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: *Exploring novel seed-treatment options to mitigate the impact of blackleg on canola*

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***
Dilantha Fernando	University of Manitoba	Co-Investigator
Ralph Lange	InnoTech Alberta	Co-Investigator

Project Start Date: **April 1, 2019** **Project Completion Date:** **March 31, 2023**

Reporting Period: **April 1, 2022** to **February 15, 2023**

CARP Project Number: **2019.10**

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: _February 15, 2023_____________

☐ **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.

It has long been suspected that early infection is important to blackleg on canola in western Canada, which can be through fresh wounds on cotyledon or lower leaves, and soil inoculum via roots. Two concurrent studies and research in Australia have confirmed the relevance, although the minimum level of soil inoculum required for root infection was still unclear. In any event, the early infection may be more readily mitigated with a seed treatment than spraying with fungicide. The objectives of study were to: **1)** understand the soil inoculum level of *Leptosphaeria maculans* (*Lm*, blackleg fungus) required to cause root infection which would result in blackleg, **2)** evaluate new commercial products for potential seed treatment against the early *Lm* infection, **3)** assess the rate effect of top candidates for maximum efficacy, and **4)** validate the efficacy in multi-site/year field trials.

Much progress has been made earlier and reported in previous reports, including: **1)** *Lm* inoculum at 10^4 to 10^5 pycnidiospores/g soil caused root infection which resulted in substantial blackleg, especially for wounded roots, **2)** the current seed-treatment standard Helix® Vibrance (HV) or Prosper® EverGol (PEG) was ineffective, while two new systemic fungicides, Fluopyram and Saltro, stood out as the most effective seed treatments against the early infection. An additional product, BION (plant growth regulator) also appeared promising, **3)** Fluopyram showed stability in efficacy with delayed emergence under controlled environment, **4)** field trials had shown that Fluopyram or Bion seed treatment reduced the blackleg severity via cotyledon infection on susceptible but not resistant canola cultivars, **5)** it was not uncommon for soil Lm inoculum to reach the 10^5 spores/g soil in short canola rotation, and the Fluopyram treatment at 75 g/100Kg seed (registered rate) showed the potential to reduce root infection at this soil inoculum level, but failed to demonstrate the efficacy in field trials at about 10^5 spores/g soil inoculum level.

The study in 2022 focused on the efficacy assessment for Fluopyram seed treatment against cotyledon, 1st-leaf and root infection field conditions. Higher product rates were also investigated against the foliar infection in greenhouse and root infection under field conditions. Data from two foliar-inoculated field trials showed that although treated plants had a tendency of slightly lower blackleg levels, the differences were insignificance (LSD, $P > 0.05$). For two soil-inoculation trials, seed treatment at 75 g rate failed to reduce blackleg ($P > 0.05$), while the rate of 300 g did so to both disease incidence and severity significantly (LSD, $P < 0.05$) on the susceptible but not resistant canola cultivars. This shows the greater efficacy associated with the higher rate of fluopyram seed treatment against the infection from the soil inoculum.

In a greenhouse study, Fluopyram seed treatment at 75 g effectively restricted the infection via fresh wounds on cotyledons but appeared insufficient against the infection on 2nd true leaves. When increasing the product rate to 150 g, the efficacy was noticeably improved on 2nd leaves.

The following are the key findings from the 3-year study: **1)** *Lm* inoculum at $>10^5$ spores/g soil can cause severe blackleg via root infection, especially if roots are wounded. This information is relevant to root injuries caused by soil insects and root-rot diseases. **2)** the current industry seed-treatment standards (HV and PEG) are ineffective against blackleg infection via leaf wounds or roots, **3)** Fluopyram, Saltro and Bion look most

promising seed treatments against the early infection, but the rates may be finetuned for extended protection and more consistent performance under field conditions. **4)** None of the treatments has shown substantial efficacy on any of the resistant canola cultivars tested under field conditions, a circumstance likely attributable to very low levels of blackleg on these cultivars already. Due to relatively low costs, these treatments may still be a good insurance policy in case any of the current cultivars losses the resistance.

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

From the perspective of canola production and export, there is a need to continue mitigating the impact of blackleg in western Canada. While the variety resistance has been the mainstay (Zhang et al. 2016; Peng 2017a; Hubbard and Peng 2018), measures such as crop rotation (Harker et al. 2017), pathogen race monitoring (Liban et al. 2016; Peng et al. 2016; Liu et al. 2021; Soomro et al. 2021) and fungicide application (Peng et al. 2016; 2017; 2021) may complement the variety resistance. Fungicide applied in seedling stages can reduce the disease impact on susceptible canola under high disease-pressure conditions, but is generally cost ineffective due to little yield returns on resistant canola cultivars (Peng et al. 2015; 2021).

