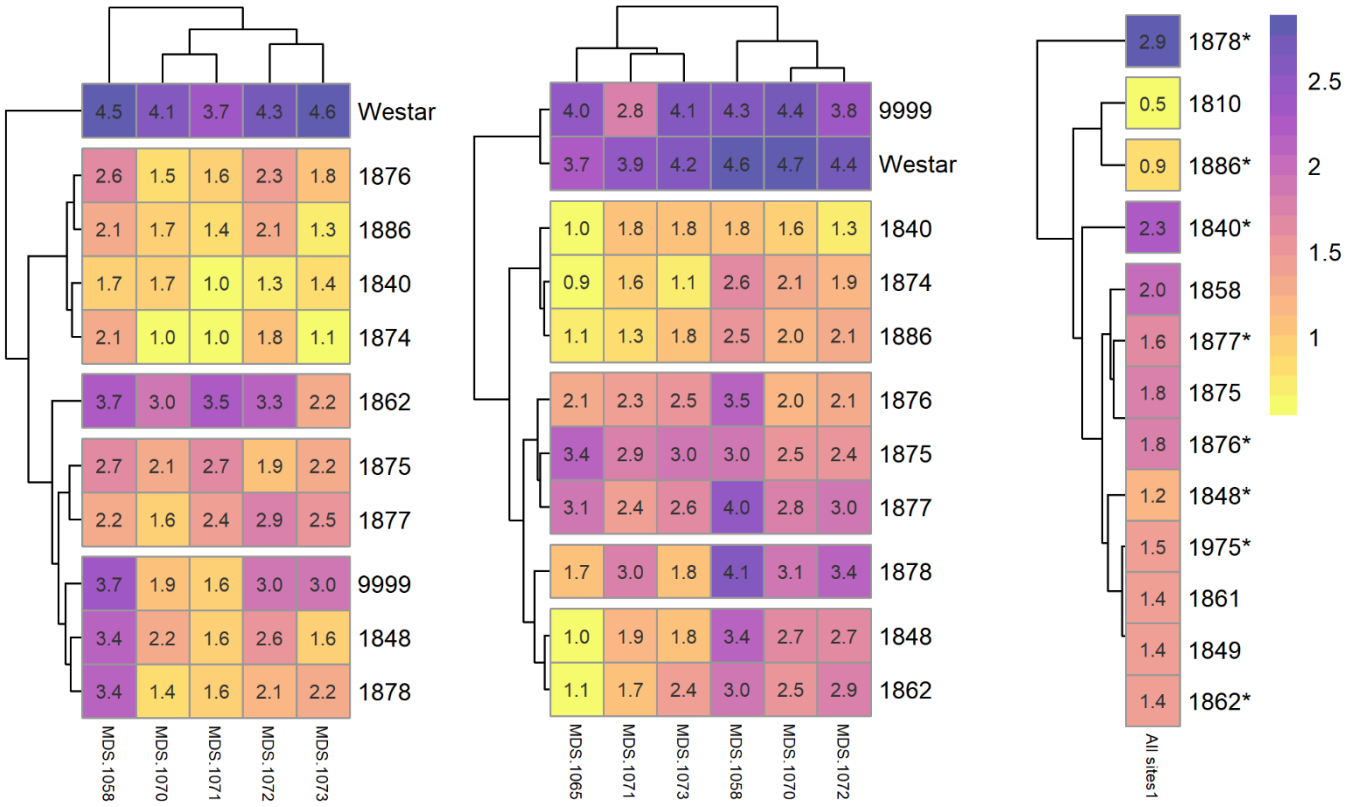

Figure 3 Response of canola cultivars exposed to populations of *Leptosphaeria maculans*. Mean disease severity (MDS) on a 0-5 scale where 0= no disease is indicated for the numbers in each square for plants inoculated in a greenhouse as seedlings (left) or adult plants (center) and in the field (right). Columns indicate pathogen populations as described in Table 1, except for field tests, in which case the mean of five locations is displayed. Rows indicate cultivars. Asterisks indicate cultivars that were included in greenhouse tests. Dendrograms illustrate hierarchical clustering by Euclidian distance using Ward's criterion. Colours indicate the range of disease severity within each of the three tests. Rows are cut to indicate the five highest-order clusters.

