Agriculture Funding Consortium

Analysis and monitoring of *Leptosphaeria maculans* race dynamics in western Canada for effective use of cultivar resistance in management of blackleg on canola

(Final Report)

Abbreviations: *Avr* –Avirulence, *R*- resistant/resistance, *MR*-moderately resistant	
Project start date: April 1, 2013	**5. Project completion date:** July 31, 2017

Research Team Information

a) Research team leader: (Requires personal data sheet only if leader has changed since last report.)

Name	*Institution*	*Expertise added*
Gary Peng	AAFC Saskatoon Research Centre	Pathology, host-pathogen interaction

b) Research team members (List names of 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*	*Expertise added*
Fengqun Yu	AAFC Saskatoon	Genetics, disease resistance
Dilantha Fernando	U of M	Plant Pathology, blackleg
Sheau-Fang Hwang	Alberta Agriculture	Disease management systems
Stephen Strelkov	U of A	Pathology, disease surveys

Executive Summary

In western Canada, cultivar resistance and crop rotation were the key strategies for successful management of blackleg of canola during 1990s and 2000s. The disease has increased in both prevalence and severity in recent years. Pathogen population shift and tighter crop rotations were suspected to be the primary cause of disease increase. In 2012, a large number of fields with R or MR cultivars were found with severe blackleg, especially in southern Manitoba and parts of Alberta. Changes in pathogen may produce virulent races that can overcome the R genes in canola cultivars, resulting in resistance breakdown. To deploy R genes effectively, the pathogen race composition (*Avr* profile) and dynamics should be determined and monitored. This work was initiated in 2007 using nine "Westar" trap plots and found that *Avrlm1* and *AvrLepR2* were generally low in the pathogen population while the other *Avr* genes were at moderate to high levels. Another study using samples collected from commercial fields in 2010 and 2011 found that *AvrLm3* and *AvrLm9* had decreased substantially from the levels observed previously. At the same time, *AvrLm7* increased. It was not clear whether the change was caused by different sampling methods used in two studies; theoretically, the pathogen population in trap plots should reflect that in surroundings, but it's unclear whether the R genes used in commercial fields can skew the data because most our canola varieties carry *Rlm3*. The objectives of this project were to analyze/monitor the *Avr* profile using Westar trap plots for regional pictures of *Avr* frequencies in the pathogen population. The study can also help identify new pathogen races capable of overcoming specific R genes and the information can help the judicious deployment of specific *R* genes in canola cultivars for effective control of blackleg.

Between 2012 and 2015, up to 35 Westar trap plots were seeded each year in all major canola production areas on the prairies. At early crop maturity, diseased stems were sampled from each plot, pathogen isolated and tested on 14 differential brassica varieties/lines carrying known *R* genes to determine the presence/absence of

specific *Avr* genes in pathogen; plants carrying an *R* gene would resist the infection only when the pathogen isolate carried the corresponding *Avr* gene. The number of pathogen isolates tested each year varied slightly depending on the blackleg occurrence in different regions; in a light disease year, fewer isolates were obtained due to a lower rate of isolation, but overall the number ranged from 202 to 626 isolates each year.

Looking across the prairies, the pathogen population changed noticeably over the four years of study; *AvrLm2*, *AvrLm4*, *AvrLm6,(8)* and *AvrLm7* were present at relatively high frequencies while the other *Avr* genes were low or even undetectable. *AvrLmS* was detected in 70% of the isolates in 2012 but was found in only 5% and 23% of the isolates collected in 2014 and 2015, respectively. Even those high-frequency *Avr* genes observed in 2012 were noticeably lower in 2015. Continued monitoring is warranted to determine if this is a new trend and where the further changes are going. When compare the data from Westar trap plots with those of commercial fields (2015) based on canola disease surveys, we notice that the prevalent *Avr* genes were similar between the respective populations; *AvrLm1*, *AvrLm3*, *AvrLm9* and *AvrLepR2* were generally very low while *AvrLm2*, *AvrLm4*, *AvrLm6,(8)* and *AvrLm7* were high in the field population. For the data in 2015, *AvrLm6,(8)* appeared noticeably more common in commercial fields than in Westar trap plots.

Among the three provinces, similarities as well as differences were observed in *Avr* profile; *AvrLm2*, *AvrLm4*, *AvrLm6,(8)* and *AvrLm7* were generally at high frequencies in each province while the other *Avr* genes were very low. *AvrLm2*, however, seemed to decrease substantially in Alberta (57% to 8%) and Manitoba (36% to 6%) between 2014 and 2015, but held steadily at about 40% in Saskatchewan. In Alberta, the frequency of *AvrLm4* was consistently lower (~40%) than those in Saskatchewan and Manitoba (60-80%) over the years of study.

The study identified the prevalence and changes of *Avr* genes in pathogen populations on the prairies between 2012 and 2015; *AvrLm2*, *AvrLm4*, *AvrLm6,(8)* and *AvrLm7* were found at high frequencies while *AvrLm1* and *AvrLm3* were generally low. This information is useful to canola breeding companies for deploying effective *R* genes against the current pathogen population on the prairies. Since only the *Rlm1* and/or *Rlm3* were found commonly in Canadian cultivars, it is likely that the major-gene resistance to blackleg is currently lacking on the prairies due to the generally low presence of *AvrLm1* and *AvrLm3* in the pathogen population. Therefore, it is postulated that the blackleg resistance by many of the R-rated canola cultivars is via other mechanisms, especially race nonspecific resistance, or quantitative/adult plant resistance. It is expected that *Rlm4*, *Rlm6* or *Rlm7* can substantially improve the blackleg resistance in most regions on the prairies, especially when used with proven background resistance.

Caution should be exercised when using this big-picture information to guide canola cultivar rotation based on specific *R* genes in a given field because the pathogen race structure can change substantially from one field to another as shown by the results of study. In the cases where severe blackleg damage occurs in a field, it would be a good practice to consider a longer crop rotation option as the first line of defense. To use a canola cultivar effective against the pathogen population in a specific field, it would be prudent to assess prominent Avr types in the field.

Overall, the project is on schedule and budget. New funding has been approved to continue the work in next 4 years to track any further change of the pathogen population, especially with potential introduction of new *R* genes and *R*-gene labelling practices in next few years.

Details of repot (be brief)

Introduction

Blackleg disease, caused by *Leptosphaeria maculans*, can be a serious constraint to the production of canola or rapeseed worldwide (Fitt et al. 2006; West et al. 2001). In western Canada, variety resistance and crop rotation are the main strategies for blackleg management and were relatively successful for many years. New pathogen races have been reported since 2002 (Chen et al. 2005, 2006; Kutcher et al. 2009). With tighter crop rotations in most of the canola growing regions (Guo et al. 2005), there were increasing reports of blackleg in past few years. In 2012, a large number of fields with a resistant (R) or moderately resistant (MR) cultivars were found with severe blackleg, especially in southern Manitoba and parts of Alberta (Peng & McCaughey 2012). Some fields showed almost 100% disease incidence. It was suspected that this sudden spike of blackleg is a result of the proliferation of new pathogen races which can overcome the resistance genes in canola cultivars.

A study led by Drs. Fernando (U of M) and Peng (AAFC Saskatoon) looked at the specific R genes in canola germplasm, including many commercial breeding lines, and found that only Rlm1 and/or Rlm3 are present in most of the lines tested (Zhang et al. 2016). The long-term use of these two R genes in most canola cultivars would likely lead to race shifts in the pathogen population. On the prairies, the blackleg pathogen races have been studied since the early 2000s, initially using a pathogenicity group (PG) system (Chen et al. 2005, 2006, Kutcher et al. 2007) and more recently, based on avirulence (*Avr*) genes in the pathogen population (Kutcher et al. 2010). Using the latter approach, Kutcher et al. (2011) conducted an extensive analysis of pathogen *Avr* frequency using samples collected from "Westar" (without R genes) trap plots at nine locations on the prairies in 2007, and showed that the *AvrLm1* and *AvrLepR2* were low across the region, *AvrLm2* and *AvrLm3* were low in southern Manitoba. In Saskatchewan and parts of Alberta, *AvrLm3* appeared at moderate levels even by 2010 (Kutcher et al. 2011; Cross et al. 2012). This work provided baseline information on the *Avr* profile in pathogen populations on the prairies. The development of new pathogen races, coupled with increasingly more intensive canola production, increases the risk of blackleg epidemics when the current R genes are overcome by the pathogen. Another study using samples collected from commercial fields in 2010 and 2011 found that *AvrLm3* and *AvrLm9* had decreased substantially from the levels observed previously, and at the same time, *AvrLm7* increased (Liban et al. 2016). It was not clear whether the changes were caused by different sampling methods used in two studies or there had been real shift in the pathogen population over the period. In theory, the pathogen population in trap plots should reflect that in the surrounding area, but it's unclear whether the R genes used in commercial fields can skew the data because most of our canola varieties would carry *Rlm3*. The objectives of the study were to continue analyzing/monitoring the *Avr* profile using primarily Westar trap plots for regional pictures of *Avr* frequencies in the pathogen population. The study can also identify new pathogen races virulent against the R genes in canola cultivars by analysing the pathogen race structure on commercial varieties with severe blackleg damage.

