Canola Agronomic Research Program (CARP)
FINAL REPORT

The Final Report should fully describe the work completed for the year and note the personnel involved. It should also note any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. A complete statement of expenses should be included. In the event of major changes within the budget, supporting notes should be included. The report should capture a complete summary of activity for the final year and an overview of the entire project.

Project Title: Monitoring the race dynamics of *Leptosphaeria maculans* for effective deployment and rotation of resistance genes for sustainable management of blackleg of canola in western Canada

Table 1. Research Team Information.

Lead Researchers		
Name	***Institution***	***Project Role***
Gary Peng	AAFC Saskatoon	Principal Investigator
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Fengqun Yu	AAFC Saskatoon	Co-Investigator

Project Start Date: **April 1, 2017** **Project Completion Date:** **December 31, 2022**

Reporting Period: **April 1, 2022** **to** **December 31, 2022**

CARP Project Number: **2017.27**

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition, a Final Extension Report/Abstract is due upon completion of the project, maximum 2-3 pages, to be used for publication on the Funders' websites and in the *Canola Digest*. Content will be used in extension material, for consumers and/or industry. Include an Executive Summary, brief project description, key findings and conclusions (with a summary graph/table or supporting image for the project), translation of key findings into best management practices and/or relevance to the canola sector and future research, and funding acknowledgment as determined in the grant award letter. The Final Extension Report is intended to support messaging to all audiences. Information needs to be clear, concise and in "grower-friendly" language.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of completion & status of activity (please check one)

Date of completion: December 31, 2022

☐ **Ahead of Schedule** ☐ **On Schedule** ☐ **Behind Schedule** ☐ **Completed**

Comments: The project was completed on schedule specified in the amended agreement.

2. Abstract/Summary - Maximum of one page. This must include project objectives, results, and conclusions for use on the Funders' websites.

Cultivar resistance and crop rotation are the key strategies for management of blackleg on canola. As the pathogen (*L. maculans*) that causes the disease has a strong ability to evolve and adapt, *R* genes can be overcome after repeated uses. A good example is *Rlm1/LepR3* used in many cultivars that is no longer effective in Alberta and Saskatchewan. Tighter crop rotations in some regions also increase the inoculum pressure, so blackleg continues to be risk to production and export of canola. For effective deployment of *R* genes, it is essential to understand the race composition and dynamics in the pathogen population. Started from 2017, this study analyzed the *L. maculans* population on the prairies annually to: 1) monitor and analyze the *Avr* profile to gain insights into pathogen race dynamics; 2) provide industry and producers with up-to-date pictures on *L. maculans* population virulence to guide deployment/recommendation of *R* genes for blackleg resistance breeding and disease management; 3) identify new races of *L. maculans* capable of overcoming common *R* genes used in canola cultivars before widespread resistance breakdown; and 4) evaluate and adopt markers for efficient *L. maculans* race monitoring.

The markers deployed since 2018 were effective in detecting *AvrLm1-2-3-4-5-6-7-9-11-S/Lep2* (10) in the pathogen population; the system also eliminated the masking effect of *AvrLm7* to *AvrLm3* and *AvrLm9* observed on host differentials carrying the corresponding *R* genes. The study showed that canola cultivars carrying any of the resistance genes *Rlm5*, *Rlm6*, *Rlm7*, *Rlm10*, *Rlm11* and *LepR1* are likely resistant to blackleg in western Canada due to the common presence of corresponding *Avr* genes in the pathogen population. The resistance genes *Rlm3* or *Rlm9* may be ineffective due to the 'masking effect' of *AvrLm7* to *AvrLm3* and *AvrLm9*. *Rlm4* may be less effective in Alberta due to low levels of *AvrLm4* in the province.

A total of 83 races were identified in the *L. maculans* population, with top-10 races accounting for 72.3%, 93.7% and 57.1% in Alberta, Saskatchewan and Manitoba. The high diversity in pathogen shows the potential for resistance erosion as virulent races had already been present for all *R* genes, except *Rlm10*. This means that no further mutation is required for the pathogen to overcome most of the *R* genes in our arsenal. Therefore, judicious deployment of *R* genes and rotation of resistant cultivars will be import for blackleg management. If an *R* gene is used repeatedly, there will be selection pressure for virulent races that can eventually break down the resistance.

When compared with the results from earlier studies, the *Avr* profile of *L. maculans* population changed only slightly between 2017 and 2021, with noticeable increases just in *AvrLep1*. However, the diversity of the pathogen population remained high, especially in Manitoba. With the introduction of new *R* genes in many canola cultivars, including *Rlm2*, *Rlm4*, *Rlm7* and *LepR2*, it will be important to continue monitoring the pathogen population for early detection of increases in the population virulence. To manage selection pressure/resistance breakdown, rotating cultivars carrying different *R* genes and extending crop rotation to reduce the inoculum pressure may be warranted .

3. Introduction – Brief project background, rationale, and objectives.

In western Canada, cultivar resistance and crop rotation are the key strategies for control of blackleg on canola. The pathogen (*L. maculans*) that causes the disease has a strong ability to evolve and adapt, with many new races found on the prairies in recent years (Liban et al. 2016). Tighter crop rotations still happen in some regions (Arnason 2016), and blackleg has continuously been present on the prairies despite the common use of resistant canola cultivars, with severe cases reported occasionally. The disease is a risk factor for canola production, as well as export. There is certainly room for continued improvement on variety resistance, including use of new specific R genes and better quantitative resistance (QR) background. Most of our cultivars carry only the resistance genes *Rlm1/LepR3* and/or *Rlm3* (Zhang et al. 2015), generally ineffective against the current pathogen population. To deploy new *R* genes, it is essential to understand the pathogen race dynamics on the prairies.