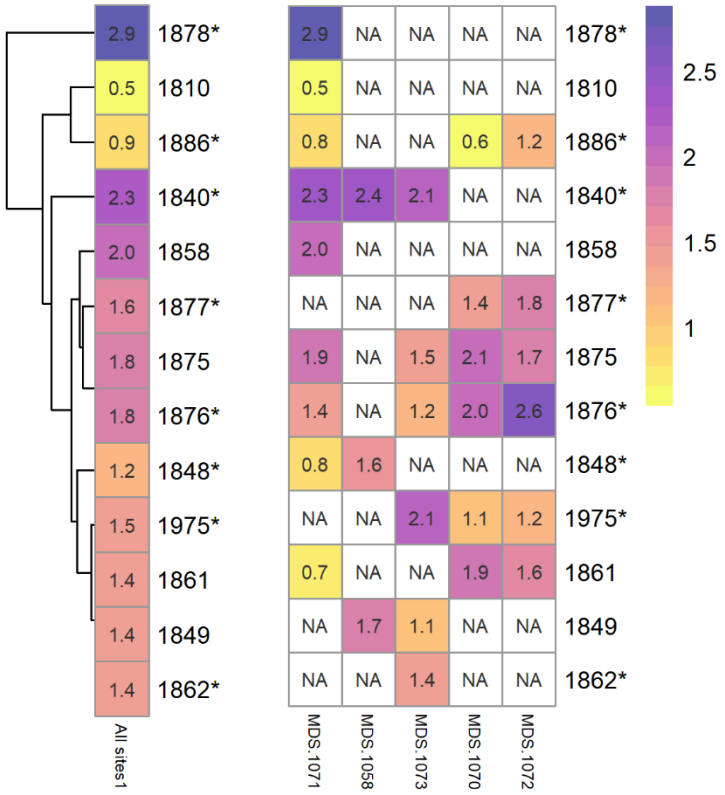

Figure 4 In-field response of canola cultivars when exposed to populations of *Leptosphaeria maculans*. Mean disease severity (MDS) on a 0-5 scale is indicated for the numbers in each square for plants exposed to natural field populations at five locations in Alberta. Columns indicate the mean response over five locations (left) or the response at each site (right). Rows indicate cultivars. Asterisks indicate cultivars that were included in greenhouse tests (Figure 3). Dendrogram illustrates hierarchical clustering by Euclidian distance using Ward's criterion. Colours indicate the range of disease severity. NA indicates sites where the particular cultivar was not present.