In western Canada, early infection through fresh wounds on cotyledon and lower leaves, as well as through roots caused by soil inoculum (Sosnowski et al. 2001; Sprague et al. 2007; Huang et al. 2022), appears important to blackleg impact possibly due to a short growing season. The root infection was still not well understood in terms of the minimum soil inoculum required for infection and the potential soil inoculum levels in western Canada, although the soil moisture condition is less fluctuating than that on the leaf surface in early spring. In Australia, the fungicide fluquinconazole has been used for years, as a seed treatment or in-furrow application, to control blackleg (Macroft and Potter2003; Sprague et al. 2010). The current seed treatments in Canada (HV or PEG) target primarily damping off/root rot, as well as flea beetles (Sekulic and Rempel 2016), and their efficacy against the early blackleg infection via either lower leaves or roots was unknown/unexpected.

In Canada, Fluopyram and Saltro have been registered for seed treatment against blackleg. Preliminary trials showed that both products can restrict the blackleg infection development via fresh wounds on cotyledons. However, field results were limited for validation of the efficacy. Life science companies and new startups have been actively developing seed-treatment formulations with novel ingredients, including a plant regular called Bion 500^{TM} FS by Syngenta. These candidates may be assessed against early blackleg infection using a common protocol. If successful, a seed treatment would be more cost-effective than fungicide spraying. The objectives of study were to: **1)** understand the soil *Lm* inoculum level required to cause root infection which would result in severe blackleg, **2)** evaluate new commercial products for seed treatment against early *Lm* infection, **3)** validate the efficacy of promising candidates using multi-site/year field trials, and **4)** assess the rate effect and stability in soil for top candidates against foliar and root infection.

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.

Westar (susceptible) and InVigor L255PC seeds were treated with Fluopyram at 75 g a.i./100 kg (registered in Canada) rate. Neither variety carries any specific *R* genes but the L255PC has been down with a level of quantitative resistance (QR) against blackleg, which seems common with many Canadian canola cultivars. For field trials with leaf inoculation, the following treatments were used:

Table 1. Treatments for field trials involving leaf inoculation

Treatment No	Products applied for seed treatment	Stage of inoculation
1	Westar -Control	Cotyledon
2	Westar - Fluopyram 75 g	
3	InVigor L255PC -Control	
4	InVigor L255PC Fluopyram75 g	

5	Westar -Control	1st Leaf
6	Westar - Fluopyram 75 g	

Each treatment was seeded in single-meter rows with about 50 seeds, and each trial arranged in a CRBD with three replicates. Each row was thinned to about 30 plants when cotyledons were fully open. At the cotyledon or 1st-leaf stage, one cotyledon or the true leaf was prick wounded for each plant and inoculated with a mixture of three pathogen isolates capable of evading all known *R* genes. Two field trials were carried out on the AAFC Research Farms near Saskatoon seeded about a week apart. Treatment effects were assessed at early crop maturity by uprooting up to 25 inoculated plants per row, cut at the crown and rated for blackleg severity using a 0-5 scale. Disease severity index (DSI) was calculated for the rating data using the following formula:

$$\text{DSI (\%)} = \frac{\sum [(\text{rating class}) \times (\text{number of plants in class})]}{(\text{Total number of plants}) \times (5)} \times 100\%$$

For trials involving soil inoculation, the *L. maculans* isolate mixture described above was applied as a soil drench to designated rows at about a week after plant emergence to achieve >10^5/g soil inoculum in the vicinity of canola roots. Control rows were drenched with the same volume of tap water. Based on 2021 results, fields with intensive canola production would easily reach this level of soil inoculum. The experimental setup was similar to that of leaf inoculation and the trial was repeated by a second seeding one week later. The plot area had not had any canola crop for the past five years, although the area had been surrounded by canola on the farm for years. The objective was to assess efficacy of fluopyram seed treatment at the registered and a higher rate against root infection. Up to 25 plants were uprooted from each row at early crop maturity and rated for blackleg severity using the 0-5 scale. DSI was calculated for each replicate and used for statistical analysis. The treatments were as follows:

Table 2. Treatments for field trials involving soil inoculation

Variety	**Treatment**
Westar	Control
	Fluopyram 75 g
	Base + Fluopyram 300 g
InVigor L255PC	Base
	Base + Fluopyram 75g

In a greenhouse study, the susceptible Westar seed was treated with Fluopyram at 75 g or 150 g. Fully expanded cotyledons or 2nd true leaves were prick inoculated with the mixed inoculum of three *Lm* isolates, and infection development was assessed using a 0-9 scale at 12-14 days after inoculation. Untreated plants were used as controls. The objective was to investigate whether a higher rate of treatment would extend the protection beyond the cotyledon stage. The experiment was run three times using a CRD, with 8-10 plants (replicates) per run. All materials were prepared independently for each experiment run.