Monitoring and analysis of *L. maculans* populations on the prairies since 2007 had shown that the avirulence (*Avr*) genes *AvrLm1* had decreased to very low levels in most regions while *AvrLm7* increased substantially. The latter would have also masked the effect of *Avrlm3* in response to the R gene *Rlm3* (Plissonneau et al. 2016). During the same period, *AvrLm2*, *AvrLm4* and *AvrLm6* were found commonly in the pathogen population. This *Avr* monitoring program provided critical information for seed companies to select and incorporate effective *R* genes in new canola hybrids, and it is important to continue the work for early detection of changes in the pathogen population that would affect the cultivar resistance.

The current project started in 2017, and the analysis of *L. maculans* population on the prairies has been carried out annually. The objectives were to: 1) monitor and analyze changes in the pathogen population to gain insights into pathogen race dynamics and population virulence; 2) provide industry and producers with up-to-date pictures on *L. maculans* virulence characteristics in prairie provinces to guide the selection and recommendation of effective R genes for blackleg management; 3) identify new races of *L. maculans* capable of overcoming common *R* genes in canola cultivars before widespread breakdown of the resistance; and 4) evaluate and adopt markers for efficient *L. maculans* race monitoring. 2022 was the last year for the project, and this report also include some of the earlier results to show the trend of population virulence over the 5 years.

4. Methods – Include approaches, experimental design, methodology, materials, sites, etc. Major changes from original plan should be cited and the reason(s) for the change should be specified.

Canola stubble with blackleg symptoms were collected during 2021 canola disease surveys organized by the provincial Pathologists/Coordinators in Alberta, Saskatchewan and Manitoba, respectively. These samples were processed to obtain > 500 *L. maculans* isolates for testing the presence/absence of target *Avr* genes using KASP markers (*AvrLm1,2,3,4,5,6,7,9,11,S-Lep2*) or host differentials. Similar numbers of isolates had been tested each of the prior four years, and a total of 2,600 isolates were tested in the 5-year study. Due to pandemic shutdowns, the project was extended for eight months to allow the completion of all testing by end of 2022.

5. Results – Present and discuss project results, including data, graphs, models, maps, design, and technology development.

A total of 520 *L. maculans* isolates (159 from AB, 207 from SK, and 154 from MB) were obtained in 2021 and analyzed for the presence of 11 *Avr* genes. In general, *AvrLm3*, *AvrLm5*, *AvrLm6*, *AvrLm7, AvrLm11* and *AvrLep1* were the most abundant *Avr* genes found, with greater than 80% of detection frequencies in the pathogen populations from each of the three provinces (**Figure 1**). *AvrLm2*, *AvrLm9*, *AvrLep1* and *AvrLmS/Lep2* were at moderate levels and detected in > 50% of the isolates. The presence of several *Avr* genes appeared variable among provinces; *AvrLm1* was rarely seen (<5%) in isolates from Alberta or Saskatchewan, but fairly common in isolates from Manitoba (51.3%). Additionally, more than 90% of the isolates from Alberta and Saskatchewan harbored *AvrLm2*, while only 53.2% of the isolates from MB carried this *Avr* gene. *AvrLm4* and *AvrLm9* were noticeably more prevalent in Saskatchewan than the other two provinces, and were found in >80%

of the isolates. In contrast, *AvrLm4* was particularly low in Alberta, with just above 20% presence in the pathogen population there. The high frequency of an *Avr* gene in the pathogen population would indicate a greater chance for the corresponding *R* gene in canola cultivars to be resistant to blackleg.

Based on the *Avr* analysis of the 2021 *L. maculans* population, canola cultivars carrying any of the following *R* genes will likely be highly resistant to blackleg across western Canada, including *Rlm5*, *Rlm6*, *Rlm7*, *Rlm11* and *LepR1*. Due to the common presence of *AvrLm7* in the pathogen population, the effect of *AvrLm3* and *AvrLm9* in the resistance of *Rlm3* or *Rlm9* will be masked (Plissonneau et al. 2016). Therefore, these two *R* genes would not be effective against blackleg in western Canada.

The *L. maculans* isolates varied noticeably in number of *Avr* alleles carried; most of them carried 7-10 *Avr* genes (>80%) and only a small number of isolates carried fewer than 7 or more than 10 *Avr* genes (**Figure 2**). There was also some variation geographically; a large portion of isolates from Saskatchewan (~80%) carried 9-10 *Avr* genes, whereas most isolates from Alberta (~60%) carried 6-7 *Avr* genes. In contract, about 90% of isolates from Manitoba carried 7-10 *Avr* genes. This variability contributed to different races of *L. maculans* found in the provinces.