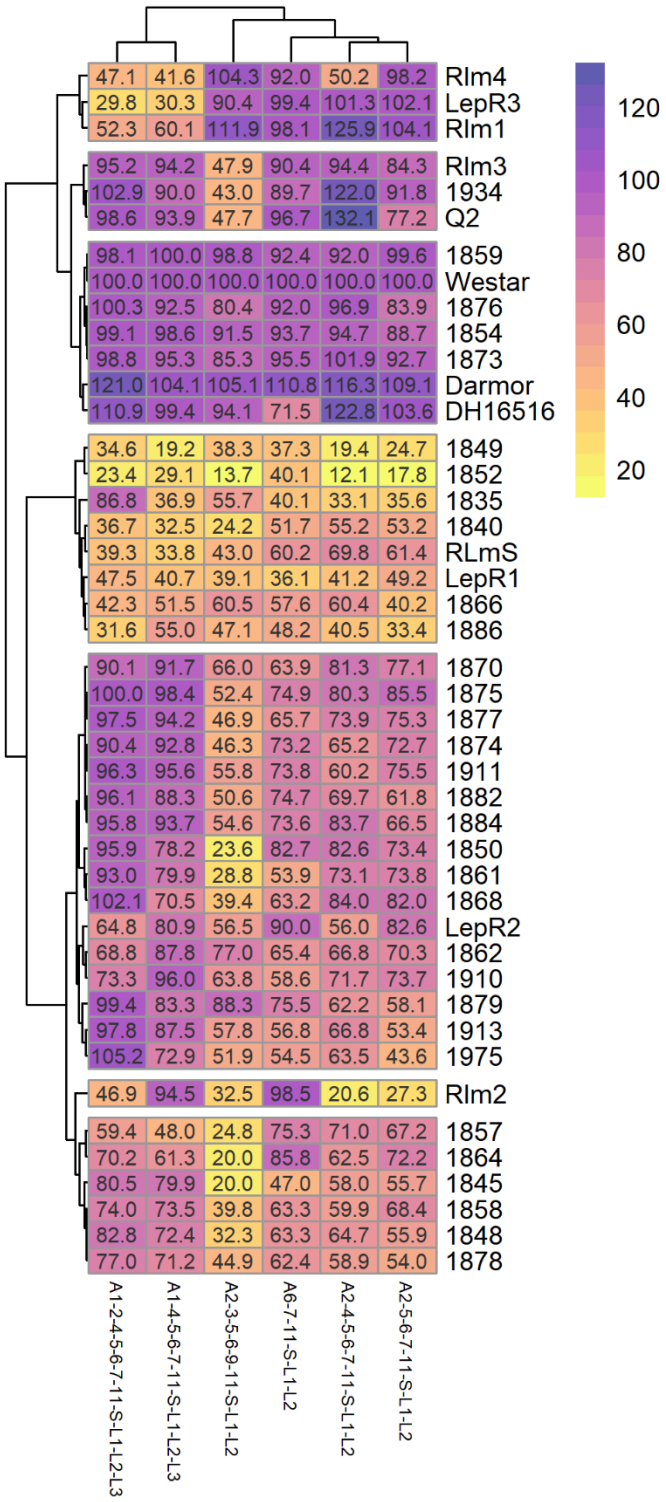

Figure 5 Severity of blackleg disease on *Brassica napus* cultivars inoculated with representative *Leptosphaeria maculans* isolates. Numbers in cells and cell colours indicate mean disease severity expressed as a percentage of severity on Westar plants. Dendrograms indicate hierarchical clustering by Ward's minimum variance method.

6. Literature cited

Bosland, P. W., and P. H. Williams. 1986. “Crucifer Genetics Cooperative Resource Book.” In *Crucifer Genetics Cooperative Resource Book. I.D. DF0C2R 5-22-87*. Madison, Wisconsin: University of Wisconsin.

Harding, M.W., T.B. Hill, G.C. Daniels, D.C. Rennie, K. Zuzak, J. Feng, and J. McDonald. 2017. “A SURVEY FOR BLACKLEG AND SCLEROTINIA STEM ROT ON CANOLA IN ALBERTA IN 2016.” *Canadian Plant Disease Survey* 97: 168–73.

Huang, Yong-Ju, Marie-Hélène Balesdent, Zi-Qin Li, Neal Evans, Thierry Rouxel, and Bruce D. L. Fitt. 2009. “Fitness Cost of Virulence Differs between the AvrLm1 and AvrLm4 Loci in Leptosphaeria Maculans (Phoma Stem Canker of Oilseed Rape).” *European Journal of Plant Pathology* 126 (2): 279–91. https://doi.org/10.1007/s10658-009-9539-7.

Kolde, Raivo. 2015. *Pheatmap: Pretty Heatmaps*. https://CRAN.R-project.org/package=pheatmap.

Lancashire, P. D., H. Bleiholder, T. Van den Boom, P. Langelüddeke, R. Stauss, E. Weber, and A. Witzenberger. 1991. “A Uniform Decimal Code for Growth Stages of Crops and Weeds.” *Annals of Applied Biology* 119 (3): 561–601.

Marcroft, Steve J., Vicki L. Elliott, Anton J. Cozijnsen, Phillip A. Salisbury, Barbara J. Howlett, and Angela P. Van de Wouw. 2012. “Identifying Resistance Genes to Leptosphaeria Maculans in Australian Brassica Napus Cultivars Based on Reactions to Isolates with Known Avirulence Genotypes.” *Crop and Pasture Science* 63 (4): 338. https://doi.org/10.1071/CP11341.

McLaren, D.L., A. Kubinec, J. Graham, E. Bargen, A. Brackenreed, D. Brar, D. Chomokovski, et al. 2017. “Survey of Canola Diseases in Manitoba In 2016.” *Canadian Plant Disease Survey* 97: 180–84.