Literature cited

1. Chen Y, Fernando WGD. 2005. First Report of Canola Blackleg Caused by Pathogenicity Group 4 of *Leptosphaeria maculans* in Manitoba. Plant Dis. 89:339.
2. Chen Y, Fernando WGD. 2006. Prevalence of pathogenicity groups of Leptosphaeria maculans in western Canada and North Dakota, USA. Can. J. Plant Pathol. 28:533-539.
3. Cross D, Peng G, Liban SH, Fernando WGD, Kirkham C, Yu F, Kutcher HR, Dokken-Bouchard FL, McLaren D. Lange R. Jurke C, and Kubinec A. 2012. The Race structure of *Leptosphearia maculans* in commercial canola fields based on 2010 disease surveys (poster). Sask-Albt. joint Ann. Plant Pathology Meeting, Lloydminster, SK.

4. Daverdin G, Rouxel T, Gout L, Aubertot J-N, Fudal I, et al. 2012. Genome Structure and Reproductive Behaviour Influence the Evolutionary Potential of a Fungal Phytopathogen. PLoS Pathog 8(11): e1003020. doi:10.1371/journal.ppat.1003020
5. Fitt BDL, H Brun, MJ Barbetti and SR Rimmer. 2006. World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). European J. of Plant Pathol. 114: 3-15.
6. Fudal I, Ross S, Gout L, Blaise F, Kuhn ML, Eckert MR, Cattolico L, Bernard-Samain,S., Balesdent MH and Rouxel T. 2007. Heterochromatin-like regions as ecological niches for avirulence genes in the *Leptosphaeria maculans* genome: map-based cloning of *AvrLm6*. Mol. Plant Microbe Interact. 20, 459-470.
7. Gout L, Fudal I, Kuhn ML, Blaise F, Eckert M, Cattolico L, Balesdent MH and Rouxel T. 2006. Lost in the middle of nowhere: the *AvrLm1* avirulence gene of the Dothideomycete *Leptosphaeria maculans*. Mol. Microbiol. 60, 67-80.
8. Guo XW, Fernando WGD, Entz MH. 2005. Effects of crop rotation and tillage on the blackleg disease of canola. Can J. Plant Pathol. 27: 53-57.
9. Kutcher HR, Keri M, McLaren DL, Rimmer SR. 2007. Pathogenicity of *Leptosphaeria maculans* in western Canada. Can. J. Plant Pathol. 29: 388-393.
10. Kutcher HR, Yu F. 2009. Blackleg revisited – races and resistance. Proc. Man. Agron. Conf. http://www.umanitoba.ca/afs/agronomistsconf/2009/proceedings2009.html.
11. Kutcher HR, Balesden, MH, Rimmer SR, Rouxel T, Delourme R, Chèvre AM, Brun H. 2010. Frequency of avirulence genes among isolates of *Leptosphaeria maculans* in western Canada. Can. J. Plant Pathol. 32: 77-85.
12. Kutcher, H.R., Yu, F.Q., and Brun, H. 2010. Improving blackleg disease management of *Brassica napus* from knowledge of genetic interactions with *Leptosphaeria maculans*. Can. J. Plant Pathol. 32 (1): 29-34.
13. Liban SH, Cross DJ, Fernando WGD, Kutcher HR, Peng G. 2016. Race structure and frequency of avirulence genes in the western Canadian Leptosphaeria maculans pathogen population. Plant Pathol 65:1161–1169.
14. Parlange F, Daverdin G, Fudal I, Kuhn ML, Balesdent MH, Blaise F, Grezes-Besset B and Rouxel, T (2009) *Leptosphaeria maculans* avirulence gene *AvrLm4-7* confers a dual recognition specificity by the *Rlm4* and *Rlm7* resistance genes of oilseed rape, and circumvents *Rlm4*-mediated recognition through a single amino acid change. Mol. Microbiol. 71, 851-863.
15. Peng G, McCaughey PW. 2012. Blackleg of canola in Manitoba. Ministerial Briefing Notes.
16. Van de Wouw AP, Stonard JF, Atkins SD, Howlett BJ, West JS and Fitt DL. 2009. Determining frequencies of avirulent alleles in airborne inoculum of *Leptosphaeria maculans* using molecular assays 16th Australian Research Assembly on *Brassicas*. Ballarat Victoria
17. West JS, Kharbanda PD, Barbetti MJ, Fitt BDL, 2001. Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. Plant Pathology 50, 10–27.
18. Zhang XH, Peng G, Kutcher HR, Balesdent MH, Delourme R, Fernando WGD. 2016. Breakdown of Rlm3 resistance in the Brassica napus - Leptosphaeria maculans pathosystem in western Canada. Eur J Plant Pathol. 145:659–674

Materials and Methods

Each year, up to 35 plots of Westar were seeded in conjuncture with mostly variety trials across the Prairies to collect pathogen inoculum from the surrounding areas. At about 5.2 growth stage (Harper-Berkenkamp scale), plants were assessed for blackleg by cutting through hypocotyls and/or tap roots. Diseased stems were used for pathogen isolation in the lab and up to 50 isolates of *L. maculans* were kept for each location and verified for the identity (*L. maculans*) by the inoculation of Westar cotyledons. The group of isolates from each field site (location) was used as an indicator of *Avr* profile in the area, and the data was also pooled over the province or prairies a big pictures. A total of 12-15 locations were targeted each year to represent the whole prairie region, but the number varied slightly due to different levels of blackleg occurred in trap plots. Overall the number of isolates ranged from 202 to 626 each year, depending mostly on blackleg incidence and severity at different field sites.

The *L. maculans* isolates from each field location were tested on 14 differential brassica lines carrying known R genes to determine the presence and frequency of corresponding *Avr* genes in the pathogen population. The differential set is capable of differentiating up to 10 *Avr* alleles. The presence of *AvrLm1*, *AvrLm3*, *Avrlm4-7* and *Avrlm6* was further checked using a PCR-based assay. The frequency and regional distribution of *Avr* alleles were summarized comparatively on the annual basis to show changes within the period of study.

Commercial canola fields with either R or MR cultivars showing severe blackleg were sampled in 2012 and 2013 and up to 25 pathogen isolates from each field were analyzed for *Avr* profile using the same protocol

described above. The information was analyzed against R genes in the cultivar grown, as well as with the regional *Avr* pictures obtained using the Westar trap plots to determine the cause of blackleg outbreak in these commercial fields.

In 2015, isolates of *L. maculans* from Westar trap plots (304) and random samples of commercial canola fields (322) were compared for *Avr* profile to assess the results associated with the two sampling methods. The same testing protocol was used for these isolates, and a total of 626 *L. maculans* isolates were tested over the 2015 samples.

Preparation of *L. maculans* isolates: Diseased stubble was plated on V8-juice agar to which 1% streptomycin sulphate and 40 mg l-1 Rose Bengal were added to restrict bacterial growth. From single pycnidia, a small amount of exudate was transferred onto V8-juice agar and the culture grown for approximately 10 days at 20ºC under 12-h lighting. These plates were then harvested by flooding with 7 mL of sterile distilled water and the surface of the plates scraped gently with a stain-less steel rod to dislodge pycnidiospores. The spore suspension was filtered through sterile cheesecloth or a 70-µm mesh cell strainer into sterile centrifuge tubes. For cotyledon inoculation, spore suspensions were estimated for concentration using a haemocytometer and diluted to 1×10^7 spores/ml.

Host differentials: A set of 14 Brassica varieties/lines with known R genes were used as differentials. Several of them are winter types and would require vernalization for seed increase. These lines were grown under controlled conditions for 4-6 weeks, vernalized at about 10ºC for 6-8 weeks, and transplanted in a field plot on the AAFC Melfort Research Farm to increase seed. The plants were grown individually in 'cages' to prevent cross-pollination, thereby ensuring homozygous seed lots for each variety.

These differentials would allow to determine the presence of *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4*, *AvrLm6,(8)*, *AvrLm7*, *AvrLm9*, *AvrLmS/LepR3, AvrLepR1*, *AvrLepR2* and *AvrLepR3* in *L. maculans* isolates.

Host inoculation and resistance assessment: Each variety/line was planted directly into soil-less potting mix (Sunshine Mix #3) in flats are grown in growth chambers at 22/16°C day/night temperature and a 16 h photoperiod. True leaves were removed weekly after inoculation to keep the cotyledons from senescing too quickly. Plant nutrients (20-20-20 fertilizer) were added at planting at about 4 g per L and 4 L per flat.