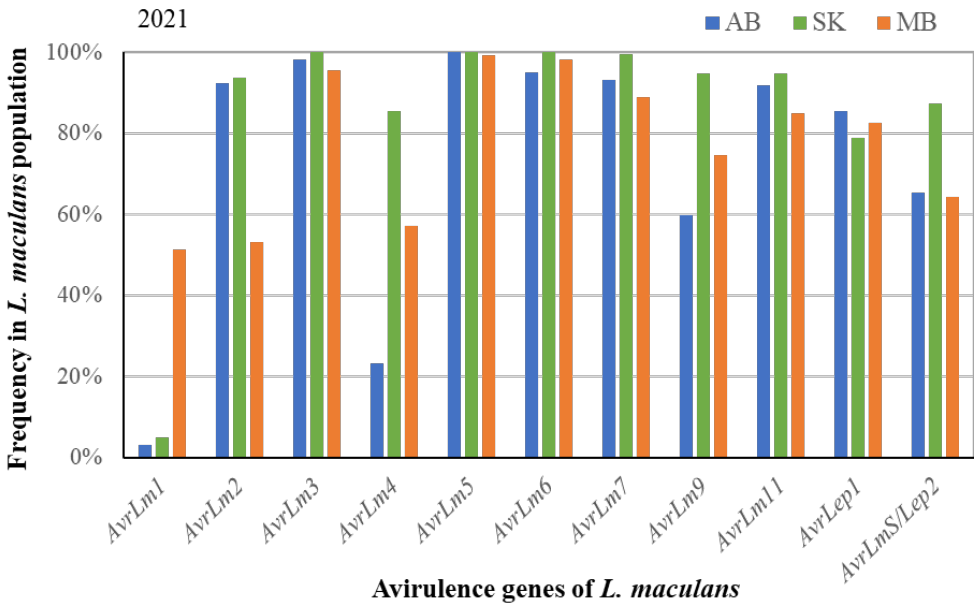

Figure 1. Avirulence (*Avr*) gene frequencies in the population of *Leptosphaeria maculans* on the Canadian prairies in 2021.

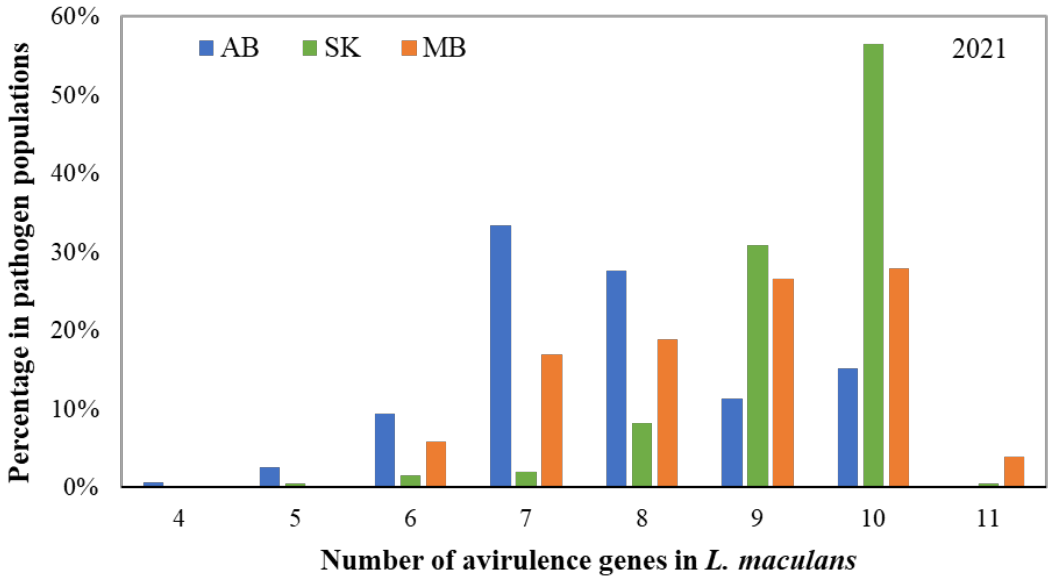

Figure 2. The frequency of *Leptosphaeria maculans* isolates carrying different numbers of *Avr* alleles in the pathogen population on the prairies (2021).

Based on *Avr* combinations, a total of 83 races were identified from the 2021 *L. maculans* population, with 40 races found in Alberta, 25 in SK, and 56 in MB (**Table 1**). Only nine races (*AvrLm2-3-4-5-6-7-9-11-Lep1-AvrLmS/Lep2*, *AvrLm3-5-6-7-9-11-Lep1-AvrLmS/Lep2*, *AvrLm3-5-6-7-11-Lep1*, *AvrLm2-3-5-6-7-9-11-Lep1-AvrLmS/Lep2*, *AvrLm2-3-5-6-7-9-11-Lep1*, *AvrLm2-3-5-6-7-11-Lep1*, *AvrLm2-3-4-5-6-7-9-11-S/Lep2*, *AvrLm2-3-5-6-7-9-11-S/Lep2* and *AvrLm2-3-4-5-6-7-9-11-Lep1*) overlapped among the three provinces; these shared races made up 87.4%, 88.4% and 38.3% of the populations collected from Alberta, Saskatchewan and Manitoba, respectively. *AvrLm2-3-4-5-6-7-9-11-Lep1-LmS/Lep2* was the most prevalent race in all provinces, accounting for 15.1%, 55.1% and 14.9% of the population in Alberta, Saskatchewan and Manitoba (**Figure 3, Table 1**). Top-10 races in these provinces accounted for 72.3%, 93.7% and 57.1% of the pathogen populations, respectively. The top-10 races varied among the provinces. For example, the second most prevalent race *AvrLm3-5-6-7-11-Lep1-LmS/Lep2* in Alberta (10.7%), however, was scarce in SK (0.5%), and not detected at all in MB. Similarly, the second most common race *AvrLm2-3-4-5-6-7-9-11-S/Lep2* in SK (14.5%) showed quite low frequencies in Alberta (2.5%) and Manitoba (2.6%), and the second most common race *AvrLm1-3-4-5-6-7-9-11-Lep1-LmS/Lep2* in Manitoba (9.1%) was not detected in either AB or SK. Based on the number of races found in different provinces, the diversity of *L. maculans* population seemed highest in Manitoba and lowest in Saskatchewan.