Murtagh, Fionn, and Pierre Legendre. 2014. “Ward’s Hierarchical Agglomerative Clustering Method: Which Algorithms Implement Ward’s Criterion?” *Journal of Classification* 31 (3): 274–95. https://doi.org/10.1007/s00357-014-9161-z.

R Core Team. 2017. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Western Canada Canola and Rapeseed Recommending Committee. 2009. “Procedures of the Western Canada Canola/Rapeseed Recommending Committee Incorporated for the Evaluation and Recommendation for Registration of Canola/Rapeseed Candidate Cultivators in Western Canada.” WCC/RCC, Saskatoon, SK, Canada.

7. Project team (max ½ page)

Ralph Lange: Project management, principle researcher: experimental design, statistical analysis.

Wendi Dmytriw: Chief Technologist.

Per the original proposal, we intended to establish field plots in Beaverlodge (Henry Klein-Gebbinck), Saskatchewan (Gary Peng) and Manitoba (Dilantha Fernando) and Vegreville (Ralph Lange. However, difficulties in obtaining test cultivars in the first year of the project prevented this, because the sites would have had to be established in year 1 to generate residue for subsequent testing. So to compare field and greenhouse results, we collected residues from cultivars could be identified, quantified disease severity at those sites and then test as many of

the same cultivars in greenhouse tests as possible. This means that project activities and expenditure of funds were conducted by InnoTech Alberta.
We obtained significant assistance from Dr. M. Hossein Borhan (AAFC – Saskatoon)and Dr. Nick Larkan (Armatus Genetics) in characterizing *L. maculans* isolates. The work of Drs. Borhan and Larkan was not done with consortium funds.

8. Benefits to the industry

a) **Project impact** At the time that this project was begun, there was debate within the canola research and extension communities surrounding what would be the most effective tool for enabling stewardship of blackleg resistance genes by producers and agronomists. In our original application, we described the opposing opinions as being between an "Australian-style tool" (grouping of cultivars based on cultivar performance when challenged with blackleg infected crop residues) versus a "French model" (classification based on known combinations of pathogen and host gene packages). This study demonstrates that the former method is impractical at best, and if implemented, likely to be severely confounded by the effects of quantitative resistance and environmental effects. This information was communicated to the Blackleg Steering Group and the WCCRRC pathology subcommittee as this project progressed, forming part of the reasoning behind the gene-specific cultivar labelling system being implemented by the Canola Council of Canada and canola genetics companies.

   This study also highlights the importance of quantitative resistance to blackleg by implication: major resistance genes interacting with defined avirulence genes do not generate discrete cultivar resistance groups, ergo quantitative resistance plays an important role in determine cultivars' blackleg resistance. The next important research direction should include elucidation of the genetics of quantitative blackleg resistance.

   Finally, it can be seen in the various figures of this report that there is little variability among cultivars in relation to blackleg resistance. Many cultivars were susceptible to multiple pathogen populations. We hope that this project will highlight the need for a larger suite of blackleg resistance genes in the Canadian cultivar inventory.

b) **Potential economic impact** The information collected in this project informed the cultivar-labelling scheme now being implemented by the Canadian canola industry. At present, severe blackleg disease affects something like 3% of canola fields. Assuming a disease severity of 2.5 in these fields, we would expect a yield loss of approximately 43% per severely affected field, based on recent work by Strelkov et. al. This translates to aggregate yield losses of roughly 130 million tonnes in Alberta, Saskatchewan and Manitoba, which equals the farm-gate value that could be saved by this project. Resulting decreases in blackleg incidence over time can also be used to mitigate trade restrictions currently being imposed on Canadian canola exports by China, since an effective labelling system would demonstrably reduce risk to China from blackleg in Canadian canola exports.

9. Contribution to training of highly qualified personnel (max ½ page)

Project outputs formed part of extension presentations to producers and agronomists.

10. Knowledge transfer/technology transfer/commercialisation (max 1 page)

Scientific presentation

Lange, R. M. and H. Klein-Gebbinck. July 2015 Development of canola cultivar blackleg resistance groups for Canada: early results. International Rapeseed Congress Blackleg Summit, Saskatoon SK.