Each isolate was tested on 2-3 plants of each variety in 2 replicates (4-6 plants in total), and each of cotyledon lobes (2) was inoculated for a total of 4 inoculation sites per plant. The cotyledons were inoculated with the pycnidiospore suspension 7 days after seeding, with each cotyledon lobe receiving a 0.5 mm diameter wound made with a modified tweezers and a 10-µl droplet of inoculum. Plants were rated for infection severity at 14 days after inoculation using a 0-9 scale that considers lesion size, tissue collapse, necrosis or chlorosis and pycnidia formation. The interaction between the isolate and host genotype was classified based on the average score as avirulent (Avr, 0 – 4.9) or virulent (avr, 5.0 – 9.0).

Identification of *Avr* genes using a PCR-based assay: *Avrlm1, Avrlm6* and *Avrlm4-7* had been cloned from *L. maculans* prior to the start of this project (Gout et al. 2006; Fudal et al. 2007; Parlange et al. 2009). Primers were designed and validated for detection of these *Avr* genes in pathogen isolates using PCR described by Van de Wouw et al (2009) and Daverdin et al (2012).

Results and Discussion

***Avr* profile over the prairies**: Looking across the prairies, the pathogen population changed noticeably over the period of study; *AvrLm2*, *AvrLm4*, *AvrLm6,(8)* and *AvrLm7* were present at relatively high frequencies

but the other *Avr* genes were low or even undetectable (**Figure 1**). *AvrLmS* was detected in 70% of the isolates in 2012 samples but was found in only 5% and 23% of the isolates collected in 2014 and 2015. There appeared to be a declining trend for many of the *Avr* alleles, especially between the 2014 and 2015 results; even the high-frequency *Avr* alleles observed at the beginning of project were noticeably lower in 2015. Continued study is warranted to determine if there is a real trend of decline. When compare the data from Westar trap plots with those from commercial fields in 2015, we noticed that the prevalent *Avr* genes were similar (**Figure 2**). It was also noticeable that *AvrLm1*, *AvrLm3*, *AvrLm9* and *AvrLepR2* were not detectable while *AvrLm6,(8)* was often noticeably higher in commercial fields.

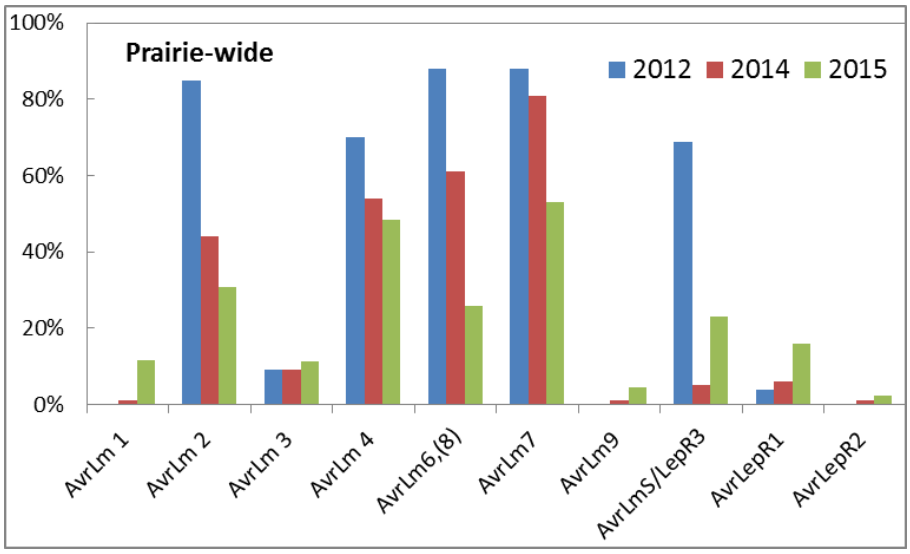

Figure 1 Comparisons of *Avr* frequencies (%) in 2012, 2014 and 2015 *L. maculans* populations on the prairies (The 2013 data was not included here due to extremely low blackleg incidence at all Alberta locations; only a few pathogen isolates were obtained and insufficient for generating an *Avr* profile).

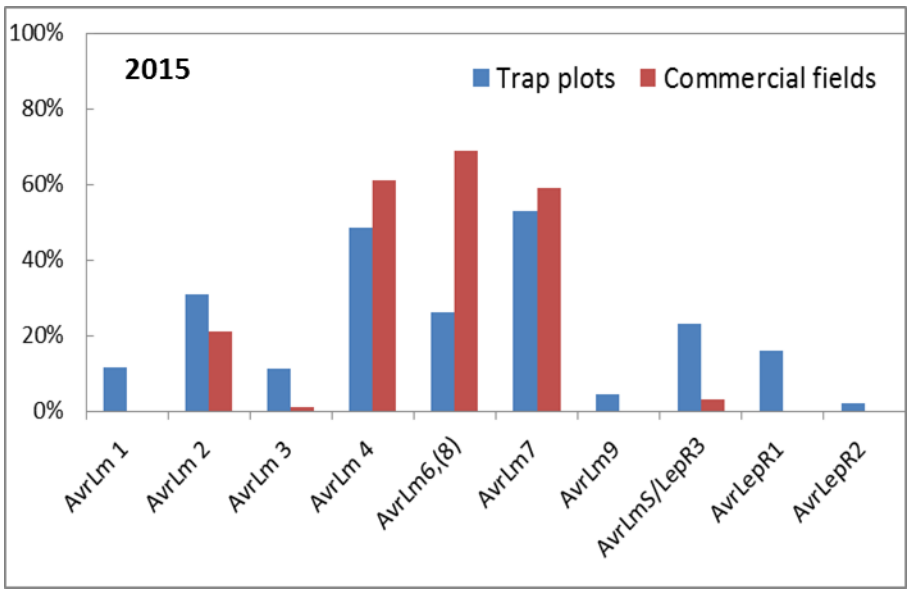

Figure 2 Comparison of *Avr* frequencies (%) in *L. maculans* populations obtained from Westar trap plots and commercial fields on the prairies (2015).

***Avr* profile in the prairie provinces (isolates from Westar trap plots)**: Similarities as well as differences were observed in the *Avr* profile for the isolates collected from three provinces; *AvrLm2*, *AvrLm4*, *AvrLm6,(8)* and *AvrLm7* were generally common in each province while the other *Avr* genes were low (**Figure 3**). *AvrLm2* appeared to decrease rapidly in Alberta (57% to 8%) and Manitoba (36% to 6%) between 2014 and 2015, while held steadily at about 40% in Saskatchewan. In Alberta, the frequency of *AvrLm4* was consistently lower (~40%) than those in Saskatchewan and Manitoba (60-80%).