Table1. Races of *L. maculans* identified from the pathogen populations collected in western Canada*

Race	No. *Avr* genes carried	No. isolates (frequency %)		
		Alberta	Saskatchewan	Manitoba
AvrLm2-3-4-5-6-7-9-11-Lep1-LmS/Lep2	10	24 (15.1)	114 (55.1)	23 (14.9)
AvrLm3-5-6-7-11 -Lep1-LmS/Lep2	7	17 (10.7)	1 (0.5)	0 (0)
AvrLm3-5-6-7-9-11 -Lep1-LmS/Lep2	8	13 (8.2)	1 (0.5)	3 (2.0)

AvrLm3-5-6-7-11-Lep1	6	12 (7.6)	3 (1.5)	2 (1.3)
AvrLm2-3-5-6-7-9-11-Lep1-LmS/Lep2	9	11 (6.9)	12 (5.8)	7 (4.6)
AvrLm2-3-5-6-7-9-11-Lep1	8	10 (6.3)	4 (1.9)	4 (2.6)
AvrLm2-3-5-6-7-11-Lep1	7	9 (5.7)	1 (0.5)	5 (3.3)
AvrLm3-5-6-7-9-11-Lep1	7	7 (4.4)	1 (0.5)	0 (0)
AvrLm2-3-5-6-7-11-Lep1-LmS/Lep2	8	7 (4.4)	0 (0)	3 (2.0)
AvrLm2-3-5-6-7-9-Lep1-LmS/Lep2	8	5 (3.1)	0 (0)	1 (0.7)
AvrLm2-3-4-5-6-7-9-11-S/Lep2	9	4 (2.5)	30 (14.5)	4 (2.6)
AvrLm2-3-5-6-7-11-S/Lep2	7	4 (2.5)	0 (0)	0 (0)
AvrLm3-5-6-7-9-11-S/Lep2	7	3 (1.9)	0 (0)	1 (0.7)
AvrLm2-3-5-6-7-9-11-S/Lep2	8	2 (1.3)	3 (1.5)	3 (2.0)
AvrLm2-3-4-5-6-7-11-Lep1	8	2 (1.3)	1 (0.5)	0 (0)
AvrLm2-3-5-6-7-9-11	7	2 (1.3)	1 (0.5)	0 (0)
AvrLm1-2-3-4-5-6-7-S/Lep2	8	2 (1.3)	0 (0)	1 (0.7)
AvrLm2-3-5-6-7-9-Lep1	7	2 (1.3)	0 (0)	0 (0)
AvrLm5-6-7-11-Lep1	5	2 (1.3)	0 (0)	0 (0)
AvrLm2-3-4-5-6-7-9-11-Lep1	9	1 (0.7)	11 (5.3)	2 (1.3)
AvrLm2-3-5-6-9-11-Lep1-LmS/Lep2	8	1 (0.7)	1 (0.5)	0 (0)
AvrLm1-3-5-6-7-11-Lep1-LmS/Lep2	8	1 (0.7)	0 (0)	5 (3.3)
AvrLm1-2-3-5-6-7-9-11-Lep1	9	1 (0.7)	0 (0)	2 (1.3)
AvrLm2-5-6-9-11-Lep1-LmS/Lep2	7	1 (0.7)	0 (0)	1 (0.7)
AvrLm3-5-7-11-Lep1-LmS/Lep2	6	1 (0.7)	0 (0)	1 (0.7)
AvrLm1-3-5-7-9-11-Lep1	7	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-4-5-6-7-11-Lep1-LmS/Lep2	9	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-4-5-6-7-9	7	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-4-5-6-7-Lep1-LmS/Lep2	8	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-5-6-7-9-S/Lep2	7	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-5-6-7-Lep1-LmS/Lep2	7	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-5-7-11	5	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-5-7-11-Lep1-LmS/Lep2	7	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-5-7-11-S/Lep2	6	1 (0.7)	0 (0)	0 (0)
AvrLm2-5-7-11	4	1 (0.7)	0 (0)	0 (0)
AvrLm3-4-5-6-7-11-Lep1	7	1 (0.7)	0 (0)	0 (0)
AvrLm3-5-6-7-9-11	6	1 (0.7)	0 (0)	0 (0)
AvrLm3-5-7-11-Lep1	5	1 (0.7)	0 (0)	0 (0)
AvrLm3-5-7-9-11-Lep1-LmS/Lep2	7	1 (0.7)	0 (0)	0 (0)
AvrLm3-6-7-9-11-Lep1-LmS/Lep2	7	1 (0.7)	0 (0)	0 (0)
AvrLm2-3-4-5-6-7-9-Lep1-LmS/Lep2	9	0 (0)	8 (3.9)	1 (0.7)
AvrLm1-2-3-4-5-6-7-9-11-S/Lep2	10	0 (0)	3 (1.5)	1 (0.7)
AvrLm1-3-4-5-6-7-11-Lep1-LmS/Lep2	9	0 (0)	2 (1.0)	3 (2.0)
AvrLm2-3-4-5-6-7-9-11	8	0 (0)	2 (1.0)	1 (0.7)
AvrLm1-3-4-5-6-7-11-S/Lep2	8	0 (0)	2 (1.0)	0 (0)
AvrLm1-2-3-4-5-6-7-9-11-Lep1-LmS/Lep2	11	0 (0)	1 (0.5)	6 (3.9)
AvrLm1-3-5-6-7-9-11-LmS/Lep2	8	0 (0)	1 (0.5)	2 (1.3)
AvrLm1-3-4-5-6-7-9-11-LmS/Lep2	9	0 (0)	1 (0.5)	1 (0.7)
AvrLm2-3-4-5-6-7-9-Lep1	8	0 (0)	1 (0.5)	0 (0)
AvrLm2-3-4-5-6-7-9-LmS/Lep2	8	0 (0)	1 (0.5)	0 (0)