Industry-oriented presentations

Canola Council of Canada. 17 September 2017. Meeting with Bayer CropScience to discuss objections to cultivar resistance labelling. Data from this project were contributed to the Canola Council's slide deck. Aberdeen, SK.

Lange, R. M. and H. Klein-Gebbinck. February 2016. Development of canola cultivar blackleg resistance groups for Canada: Preparation for field study. Presentation to Western Canadian Canola/Rapeseed Recommending Committee.

Lange, R. M. January 21, 2015. "Blackleg: A re-emerging threat in canola." Agronomy Update Conference. http://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/crop15187/$FILE/ralph-lange-blackleg-re-emerging-threat-2015.pdf

Lange, R. M. April 6, 2016."Blackleg pathogen variation & developing a "tub test"". Edmonton.

Lange, R. M. October 2016 "Canola Resistance Groups: Do they exist, and how useful can they be?" Presentation to agronomists, producers, AgProve Forum, Glaslyn SK.

Lange, R. M. and H. Klein-Gebbinck. October 2016. Stewardship of a precious resource: is durable resistance to canola diseases still possible? Presentation to agronomists, producers, AgProve Forum, Glaslyn SK.

Media activities

Arnason, R. Nov. 3, 2016. "Alberta sees blackleg cases skyrocket". Western Producer https://www.producer.com/2016/11/alberta-sees-blackleg-cases-skyrocket/

Booker, R. April 21, 2016. "Blackleg pathogens studied to rate canola resistant cultivars." Western Producer. https://www.producer.com/2016/04/blackleg-pathogens-studied-to-rate-canola-resistant-cultivars/

Section D: Project resources

1. **Provide a detailed listing of all cash revenues to the project and expenditures of project cash funds in a separate document certified by the organisation's accountant or other senior executive officer, as per the investment agreement.** Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage (CDL); and overhead (if applicable).
2. **Provide a justification of project expenditures and discuss any major variance (*i.e.*, ± 10%) from the budget approved by the funder(s).**
3. **Resources:**
   Provide a list of all external cash and in-kind resources which were contributed to the project.

Total resources contributed to the project		
Source	**Amount**	**Percentage of total project cost**
Agriculture Funding Consortium	$255,000	78.97%
Other government sources: Cash	$67,891.13	21.03%
Other government sources: In-kind		%
Industry: Cash		%
Industry: In-kind		%
Total Project Cost	**$322,891.13**	**100%**

External resources (additional rows may be added if necessary)		
Government sources		
Name (no abbreviations unless stated in Section A3)	Amount cash	Amount in-kind
InnoTech Alberta Inc	$67,891.13	
Industry sources		
Name (no abbreviations unless stated in Section A3)	Amount cash	Amount in-kind

Section E: Research Team Signatures and Authorised Representative's Approval

The Principal Investigator and an authorised representative from the Principal Investigator's organisation of employment MUST sign this form.

Research team members and an authorised representative from their organisation(s) of employment MUST also sign this form.

By signing as an authorised representative of the Principal Investigator's employing organisation and/or the research team member's(s') employing organisation(s), the undersigned hereby acknowledge submission of the information contained in this final report to the funder(s).

Principal Investigator

Principal Investigator	
Name: Ralph Lange	**Title/Organisation:** InnoTech Alberta
Signature:	**Date:** 31 January, 2018
Principal Investigator's Authorised Representative's Approval	
Name: Jean-Paul Tetreau	**Title/Organisation:** InnoTech Alberta
Signature:	**Date:** 31 January, 2018

Section E: Research Team Signatures and Authorised Representative's Approval

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Principal Investigator

Principal Investigator	
Name: Ralph Lange	**Title/Organisation:** InnoTech Alberta
Signature:	**Date:** 31 January, 2018
Principal Investigator's Authorised Representative's Approval	
Name: Jean-Paul Tetreau	**Title/Organisation:** InnoTech Alberta
Signature:	**Date:** 31 January, 2018

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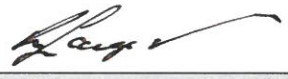
Principal Investigator

Principal Investigator	
Name: Ralph Lange	**Title/Organisation:** InnoTech Alberta
Signature: 	**Date:** 31 January, 2018
Principal Investigator's Authorised Representative's Approval	
Name: Jean-Paul Tetreau	**Title/Organisation:** InnoTech Alberta
Signature:	**Date:** 31 January, 2018

Section F: Suggested reviewers for the final report

Provide the names and contact information of four potential reviewers for this final report. The suggested reviewers should not be current collaborators. The Agriculture Funding Consortium reserves the right to choose other reviewers. Under *Section 34* of the *Freedom of Information and Protection Act (FOIP)* reviewers must be aware that their information is being collected and used for the purpose of the external review.