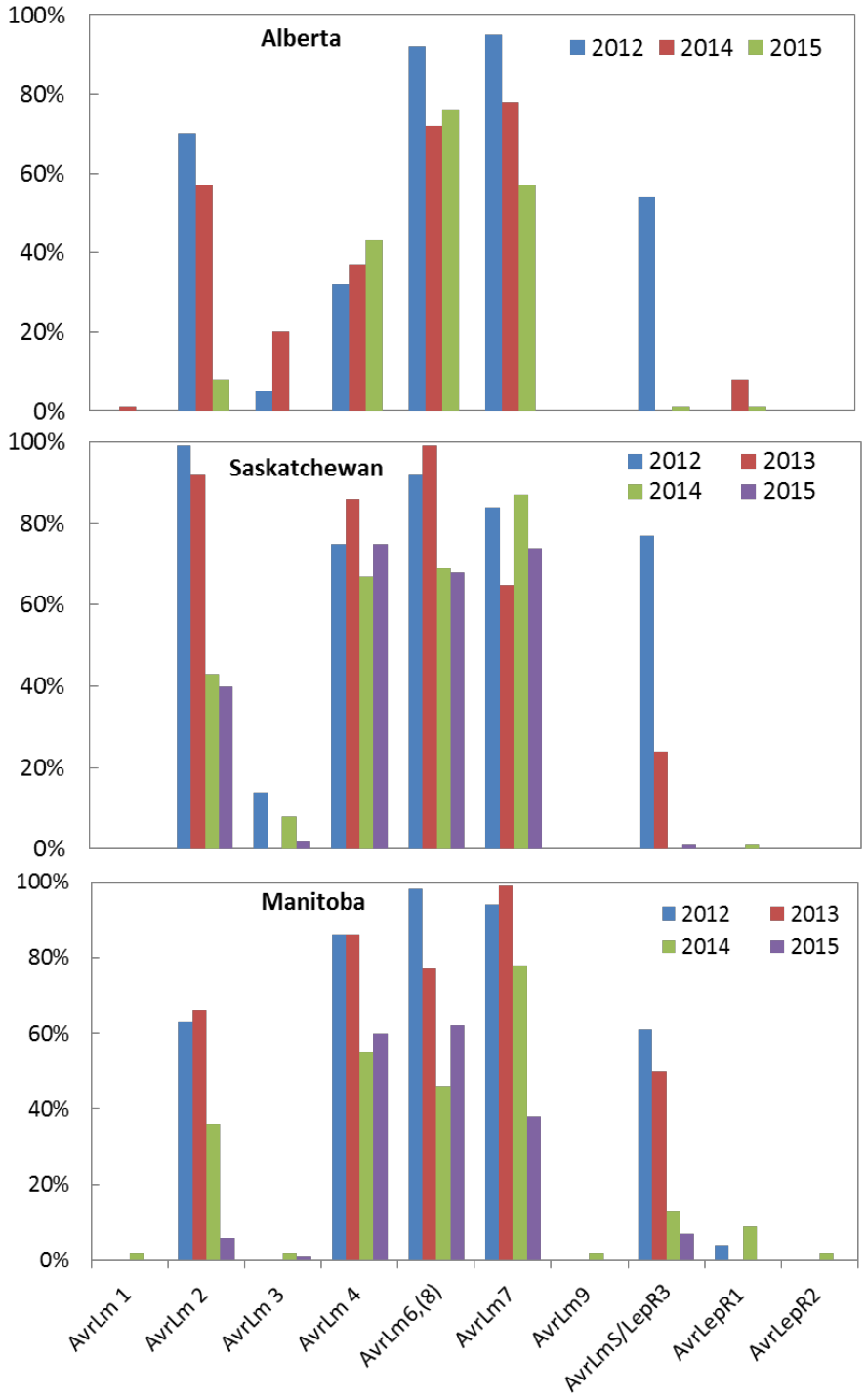

Figure 3 *Avr* frequencies (%) found in *L. maculans* populations in Alberta, Saskatchewan and Manitoba between 2012 and 2015. No data from Alberta in 2013 due to general absence of blackleg in trap plots.

***Avr* profile in severely diseased commercial fields**: Diseased stubble was collected from commercial canola fields in central Alberta and southern Manitoba where the incidence of blackleg was higher than 30% in 2012 and 2013. In each field, about 100 plants were pulled at 5 random locations along a "W" path, and the diseased stems were kept for pathogen isolation. The same set of host differentials described earlier was used for *Avr* gene identification in *L. maculans* isolates.

The isolates from five 2012 fields in Alberta (2) and Manitoba (3) were tested for *Avr*-gene profile. In Alberta, the field near Trochu showed >70% disease incidence at early crop maturity relative to ~30% in the field near Olds. *AvrLm6* and *AvrLm7* were common in both fields (**Figure 4**), while *AvrLm1* and *AvrLm9* were absent in Olds field but still detectable in the Trochu field. *AvrLm2* and *AvrLmS* were noticeably higher in Trochu field, but the most striking difference was *AvrLm4*; it was found in nearly 60% of pathogen isolates in Trochu field but not at all in the Olds field. The *Avr* profile of *L. maculans* in these two fields was not similar to that in the greater area of Alberta (**Figure 3**) other than the absence of *AvrLm4* at the Olds location. The difference in *Avr* profile between the two fields can't seem to explain the substantially higher blackleg incidence in the Trochu field because almost each *Avr* gene there showed a similar or higher frequency relative to that in the Olds field. The higher frequencies of *AvrLm2*, *AvrLm4* and *AvrLmS* in the Trochu field did not help lower blackleg incidence there. This also shows that these *Avr* genes are irrelevant to the major-gene resistance at this point because only *Rlm1* and/or *Rlm3* are present commonly in Canadian canola cultivars (Zhang et al. 2016).

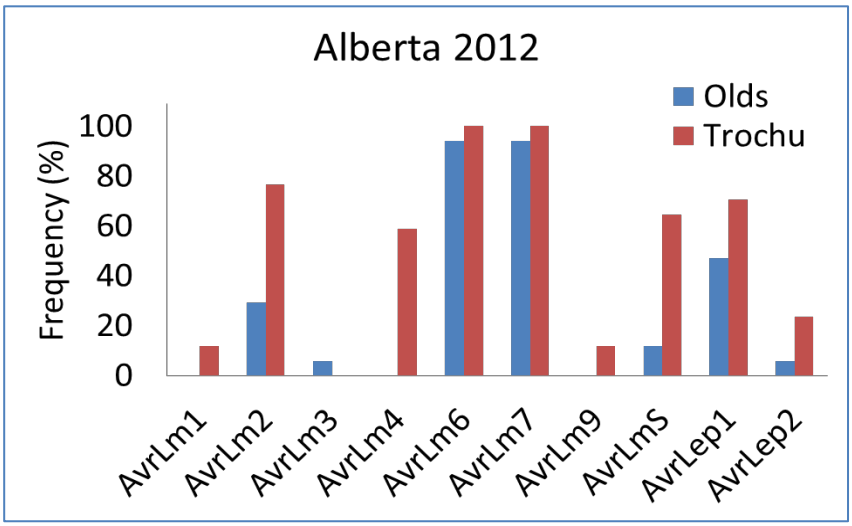

Figure 4 *Avr* allele frequencies of *L. maculans* from two commercial canola fields with high incidence of blackleg near Trochu and Olds, Alberta.

In Manitoba, the *Avr*-gene profile appeared similar among the three 2012 commercial fields examined with only minor variations (**Figure 5**); the frequencies for *AvrLm4*, *AvrLm6* and *AvrLm7* were high except at the Holland location (about 50%). *AvrLmS* was noticeably lower in the Cartwright field, while *AvrLm1, AvrLm3* and *AvrLm9* were generally low in each of these fields. Relative to that observed in the Alberta fields (**Figure 4**), *AvrLm4* appeared to be at higher levels in Manitoba, ranging between 72% and 91%. It is noteworthy that *AvrLep2* was present in almost 40% of pathogen isolates in the Holland field but absent in the other two fields. In the monitoring of large areas on the prairie using Westar trap plots, *AvrLep2* has been consistently low or undetectable. The *Avr* profile in these three commercial fields was similar to that found over the greater area of Manitoba (**Figure 3**).

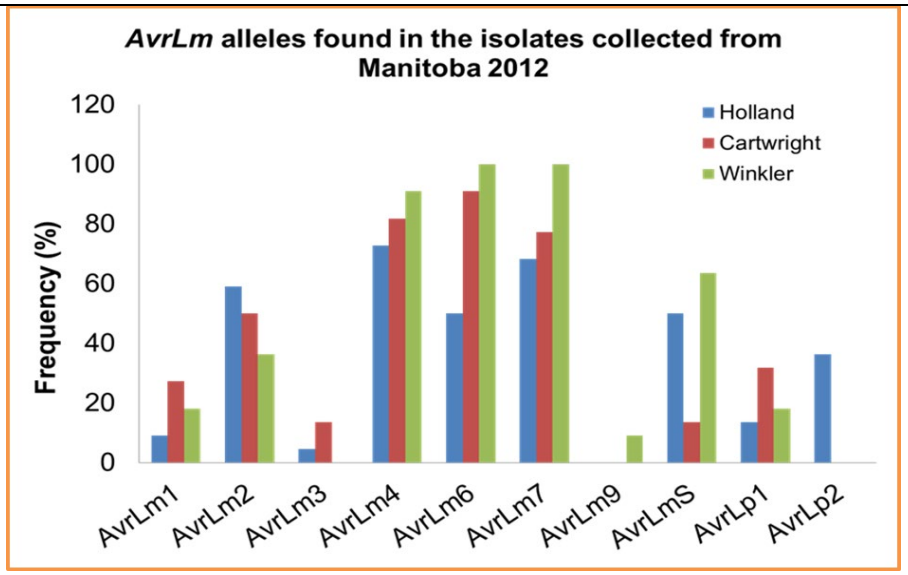

Figure 5 *Avr* allele frequencies of *L. maculans* from three commercial canola fields with high incidence of blackleg in Manitoba (2012).

In 2013, additional four commercial canola fields were sampled in southern Manitoba near Killarney, Lowe Farm (2) and Winker where the disease incidence was at about >75%, 10%, 20% and ~50%, respectively. The two fields near Lowe Farm with lower levels of blackleg were used for comparisons to assess if the pathogen Avr profiles are different in fields with substantially different blackleg inicidence. There was much similarity in pathogen *Avr* profile among these fields; *AvrLm4*, *AvrLm*6 and *AvrLm*7 were generally >90% while *AvrLm2* and *AvrLmS* were >60%, except in a Lowe Farm field (**Figure 6**). *AvrLm3*, *AvrLm*9 or *AvrLep2* were at very low levels. At a Lowe farm (1) site, *AvrLm2* was noticeably lower than in other fields. Relative to the picture over Manitoba (**Figure 3**), *AvrLm1* was noticeably more common in these commercial fields, often surpassing 20%. Considering the common presence of *Rlm1* in our canola varieties, *AvrLm1* would be expected to be at very low levels in commercial canola fields.