AvrLm3-5-6-7-Lep1	5	0 (0)	1 (0.5)	0 (0)
AvrLm1-3-4-5-6-7-9-11-Lep1-LmS/Lep2	10	0 (0)	0 (0)	14 (9.1)
AvrLm1-3-4-5-6-7-9-Lep1-LmS/Lep2	9	0 (0)	0 (0)	12 (7.8)
AvrLm1-3-5-6-7-11-S/Lep2	7	0 (0)	0 (0)	8 (5.2)
AvrLm1-2-3-5-6-9-11-Lep1-LmS/Lep2	9	0 (0)	0 (0)	3 (2.0)
AvrLm1-3-4-5-6-7-9-S/Lep2	8	0 (0)	0 (0)	3 (2.0)
AvrLm1-2-3-4-5-6-7-9-11-Lep1	10	0 (0)	0 (0)	2 (1.3)
AvrLm2-3-4-5-6-7-11	7	0 (0)	0 (0)	2 (1.3)
AvrLm3-4-5-6-7-9-11-Lep1-LmS/Lep2	9	0 (0)	0 (0)	2 (1.3)
AvrLm3-5-6-9-11-Lep1-LmS/Lep2	7	0 (0)	0 (0)	2 (1.3)
AvrLm1-2-3-4-5-6-7-11-Lep1-LmS/Lep2	10	0 (0)	0 (0)	1 (0.7)
AvrLm1-2-3-4-5-6-7-9-11	9	0 (0)	0 (0)	1 (0.7)
AvrLm1-2-3-4-5-6-7-9-Lep1-LmS/Lep2	10	0 (0)	0 (0)	1 (0.7)
AvrLm1-2-3-4-5-6-7-Lep1-LmS/Lep2	9	0 (0)	0 (0)	1 (0.7)
AvrLm1-2-3-5-6-Lep1-LmS/Lep2	7	0 (0)	0 (0)	1 (0.7)
AvrLm1-2-4-5-6-7-9-11-Lep1-LmS/Lep2	10	0 (0)	0 (0)	1 (0.7)
AvrLm1-3-5-6-11-Lep1-LmS/Lep2	7	0 (0)	0 (0)	1 (0.7)
AvrLm1-3-5-6-11-S/Lep2	6	0 (0)	0 (0)	1 (0.7)
AvrLm1-3-5-6-7-9-11-Lep1	8	0 (0)	0 (0)	1 (0.7)
AvrLm1-3-5-6-9-Lep1-LmS/Lep2	7	0 (0)	0 (0)	1 (0.7)
AvrLm1-3-5-6-9-S/Lep2	6	0 (0)	0 (0)	1 (0.7)
AvrLm1-3-6-7-9-11-Lep1	7	0 (0)	0 (0)	1 (0.7)
AvrLm1-4-5-6-7-9-11-Lep1-LmS/Lep2	9	0 (0)	0 (0)	1 (0.7)
AvrLm1-5-6-7-11-S/Lep2	6	0 (0)	0 (0)	1 (0.7)
AvrLm1-5-6-9-11-Lep1-LmS/Lep2	7	0 (0)	0 (0)	1 (0.7)
AvrLm2-3-5-6-11-Lep1-LmS/Lep2	7	0 (0)	0 (0)	1 (0.7)
AvrLm2-3-5-6-7-11	6	0 (0)	0 (0)	1 (0.7)
AvrLm2-4-5-6-7-9-11-Lep1-LmS/Lep2	9	0 (0)	0 (0)	1 (0.7)
AvrLm2-5-6-7-9-S/Lep2	6	0 (0)	0 (0)	1 (0.7)
AvrLm3-4-5-6-7-9-11	7	0 (0)	0 (0)	1 (0.7)
AvrLm3-4-5-6-7-9-11-Lep1	8	0 (0)	0 (0)	1 (0.7)
AvrLm3-4-5-6-7-9-11-S/Lep2	8	0 (0)	0 (0)	1 (0.7)
AvrLm3-5-6-11-Lep1-LmS/Lep2	6	0 (0)	0 (0)	1 (0.7)

* Races as defined by the combination of 11 *Avr* alleles (*AvrLm1-7*, *AvrLm9*, *AvrLm11* and *AvrLep1-2*).