Reviewer #1
Name: Dr. Curtis Rempel
Position: Vice President, Crop Production and Innovation
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Reviewer #2
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Reviewer #3

Name: Dr. Nicholas Larkan
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Institution: Armatus Genetics Inc.
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Fax Number:
Email Address: nlarkan@armatus.ca

Reviewer #4

Name:
Position:
Institution:
Address:
Phone Number:
Fax Number:
Email Address:

Summary of Expenditures
Alberta Crop Industry Development Fund Ltd.,
AITF Contract # C2014000352000328- ACIDF Reference # 2014F119R
For the Period: Aprl 1, 2014 - March 31, 2017

Development of Canola Cultivar Blackleg Resistance Groups: Feasibility Evaluation
Job#: 13910172

Expenditures	**ACIDF**	**AITF**	**Total**
Personnel	$ 212,734.37	$ 52,954.26	$ 265,688.63
Overhead	$ 31,910.33	$ 7,943.18	$ 39,853.51
Materials and Supplies	$ 8,628.85	$ 6,993.69	$ 15,622.54
Travel	$ 1,726.45		$ 1,726.45
Total Expenditures	**$ 255,000.00**	**$ 67,891.13**	**$ 322,891.13**

Funding	
Alberta Crop Industry Development Fund	$ 85,000.00
Western Grains Research Foundation	$ 85,000.00
Alberta Canola Producers Commission	$ 85,000.00
InnoTech Alberta Inc	$ 67,891.13
Total Funding	**$ 322,891.13**

P.P.

Brenda Dziwenka
Financial Analyst - AITF

Jan 31/18
Date

Appendix: Revenues and Expenses

Reporting period	Source	Type	Personnel	Travel	Capital Assets	Supplies	CDL*	Other	Total
Cash Received Year 1 Dates: 2013/04/01 to 2014/03/31									**85,001.00**
	AFC	Budgeted	$52,397.26	$3,500.00		$8,825.34		$250.00	$64,972.60
		Spent/actual	$48,797.44	$14.29		$5,501.95		$7,319.49	$61,633.17
	Gov't	Cash							$0.00
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Year 1			**$48,797.44**	**$14.29**	**$0.00**	**$5,501.95**	**$0.00**	**$7,319.49**	**$61,633.17**
Carry-over Year 2 Dates: 2014/04/01 to 2015/03/31									**23,367.83**
	AFC	Budgeted	$75,684.93	$3,500.00		$15,578.77		$250.00	$95,013.70
		Spent/Actual	$76,705.83	$1,680.70		$6,775.90			$85,162.43
	Gov't	Cash	$3,088.89					$11,969.11	$15,058.00
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Year 2			**$79,794.72**	**$1,680.70**	**$0.00**	**$6,775.90**	**$0.00**	**$11,969.11**	**$100,220.43**
Carry-over Year 3 Dates: 2015/04/01 to 2016/03/31									
	AFC	Budgeted	$75,684.93	$3,500.00		$15,578.77		$250.00	$95,013.70
		Spent	$84,237.00	$31.46		$3,344.69		$20,564.91	$108,178.06
	Gov't	Cash	$52,859.47						$52,859.47
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Year 3			**$137,096.47**	**$31.46**	**$0.00**	**$3,344.69**	**$0.00**	**$20,564.91**	**$161,037.53**
Carry-over Year 4 Dates: 2016/04/01 to 2017/03/31									
	AFC	Budgeted							$0.00
		Spent							$0.00
	Gov't	Cash							$0.00
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Year 4			**$0.00**	**$0.00**	**$0.00**	**$0.00**	**$0.00**	**$0.00**	**$0.00**

CUMULATIVE AFC CASH SPENT	$265,688.63	$1,726.45	$0.00	$15,622.54	$0.00	$39,853.51	$322,891.13