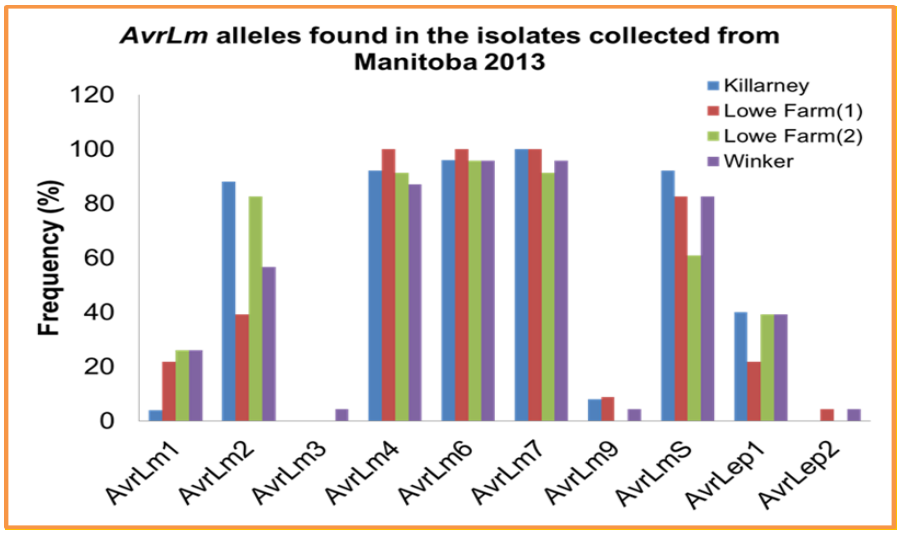

Figure 6 *Avr* allele frequencies of *L. maculans* in Manitoba commercial canola fields with low to high levels of blackleg (2013).

Identification of pathogen races: A total of 372 *L. maculans* isolates collected from commercial canola fields in Alberta, Saskatchewan and Manitoba during 2012 and 2013 were categorized into 90 races based on the *Avr* genes carried by each isolate (**Figure 7**). Of these, 22 were more common, each accounting for >1% of the pathogen population. The race *AvrLm2,4,6,7,(8)* had the highest overall representation (12%), and the other races also found frequently in both years included *AvrLm2,4,6,7,(8)*,S (11%) and *AvrLm2,4,6,7,(8)*,S,*Lep1* (7%) (**Figure 8**). There were slight variations in the frequency between the years; *AvrLm2,4,6,7,(8)* was the most frequent race in 2012 (11%) whereas *AvrLm2,4,6,7,(8),S* was the most common race (21%) in 2013.

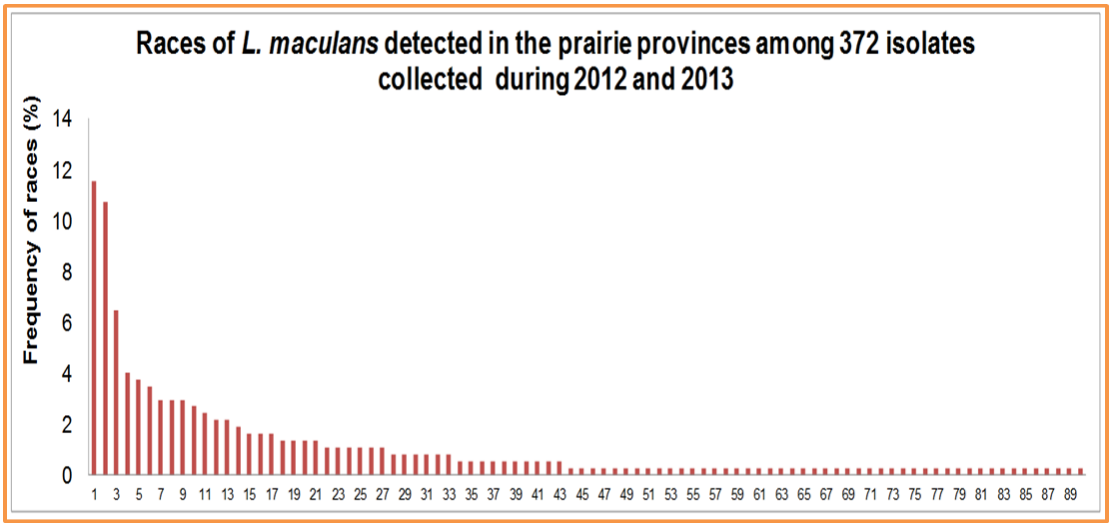

Figure 7 The frequency of *L. maculans* races based on 2012 and 2013 samples collected from commercial canola fields in Alberta, Saskatchewan and Manitoba.

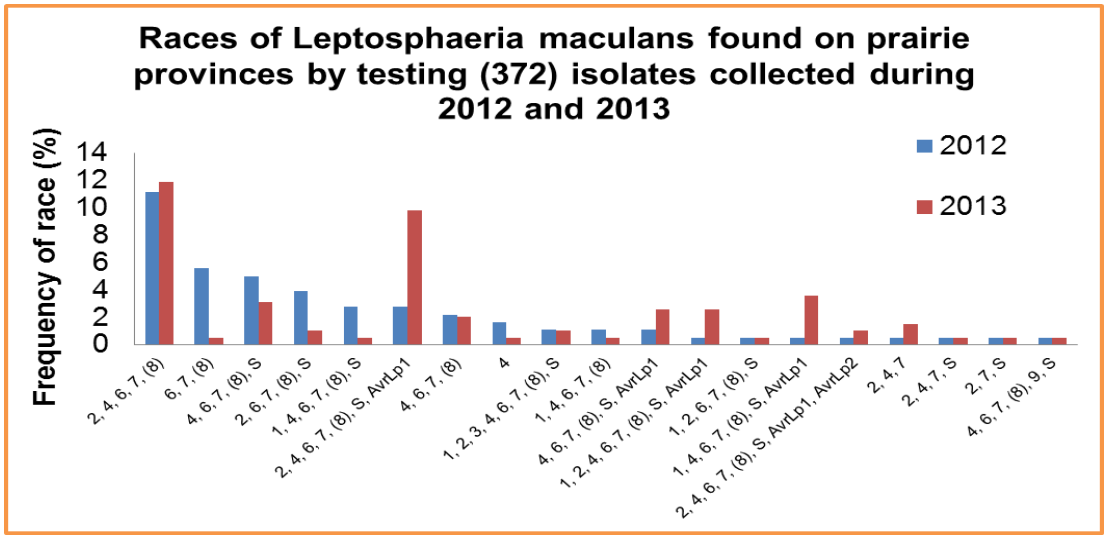

Figure 8 The common races of *L. maculans* in commercial fields on the prairies between (2012, 2013).

Developing a molecular assay for efficient detection of *Avr* genes in *L. maculans* isolates: The objective of this initiative was to explore a PCR-based approach to detect *AvrLm1*, *AvrLm6* and *AvrLm4-7* in the pathogen population. Gene-specific primer sets were designed for *AvrLm1* and *AvrLm6* based on the coding sequences and 30 *L. maculans* isolates with (15) and without (15) the respective *Avr* function were subjected to PCR screening. Only the 15 isolates carrying *AvrLm1* yielded expected *AvrLm1*-specific PCR products, while the other 15 isolates carrying no *AvrLm1* showed no amplicon. In contrast, the *AvrLm6*-specific amplicon was detected in isolates with and without *AvrLm6*. External primers targeting the promotor and 3' regulatory region of *AvrLm6* was further developed, which amplified DNA from the isolates carrying *AvrLm6* 100%, but also produced an amplicon on 6.7% of the isolates without *AvrLm6*. For *AvrLm4*, the SNP mutation (G^{358} to C^{358}) lead to a G^{120} to R^{120} amino acid substitution which confers the virulence towards *Rlm4* was used to develop a KASP (Kompetitive Alelle Specific PCR) assay. Genotyping with the KASP primers found that the G^{358} to C^{358} mutation was present in all 15 *L. maculans* isolates carrying no *AvrLm4*. For *AvrLm7*, a primer set was designed to amplify the entire coding sequence of the *Avr* allele from both avirulent and virulent isolates. Multiple sequence comparisons showed that a wide range of molecular events were involve in the loss of *Avr* effect against *Rlm7*.