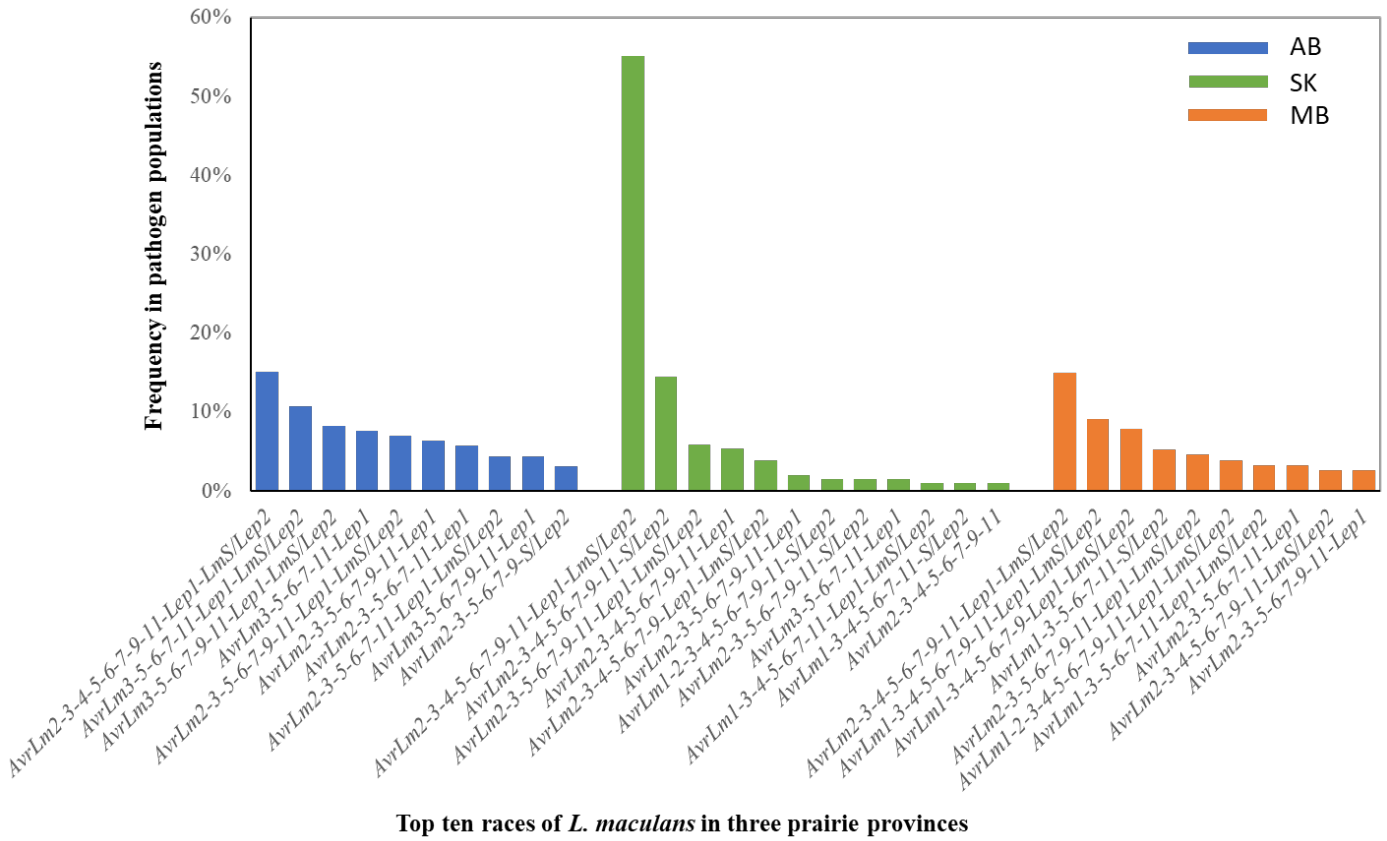

Figure 3. Top ten races of *Leptosphaeria maculans* in the pathogen population on the prairies (2021)

Based on the Margalef index of richness in species and Simpson index of diversity, there was a strong indication for the richness in species (races) and diversity in the *L. maculans* populations collected from all of the provinces (**Table 2**), especially for Alberta and Manitoba with the Simpson index of diversity was also high (>0.9). The greater Simpson index of diversity would suggest high genetic diversity in these *L. maculans* populations. This diversity in the pathogen population also showed that for most of the *R* genes, there was at least a virulent race of *L. maculans* already existing in western Canada; it also means that no new mutation is required and virulent races are present in the current pathogen population. If an *R* gene is used repeatedly, there will be selection pressure on these virulent races that will eventually break down the resistance once the virulent races have reached a population level.

Table 2. Margalef index of richness in species (races) and Simpson index of diversity for *L. maculans* isolates collected in western Canada

Province	No. isolates	No. race	Margalef index	Simpson index
Alberta	159	40	7.7	0.94
Saskatchewan	207	25	4.5	0.67
Manitoba	154	56	10.9	0.95

When comparing the results over the 5 years of the project period (2017-2021), it seems that the *L. maculans* population stayed relatively stable in terms of avirulence profile and only *AvrLep1* increased noticeably (**Figure 4**). The cause for this increase in *AvrLep1* is still unclear, but this has happened in each of the provinces. Changes in pathogen race structure is influenced primarily by *R* genes used in canola cultivars. In recent years, *Rlm2* and *Rlm4* have been introduced in western Canada, but it does not appear that the corresponding *Avr* genes have been reduced substantially yet in the pathogen population, except for *AvrLm4* in Alberta where this *Avr* gene has been consistently less common than in the other two provinces (**Figure 4**). It is unclear if this lower *AvrLm4* situation is caused by the use of resistant cultivars carrying *Rlm4* in Alberta or by other factors in the region. Across the prairies, it is likely that canola cultivars carrying these new *R* genes had not been grown widely, and the relatively high presence of these two *Avr* genes in many regions indicates continued efficacy of *Rlm2* and *Rlm4*. Cultivars carrying *Rlm7* have also been released, and the high level of *AvrLm7* across western Canada would indicate strong blackleg resistance for cultivars carrying this *R* gene. It will be important to continue regional monitoring of *L. maculans* population for early detection of pathogen population adaptation to these newly introduced *R* genes.

The data also showed dramatic increases in *AvrLm3* and *AvrLm9* after 2018, and *AvrLmS/Lep2* after 2020 (**Figure 4**). These sudden changes were due to the use of KASP markers in detection of these *Avr* genes, rather than the real genetic evolution of the pathogen population. Prior to 2018, an inoculation bioassay was used to identify *AvrLm3* and *AvrLm9* based on reactions on host differentials. Due to the common presence of *AvrLm7* in the pathogen population, the effect of *AvrLm3* and *AvrLm9* on activating the *R* genes *Rlm3* and *Rlm9* would have been masked (Plissonneau et al. 2016; Ghanbarnia et al. 2018) in the bioassay, so resulting in extremely low detection of *AvrLm3* and *AvrLm9*, respectively. This interference, however, would not happen with the marker analysis. The cloning work by Xiang Neik et al. (2022) showed that *AvrLmS* and *AvrLep2* are the same gene, so are renamed as *AvrLmS-Lep2* (*AvrLmS/Lep2*). Using the sequence cloned, we successfully developed KASP markers for detection of *AvrLmS/Lep2* and adopted the protocol for the analysis of 2020 and 2021 *L. maculans* populations. The marker detection of *AvrLmS/Lep2* was less ambiguous than the phenotyping, which identified much higher presence of *AvrLep1* in the pathogen population than before.