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

Under field conditions, data from two foliar-inoculated trials near Saskatoon showed that although the plants received a Fluopyram seed treatment had a tendency of lower blackleg disease incidence (DI) or disease severity index (DSI) on both susceptible and resistant canola cultivars, there was a general lack of statistical significance ($P > 0.05$) for the treatment. For two soil-inoculation field trials, moderate levels of blackleg were observed with non-inoculated susceptible control plants, indicating some background inoculum in the soil already (**Figure 1**). The soil- drench application of *Lm* inoculum did not increase the blackleg level significantly ($P > 0.05$) on either susceptible or resistant cultivar over respective non-inoculated controls. Seed treatment with 75 g of fluopyram did not reduce the blackleg DI or DSI ($P > 0.05$) on either cultivar, when compared to respective inoculated controls, but the treatment at 300 g reduced both DI and DSI substantially on the susceptible Westar but not the resistant InVigor L255PC (**Figure 1**). This shows greater efficacy of the higher fluopyram rate in reducing blackleg infection via roots from the soil inoculum.

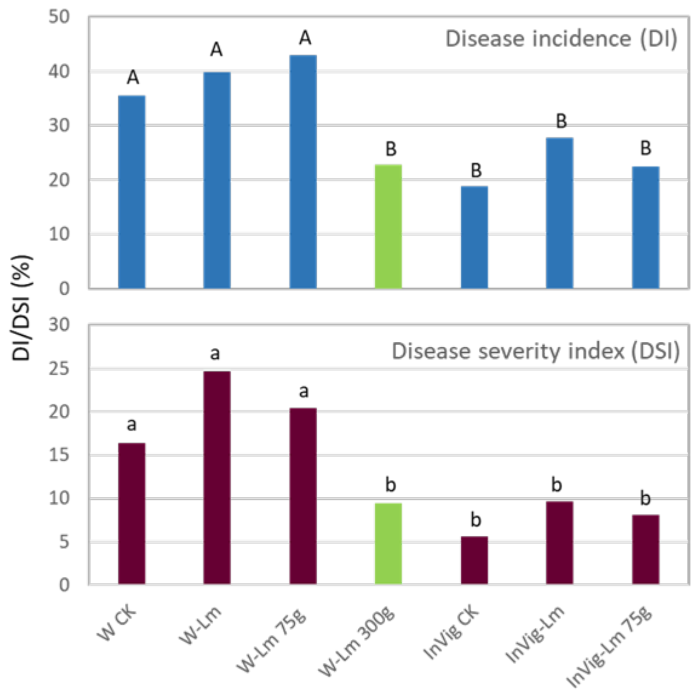

Figure 1. Effect of Fluopyram seed treatments on blackleg disease incidence (DI) and disease severity index (DSI) caused by soil inoculum of *Leptosphaeria maculans* on Westar (W) and InVigor L255PC (InVig) in field trials at Saskatoon, SK (data from two trials were pooled). 75g and 300g were the rates of seed treatment used for 100 Kg of canola seed. The treatments with the same letters do not differ (LSD, $P > 0.05$).

In a greenhouse study, Fluopyram seed treatment at 75 g effectively limited the infection via fresh wounds on cotyledons relative to the non-treated control (**Figure 2**), which was consistent with earlier results. The 75-g rate, however, appeared insufficient against the infection on 2nd leaves of canola seedlings, while a treatment at 150-g was noticeably more effective.