In general, PCR-based markers were fund to be effective in detecting *AvrLm1*, *AvrLm6* and *AvrLm4*, but the results on *AvrLm7* were inconclusive due likely to multiple events that happened during the change from *Avr* to virulence. It may take more refinement to optimize the primers and PCR conditions for accurate detection of *AvrLm7*. During this study, *AvrLm2* (Ghanbarnia et al. 2015), *Avrlm3* (Plissonneau et al. 2016), *AvrLm11* (Balesdent et al., 2013) and *AvrlmJ1/AvrLm5* (Van de Wouw et al. 2014; Plissonneau et al. 2017)/ *Avr* genes have since been cloned and including these newly cloned *Avr* genes would help develop a more useful PCR-based protocol. However, this expanded work would require more resources not planned for the project. At the same time, a new initiative was taken by a colleague (Dr. Borhan, AAFC Saskatoon) to develop a PCR-based *Avr* detection protocol. A new project funded by AAFC has also been initiated (PI: Dr. Yu AAFC Saskatoon) using next-generation sequencing and SNP array technology to develop a more robust and versatile molecular tool for efficient *Avr* differentiation in the pathogen isolates. This technology will not be depending heavily on the cloning of *Avr* genes (it's uncertain when each of the known *Avr* genes will be cloned), but using the sequence information of well characterized pathogen isolates from the current project. A large number of *L. maculans* isolates with *Avr* genes are being sequenced for the identification of unique SNP haplotype patterns associated with specific *Avr* genes or gene combinations. In continued monitoring, this technology will be validated with phenotyping data.

Further discussion/comments: The study identified the prevalence and changes of *Avr* genes in pathogen populations on the prairies; *AvrLm2, AvrLm4*, *AvrLm6,(8)* and *AvrLm7* are often found at high frequencies while *AvrLm1*, *AvrLm3* and *AvrLm9* are generally absent. This information is highly useful to canola breeders in deploying *R* genes effective against the current population of pathogen. Since only *Rlm1* and *Rlm3* are found commonly in Canadian cultivars, it is likely that the major-gene resistance has not been playing a key role in controlling blackleg on the prairies in recent years due to the general absence of *AvrLm1* and *AvrLm3* in the pathogen population. The general resistance by many canola cultivars is likely through other mechanisms, especially the race nonspecific or quantitative resistance. At the same time, it is expected that introducing *Rlm4*, *Rlm6* or *Rlm7* into canola varieties can further enhance the resistance performance against blackleg in most regions on the prairies by targeting the most prevalent *Avr* genes,

especially when these *R* genes can be combined with a superior quantitative resistance background which has been shown in many current varieties already.

It needs to be pointed out that this big-picture information is more for pathogen race distribution over a large area and may not be extrapolated for everyone field in the area because there are possibilities for substantial variations in *Avr* profile among different fields. The 2014 data from Alberta serve an example of this point; the *AvrLm3* was found in 90% of isolates from Westar stubble at the Edmonton site, while this *Avr* gene was very low or undetected at other sites (**Figure 9**). Couple with the high *AvrLm3* frequency, *AvrLm4* and *Avrlm7* were both missing in the pathogen population (**Figure 9**, arrows). The data fits the model described by Plissonneau et al. (2016) well; when *AvrLm4-7* was absent, the functions of *AvrLm3* would not be suppressed and canola carrying the *Rlm3* would be resistant. Although this type of extreme variation was infrequent during the study, it does point out possible different *Avr* profile form field to field. Therefore, this regional *Avr* data will be of limited value in guiding rotation of canola varieties carrying different *R* genes in a specific field. Additionally, possibility also exists that *AvrLm3* is more prevalent in the pathogen population that what the number show due to the shadow effect of *AvrLm4-7* that masks the function of *AvrLm3*. As such, due to a dramatic increase in *AvrLm4-7* on the prairies in the past several years, *AvrLm3* was not necessarily brought down but concealed in the pathogen population. When *AvrLm4-7* is low, as in 2014 Edmonton (**Figure 9**), *AvrLm3* would become prominently visible. There appear advantages to stack *Rlm3* and *Rlm7* in canola varieties for more durable resistance on the prairies.

The relationship of the *Avr* gene profile to blackleg incidence and severity seems more complicated in specific canola fields than originally hypothesized due largely to limited information on the *R* genes carried by canola cultivars and to complex resistance mechanisms involved in many of the current cultivars. It was hypothesized initially that severe blackleg damage on *R*-rated canola cultivars would be caused by strong selection pressure exerted by major *R* genes in the cultivar that compelled the pathogen population to adapt once the mutation or recombination resulted in a virulent population against the corresponding *R* genes. That would have rendered the *R* gene(s) in the cultivar ineffective. For example, if the cultivar carries *Rlm1* is used widely, then a shift of *AvrLm1* (avirulent) to *avrLm3* (virulent) means the pathogen population has the ability to overcome the major-gene resistance quickly, especially in short rotation practices. The information is required for the presence or absence of *Avr* genes in pathogen and the *R* genes carried by canola cultivars to determine the effectiveness of resistant cultivars. During the study, the *R* genes carried by specific canola cultivars are generally unknown. A recent study found that resistance genes *Rlm1* and *Rlm3* were present in approximately 10% and 70% of the Canadian canola cultivars and breeding lines, respectively (Zhang et al. 2016). This information, however, is not specific to commercial canola varieties because most of seed companies that participated in the study were reluctant to decode the materials tested. Our recent work found that *Rlm1* and *Rlm3*, as well as nonspecific resistance, are associated with almost all common canola cultivars sold in western Canada (Soomro 2017). It appears that there are opportunities to deploy new major R genes as well as nonspecific resistance in canola cultivars to improve blackleg management on the prairies.

The current study provides some insight into the pathogen race structure in response to current R-rated canola cultivars that suffered noticeable blackleg damage in commercial fields. *AvrLm2*, *AvrLm4*, *AvrLm6* and *AvrLm7* are generally common in most of these fields, regardless of blackleg incidence observed. It may

likely be deduced that the corresponding *R* genes are not present in the canola cultivars sampled; otherwise, the *Avr* genes would have been much lower in these fields.

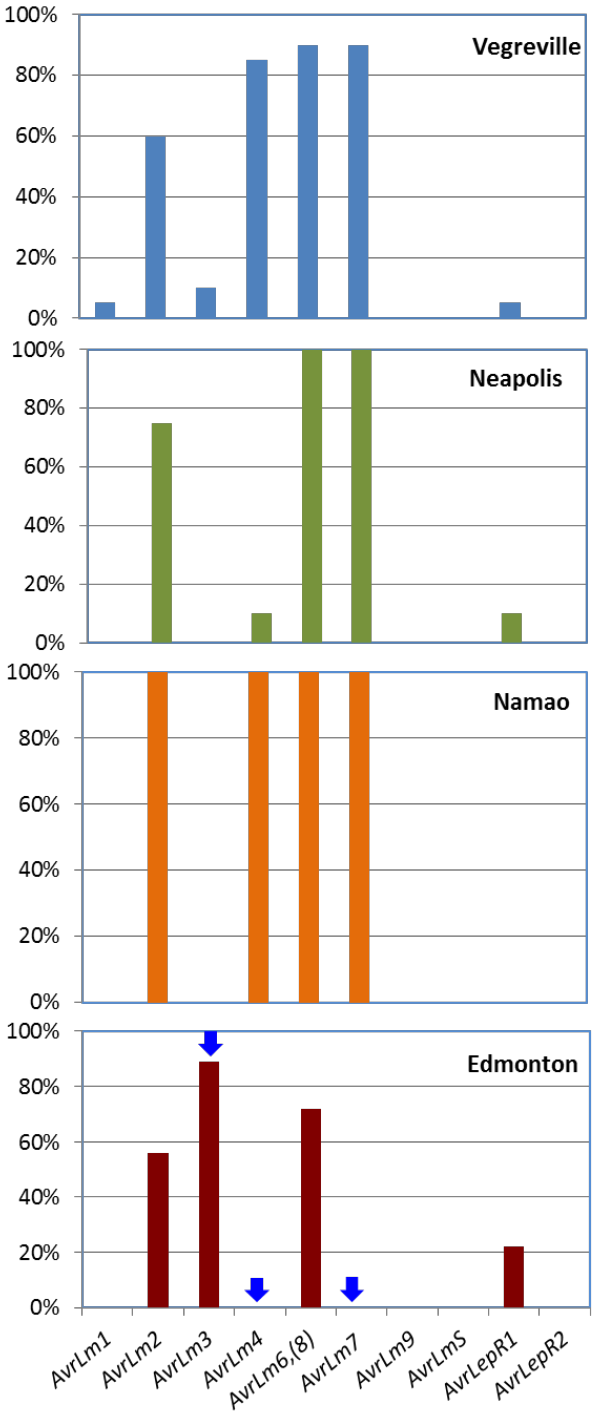

Figure 9. *Avr* (%) in *L. maculans* isolates from Westar trap plots at different locations in Alberta (2014).