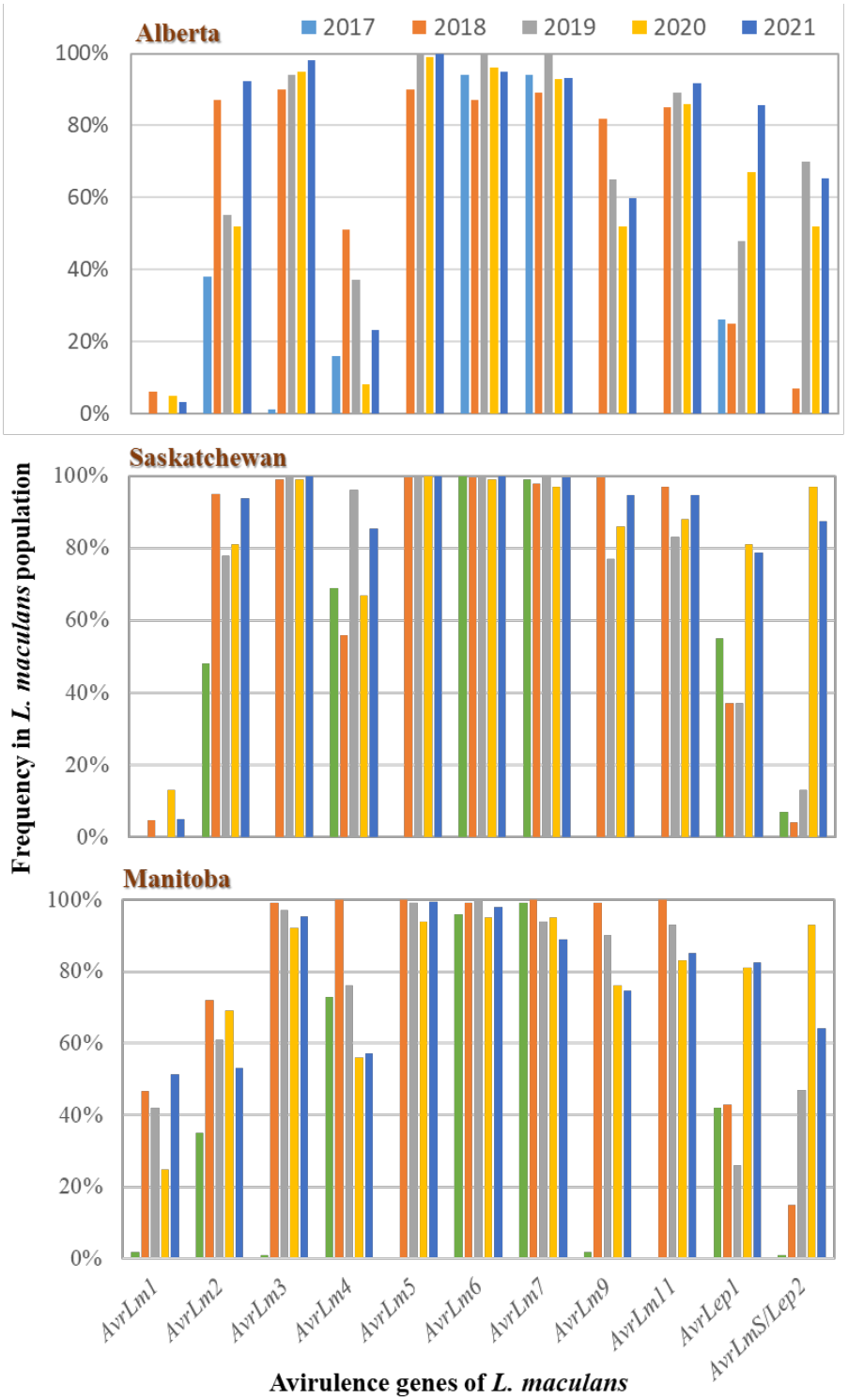

Figure 4. Avirulence (*Avr*) gene frequencies in the population of *Leptosphaeria maculans* on the Canadian prairies between 2017 and 2021.

Additionally, about 800 *L. maculans* isolates obtained from provincial canola disease surveys and WCC/RRC blackleg Coop trial nurseries in 2018 were analyzed for the presence of *AvrLm10* using a differential host from INRA France that carry *Rlm10*. In an initial analysis, over 80% of the isolates showed positive in carrying this effector gene, but further testing of '*AvrLm10*-negative' isolates indicated that all of them carry *AvrLm10.*

6. Conclusions and Recommendations – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development, and communication needs, if applicable.

When compared to earlier results (Liban et al. 2016; Soomro et al. 2020; Liu et al. 2020), the avirulence profile of *L. maculans* population stayed relatively stable for most of the *Avr* genes from 2017 to 2021; only *AvrLep1* increased noticeably. The development and adoption of KASP markers after 2018 not only enhanced efficiency of *Avr* analysis, but also expanded the scope of monitoring to include *AvrLm5*, *AvrLm11* and *AvrLmS-Lep2*. The masking effect on *AvrLm3* and *AvrLm9* due to the high presence of *AvrLm7* in *L. maculans* was also rectified because of the use of markers as opposed to host differentials. To this *L. maculans* population, cultivars carrying the *R* gene *Rlm2*, *Rlm5*, *Rlm6*, *Rlm7, Rlm10, Rlm11*, *LepR1* or *LepR2* will likely be resistant to blackleg in most prairie regions. It is noteworthy that *AvrLm4* has been consistently lower in the pathogen population in Alberta than in the other two provinces. Therefore, the performance of the corresponding resistant gene *Rlm4* should be closely monitored there.