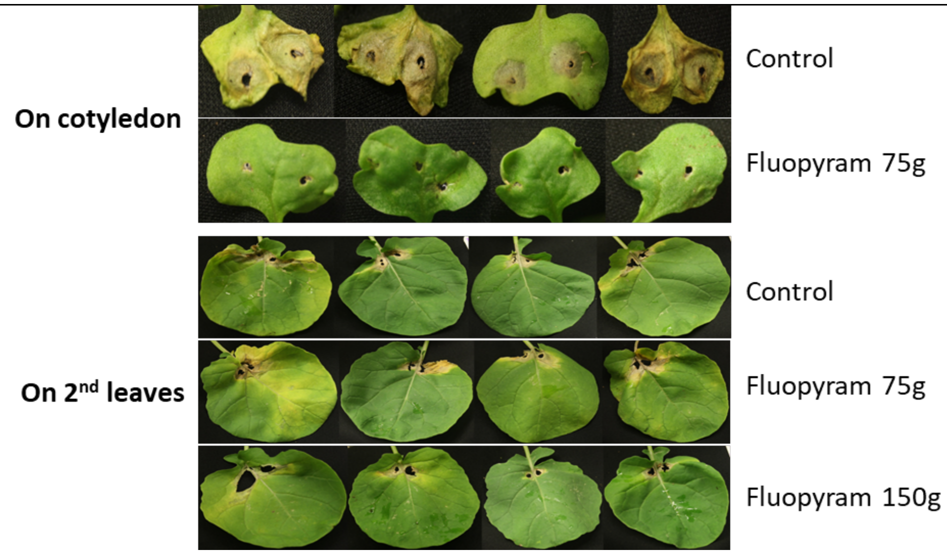

Figure 2. Typical infection severity on cotyledons and 2nd true leaves of canola at 12-14 days after inoculation.

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.

The study has found that: **1)** *Lm* inoculum at >10^5 spores/g soil can result in severe blackleg via root infection, especially if roots are wounded. This information is relevant as fields with intensive canola production can reach this level of soil inoculum and root injuries caused by soil insects/root-rot diseases can exacerbate the infection, **2)** the current seed treatments such as HV and PEG are ineffective against blackleg infection through either leaf wounds or roots, **3)** Fluopyram, Saltro and Bion seed treatments look promising against the early infection, and the rate may be finetuned for extended protection and more consistent performance under field conditions. **4)** None of these treatments has shown substantial efficacy on any of the resistant canola cultivars tested under field conditions, a circumstance likely attributable to generally low levels of blackleg on these cultivars. Due to relatively low costs, however, the treatments may still be a good insurance policy in case of resistance erosion.

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

1. 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.
2. Peng G. 2019. Participated at "Blackleg Booth" during Saskatchewan Canola PALOOZA. Saskatoon (July 9, 2019).
3. Peng G, Soomro W, Hubbard M, Zhai C, Liu X, McGregor L, Fernando WGD, Lange R, Yu F, McLaren, D. 2019. The need for an integrated approach to manage blackleg of canola in western Canada. 14th Int. Rapeseed Cong, June 16-19, 2019, Berlin (Poster).
4. Peng G. 2020. Invited by SaskCanola to give a webinar presentation on "Improving blackleg management in western Canada: what's coming down the research pipelines?" June 5, 2020.
5. Peng G, Liu X, McLaren D, McGregor L, Yu F. 2020. Fungicide seed treatment for effective control of blackleg of canola in Canada. *Can J. Plant Path*. 42: 480-492.

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

Financial support by SaskCanola has been acknowledged in each of the above presentations/publications.

9. Literature Cited

1. Huang SL, Zhai, C, Zou ZW, Liu F, Parks P, Mcgregor L, Fernando WGD, Peng G. 2022. Effect of wounding and wound age on infection of canola cotyledons by *Leptosphaeria maculans*, interacting with leaf wetness. *Can J Plant Pathol* 44: 709-722.
2. 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
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7. Peng G. 2017a. Managing blackleg in canola in Western Canada, part I. *Top Crop Manager*.
8. https://www.topcropmanager.com/diseases/managing-blackleg-in-canola-in-western-canada-part-one-20210#.
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10. Peng G, Liu C, Fernando WGD, Lange R, McLaren D, Kutcher HR, Singh G, Turkington TK, Johnson EN, Yu F. 2021. Early application of fungicide reduces the yield impact of blackleg only on susceptible canola under moderate to high disease pressure. *Can J Plant Pathol* 43: 384-393.
11. Sekulic G, Rempel CB. 2016. Evaluating the role of seed treatments in canola/oilseed rape production: Integrated pest management, pollinator health, and biodiversity. *Plants* 5, 32; doi:10.3390/plant 030032.
12. 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.
13. Sosnowski MR, Ramsey MD, Murray GM, Scott ES, Wilmshurst C. 2001. Symptoms of blackleg (Leptosphaeria maculans) on the roots of canola in Australia. *Plant Pathol* 50: 808.
14. Sprague SJ, Watt M, Kirkegaard JA, Howlett BJ. 2007. Pathways of infection of Brassica napus roots by Leptosphaeria maculans. *New Phytologist* 176: 211–222.
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17. 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.

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

A Research Biologist was hired to conduct most of the experiments associated with the study, and no graduate student was involved directly. No equipment was purchased for the study either.

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

None	
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 ☐ Yes - a modified version will be sent X ☐ No
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

Please send an electronic copy of this completed document to:

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