With generally low *AvrLm1* and *AvrLm3* in the areas monitored, *Rlm1* or *Rlm3* (common in canola cultivars), are not expected to be effective in any of these commercial fields studied. However, it would not be justified to attribute the high blackleg incidences observed solely to the low *AvrLm1* or *AvrLm3* because

both Avr genes were found very low in light-disease fields. For example, in Lowe Farm, MB, both *AvrLm1* and *AvrLm3* were <30% but the disease incidence was only 10-20%, as opposed to the field near Killarney, MB where *AvrLm1* and *AvrLm3* were at similar levels but the disease incidence was 75% (**Figure 6**). Although it cannot be ruled out that the cultivars in the Lowe Farm fields might have carried additional *R* genes, this possibility is likely low based on the studies of Zhang et al. (2016) and Soomro (2017). The quantitative resistance, interaction of *R* genes (despite ineffective when used alone) stacked in a variety, crop rotation and weather events may all contribute to the different levels of blackleg observed in these commercial fields. The quantitative resistance in our canola cultivars is not well understood; it is still being characterized and this information may be of further value to blackleg resistance due to its greater durability than major-gene resistance.

The analysis of a separate set of *L. maculans* isolates collected from commercial canola fields on the prairies in 2015 showed a similar in *Avr* profile relative to that of samples from Westar trap plots; the frequencies of *AvrLm1*, *AvrLm3*, *AvrLm9* and *AvrLep2* were generally low in most part of the region (**Figure 2**). The results indicate that both sampling methods (trap plots vs collecting broadly from commercial fields) can be used for monitoring of *Avr* dynamics in a large area. During this study, a suggestion was made, after the review of 2015 interim report, to fix the location of trop plots and track the change of pathogen population at same locations over time. Since 2015 would be the last crop year when pathogen samples were collected for this project, it would not be feasible to fix the trap-plot location for continued monitoring. Additionally, our data comparing *L. maculans* from trap plots and random commercial fields in 2015 showed fairly similar *Avr* patterns between the two sampling approaches, it is likely that random field samples from provincial canola disease surveys will be used for *Avr* monitoring. A companion project with seed companies collected samples from ten WCC/RRC coop trials across the prairies in 2015 and the *Avr* profile on these sites would be checked every 3-4 year for the pathogen race changes. This set up will address the review's comment on the need of addition information on pathogen *Avr* change over time. The *Avr* pictures among the coop sites (**Figure 10**) showed much greater variability than those among the provinces shown in **Figure 3**. *AvrLm4 AvrLm6* and *AvrLm7* were relatively more frequent at most of the coop sites, *AvrLm1 AvrLm3* and *AvrLm9* were generally low. *AvrLm2 AvrLmS* and *AvrLep1* were more variable depending on the location, and *AvrLep3* was relatively common only at the Carman and Boissevain sites in Manitoba. At Portage, MB, the same situation was seen as that of Edmonton in 2014 (**Figure 9**, arrows) where the high *AvrLm3* frequency was accompanied by missing of *AvrLm4-7* in the pathogen population. This reminds us an opportunity for more judicious use of *Rlm3* and *Rlm7* via stacking or *R* gene rotation. This aspect can be further explored for an effective *R*-gene deployment. With the continued support of SaskCanola, this studied will be carried on for additional 5 years for support our R-gene labeling strategy by providing the industry with up to date *Avr* profile on the prairies.

Additional references

Balesdent MH, Fudal I, Ollivier B, Bally B, Grandaubert J, Eber F, Chèvre AM, Leflon M, Rouxel T. 2013. The dispensable chromosome of Leptosphaeria maculans shelters an effector gene conferring avirulence towards Brassica rapa. *New Phytolog*. 198: 887–898.

Ghanbarnia K, Fudal I, Larkan NJ, Links, MG, Balesdent MH, Profotova B, Fernando WGD, Rouxel, T, Borhan, MH. 2015. Rapid identification of the *Leptosphaeria maculans* avirulence gene *AvrLm2* using an intraspecific comparative genomics approach.

Houterman PM, Cornelissen BJC, Rep M. 2008. Suppression of plant resistance gene-based immunity by a fungal effector. PLOS Pathogens 4: e1000061. Howlett BJ, Idnurm A, Pedras MSC. 2001. *Leptosphaeria maculans*, the causal agent of blackleg disease of Brassicas. Fungal Genetics and Biology 33: 1–14.

Plissonneau C, Rouxel T, Chèvre AM, Van de Wouw AP, Balesdent MH. 2017. One gene-one name: the *AvrLmJ1* avirulence gene of *Leptosphaeria maculans* is *AvrLm5*. Mol Plant Pathol. doi: 10.1111/mpp.12574

Plissonneau C, Daverdin G, Ollivier B, Blaise F, Degrave A, Fudal I, Rouxel T, Balesdent MH. 2016. A game of hide and seek between avirulence genes *AvrLm4-7* and *AvrLm3* in *Leptosphaeria maculans*. *New Phytolog*. 209: 1613-24.

Van de Wouw AP, Lowe RGT, Elliott CE, Dubois DJ, Howlett BJ. 2014. An avirulence gene, *AvrLmJ1*, from the blackleg fungus, *Leptosphaeria maculans*, confers avirulence to *Brassica juncea* cultivars. Mol Plant Path15:523-30.

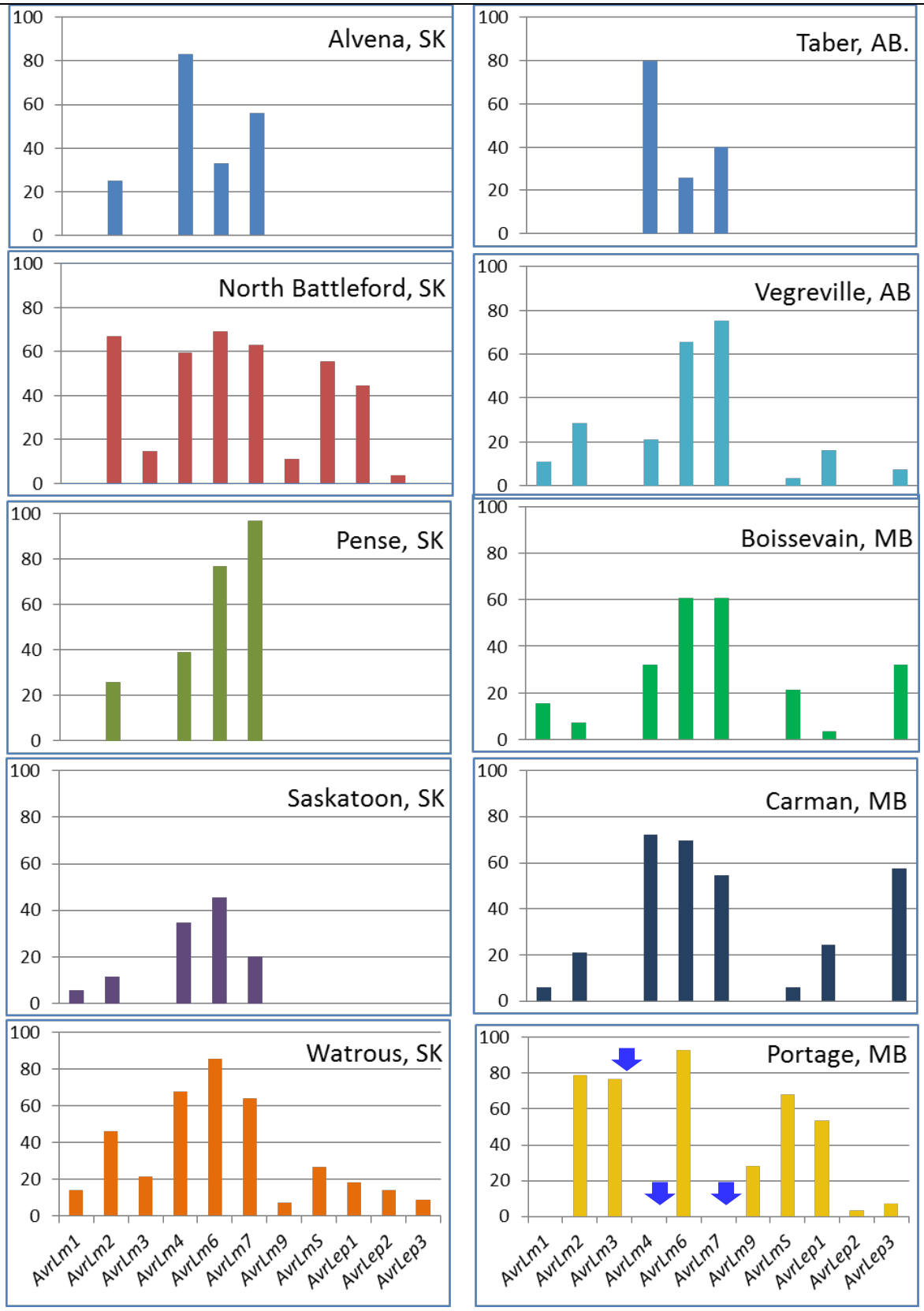

Figure 10. *Avr* (%) of *L. maculans* isolates at ten WCC/RRC co-op trial sites (2015).