Relative to earlier results (Soomro et al. 2020), the diversity of pathogen population remained high, especially in Manitoba, with more than 80 races identified in western Canada. It is important to point out that for most of the *R* genes, there was at least one virulent race of *L. maculans* already existent; this means that no new mutation is required and virulent races are present in the current pathogen population. Therefore, judicious deployment of *R* genes and rotation of resistant cultivars will be import for genetic control of blackleg. If an *R* gene is used repeatedly, there will be selection pressure for virulent races that can eventually break down the resistance.

With the introduction of new *R* genes, including *Rlm2, Rlm4*, *Rlm7* and *LepR2*, into more canola cultivars, the pathogen population will likely shift. It is important to continue the monitoring to provide the industry critical information for deploying effective *R* genes and *R*-gene rotation. With the support of canola grower groups and WGRF, the funding has been secured for the continued work in next five years. The information may be even more useful if it could be specific to smaller regions such as crop districts in each province to better assist *R*-gene rotation recommendations on a regional basis.

7. Extension and communication activities: (e.g. extension meetings, extension publications, peer-reviewed publications, conference presentations, photos, etc).

1. Peng G. 2017. Blackleg race dynamics, fungicide, resistance gene labelling and crop rotational strategies. An invited talk at the Science-O-rama, Lacombe, AB. April 5, 2017 (Expert Panel).
2. Peng G. 2017. Participated at CCC “Blackleg Booth” at the Manitoba Canola PALOOZA. Portage La Prairie, MB, June 22, 2017
3. Peng G. 2018. Managing blackleg of canola in Canada –host resistance, pathogen race dynamics, and fungicide strategies. A seminar at Centre de Versailles-Grignon, INRA, France (Jun 26, 2018).
4. Peng G. 2018. Unique aspects for blackleg infection and management in Canada. A seminar at Institut de Génétique, Environ et Protection des Plantes, INRA, Rennes, France (Jun 29, 2018).
5. Peng G provided the information for the article “Survey shows blackleg pathogen population across the Prairies” Canola Digest, Aug. 27, 2018.
6. Peng G. 2018. Managing blackleg of canola in western Canada –an integrated strategy. An invited talk at the 5th Joint Meeting of Plant Pathol Soc. Alberta and Can Phytopathol Soc.-Saskatchewan Group.

Lloydminster, AB. Oct. 17, 2018 (Expert Panel).

7. Peng G. 2018. Blackleg of canola in Saskatchewan – an update on the disease, pathogen and cultivar resistance. An invited talk at Saskatchewan Ann. Agronomy Conference, Saskatoon (Dec. 12, 2018).
8. Peng G. 2018. Blackleg of canola in western Canada –What we know and don't know? An invited talk at the Manitoba Agronomy Update. Winnipeg (Dec. 13, 2018).
9. Peng G. 2019. Managing blackleg of canola in western Canada – an integrated strategy. An invited webinar talk organized by Top Crop Manager, Feb. 9, 2019.
10. Peng G. 2019. Participated at "Blackleg Booth" during Saskatchewan Canola PALOOZA. Saskatoon (July 9, 2019).
11. Soomro W, Kutcher HR, Yu F, Hwang SF, Strelkov SE, Fernando WGD, McLaren D, Peng G. 2021. Race structure of Leptosphaeria maculans in western Canada between 2012 and 2014 and its influence on blackleg of canola. Can J Plant Path. 43: 480-493.
12. Liu F, Zou Z, Peng G, Fernando WGD. 2021. Leptosphaeria maculans isolates reveal their allele frequency in western Canada. Plant Disease 105: 1440–1447.

8. Acknowledgements – Include actions taken to acknowledge support by the Funders.

Financial support by Alberta Canola Producers Commission, SaskCanola and Manitoba Canola Growers Association has been acknowledged in each of the above presentations and publications.

9. Literature Cited

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Xiang Neik T, Ghanbarnia K, Ollivier B, Scheben A, Severn-Ellis A, Larkan NJ, Haddadi P, Fernando DWG, Rouxel T, Batley J, Borhan HM, Balesdent MH. 2020. Two independent approaches converge to the cloning of a new *Leptosphaeria maculans* avirulence effector gene, *AvrLmS-Lep2*. *Mol Plant Pathol*. 23: 733-748.

Zhang XH, Peng G, Kutcher HR, Balesdent MH, Delourme R, Fernando WGD. 2015. Breakdown of Rlm3 resistance in the Brassica napus - Leptosphaeria maculans pathosystem in western Canada. Eur J Plant Pathol. 145: 659–674.

10. Other Administrative Aspects: HQP personnel (PhD and/or MSc students) trained and involved; equipment bought; project materials developed

A Biologist was assigned to carrying out majority of the work, including KASP marker development and validation. Dr. X. Zhu (post-doc) was assigned to the project for eight months in 2022 to fast track the isolation and phenotyping using a bioassay. Both post-doc and Biologist assisted in development of the extension messaging. No graduate student has been involved in the project directly.

11. Appendices - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.

12. Financial (to be provided to each Funding Agency (at the addresses indicated in 11.2) a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to the Funders' Funding. b. Explanation of variances from budget which are greater than 10%. c. An invoice for each Funding Agency	
13. Final Report Posting Do you consent to a version of this Final Report (with sensitive information removed) to be posted on the funder's website?	☐ Yes - this version can be posted X ☐ Yes - a modified version will be sent ☐ No X
14. Research Abstract Posting Do you consent to the 2-3 Research Abstract submitted with this Final Report to be posted on the funders and the Canola Council of Canada's website?	☐ Yes X ☐ No

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