11. Technology transfer activities (max. 1 page)

I. Presentations and poster at meetings and interviews by mass media:

1. Peng G. "Canola rotation: Risk assessment". Canola Watch (Mar. 5, 2014), Issue 3.
2. Peng G. Interviewed by CJVRFM/CK750AM Melfort, SK on "Crop rotations relative to blackleg, clubroot and sclerotinia", Mar. 27, 2014.
3. Peng, G. 2014. Scouting is step one to preserve blackleg resistance. Country Guide 2014 July Issue.
4. Peng, G. Blackleg of canola -*Avr*-gene profile in pathogen population & its role in disease management. An invited talk at 2014 Canola Industry Meeting. Dec. 3, 2014, Saskatoon, SK.
5. Harker, K.N., O'Donovan, J.T., Turkington, T.K., Blackshaw, R.E., Lupwayi, N.Z., Smith, E.G., Johnson, E.N., Kutcher, H.R., Willenborg, C.J., Gan, Y.T., Dosdall, L.M., Hall, L.M., Peng, G., Irvine, R.B., Mohr, R.M. 201). Sustainable canola production increases with cropping system diversity. 14th Int. Rapeseed Cong, Saskatoon, SK, Canada, July 5-9, 2015 (Poster)
6. Soomro WM, Kutcher HR, Peng G. 2015. The occurrence of blackleg in relation to the Avr-allele profile of Leptosphaeria maculans in commercial canola fields in western Canada. Botany 2015, Edmonton, AB, Canada, July 25-29 (Poster).
7. Zhang X, Liban S, Fernando WGD, Cross DJ, Peng G and Kutcher HR. 2015. Genetic resistance to blackleg in the Canadian canola (*B. napus*) germplasm and the breakdown of the dominant *Rlm3* by the *L. maculans* Pathogen Population. 14th Int. Rapeseed Cong, Saskatoon, SK, Canada, July 5-9, 2015 (Poster).
8. Peng G, Kirkham C, Fernando WGD, Lange R, McLaren DL, Kutcher HD, Johnson EN, Turkington TK. 2015. Early fungicide application reduces blackleg impact on canola only when cultivar resistance is broken and the disease pressure is high. 14th Int. Rapeseed Cong, Saskatoon, SK, Canada, July 5-9, 2015 (Oral presentation).
9. Soomro WM, Kutcher HR, Peng G. 2015. Characterizing blackleg resistance in commercial canola cultivars. *Sask-CPS Regional Meeting*, Dec. 3, 2015. Saskatoon, SK (Oral).
10. Peng G. 2015. Is blackleg creeping back –what we know/don't know and how to mitigate the risk? Invited talk at the Soils and Crops 2015. Saskatoon, SK, March 17.
11. Peng G. 2015. Is blackleg creeping back? –what we know/don't know and how to mitigate the risk? Invited Talk at Soils and Crops. Saksatoon, SK, Mar. 17.
12. Kutcher HR, Turkington TK, Banniza S, Peng G, 2015. Top of mind disease issues for 2015. An invited talk at the 4th Annual ag Prove Forum, Battleford, SK, March 26.
13. Peng G. Interviewed by CTV Farm Gate on blackleg management broadcast on March 28, 2015.
14. Peng G, Fernando WGD, Lange R. Blackleg of canola, should you be concerned? An invited talk at Canola Day in Kenosee, SK., November 16, 2015.
15. Peng G, Fernando WGD. Avr-gene profile in *Leptosphaeria maculans* on the prairies. An invited update at WCC/RRC meeting, December 1, 2015, Saskatoon, SK.
16. Peng G. Blackleg of canola on the prairies -What do we know/don't know? An invited talk at SK Plant Pathology Subcommittee Annual Meeting, December 11, 2015, Saskatoon, SK.
17. Peng G. Interviewed by AdFarm on March 8, 2016. "Blackleg of canola –the pathogen race change and disease management" for the Research Hub, Canola Council of Canada.
18. Peng G. interviewed by Gord Gilmour – "A fine balance -Controlling crop diseases is a classic example of where short-term decisions can mean long-term problems. Country Guide Mar 36-38.
19. Peng G. Interviewed by Post Media - Joshua Santos, on blackleg of canola. Aug. 4, 2016.

20. C. ZHAI, X. LIU, T. SONG, F. YU AND G. PENG. 2016. Blackleg resistance by Rlm1 may be triggered by localized activation of salicylic acid and suppression of abscisic acid and auxin pathways. Poster at CPS 2016 Annual Meeting. June 12-15, Moncton, NB.
21. W. M. SOOMRO, H. R. KUTCHER AND G. PENG. 2016. Non-race specific resistance to blackleg by Canadian canola cultivars shows delayed or reduced pathogen spread from infected cotyledons into petioles and stems. Poster at CPS 2016 Annual Meeting. June 12-15, Moncton, NB.
22. Peng G. Interviewed by Julienne Isaacs on Sept 26, 2016. To spray or not to spray? Country Guide 2016 Sept issue.
23. Peng G. Interviewed by Robert Arnason from Western Producer on November 9, 2016 for an article on "Blackleg management –resistance gene vs crop rotation"
24. Peng G, Soomro W, Kutcher HR, Yu F. 2016. Dynamics of the *Leptosphaeria maculans* Avr-gene profile and canola cultivar resistance to blackleg in western Canada. Proc. 20th Crucifer Genetics Conf. & 19th Australian Res. Assembly on Brassicas. Oct. 3-6 2016, Melbourne, Australia, *p*42.
25. Peng G. Managing blackleg of canola on the prairies -From a research perspective. Germination Magazine Webinar seminar –Feb 23, 2017
26. Peng, G, Jurky C, Conulson J 2017. CanoLab. Blackleg - its identification and management. Regina, Feb 18, 2017.
27. Peng G. Managing blackleg of canola in western Canada - Pathogen population, host resistance & others. Invited presentation at Field Crop Disease Summit –Saskatoon, Feb 21, 2017
28. Peng G. Blackleg race dynamics, fungicide responses, resistance gene labels and rotation strategies. Science Orama -Canola Research Update. Lacombe, Alberta, April 5, 2017.
29. Peng G. Interview by Carolyn King for an article on Blackleg races & resistance: A new cultivar labelling system provides another tool to help canola growers fight this disease. Top Crop Manager
30. Peng, G. Interviewed by Robin Booker –Western Producer for an article on "Blackleg race test will aid management" Western Producer July 14, 2017.
31. Peng G. Melfort 2017 Field Day. Blackleg of Canola –R gene labelling and more. July 26, 2017
32. Peng G, Interviewed by JOSHUA SANTOS –Post Media – on Rotational Management of Blackleg, July 27, 2017.
33. Peng G. 2017. Interviewed by Bruce Barker –Top Crop Manager on Aug 26, 2017 for an article "Tools expanding for blackleg management - New risk assessment matrix, labeling system and gene test identification."

II. Scientific publication:

34. Peng G, Fernando WGD, Lange R, Kutcher HR 2014. Blackleg of canola -new management strategies against an old disease in western Canada (Abstr.). *Can. J. Plant Pathol* 36:289.
35. Harker KN, O'Donovan JT, Turkington TK, Blackshaw RE, Lupway NZ, Smith EG, Dosdall LM, Hall LM, Kutcher HR, Peng G. 2015. Canola rotation frequency impacts canola yield and associated pest species. *Can J. Plant Sci.* 95: 9-20.
36. K. N. Harker1, J. T. O'Donovan1, T. K. Turkington1, R. E. Blackshaw2, N. Z. Lupwayi2, E. G. Smith2, L. M. Dosdall3, L. M. Hall3, H. R. Kutcher4, Willenborg CJ, Peng G, Irvine RB, Mohr R. 2015. Canola cultivar mixtures and rotations do not mitigate the negative impacts of continuous canola. *Can J Plant Sci.* 95: 1085-1099.
37. Zhang XH, Peng G, Kutcher HR, Balesdent MH, Delourme R, Fernando WGD. 2016. Breakdown of *Rlm3* resistance in the Brassica napus - Leptosphaeria maculans pathosystem in western Canada. *Eur J Plant Pathol.* 145:659–674

38. Liban SH, Cross DJ, Fernando WGD, Kutcher HR, Peng G. 2016. Race structure and frequency of avirulence genes in the western Canadian Leptosphaeria maculans pathogen population. *Plant Pathol* 65:1161–1169
39. Three additional manuscripts out of this project are in preparation.

III. Extension publications:

40. A race against evolving blackleg -The blackleg pathogen is evolving to challenge blackleg resistant canola varieties. Grainews Feb18, 2016
41. Contributions to Blackleg Factsheet produced by SK SAF and SaskCanola. 2017