Canola Agronomic Research Program (CARP)
FINAL REPORT

The Final Report should fully describe the work completed over the duration of the project, including results and activities from the final 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. .

Project Title: Influence of pH on the clubroot pathogen: are there pH-insensitive strains?

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Dr. Stephen Strelkov	University of Alberta	Principal Investigator
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Dr. Sheau-Fang Hwang	University of Alberta	Co-Investigator:Collaborator, assistance with greenhouse studies and lime formulations
Dr. Yoann Aigu	University of Alberta	Postdoctoral Fellow

Project Start Date: April 1, 2019 **Project Completion Date: September 30, 2023**
Reporting Period: April 1, 2019 September 30, 2023

CARP Project Number: 2019.09

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 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 of the specific CARP funders and other funders 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: ___Sept. 30, 2023_____

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

Comments: The project is now complete.

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

Introduction and Objectives: Clubroot, caused by *Plasmodiophora brassicae*, is an important soilborne disease of canola that is managed mainly by planting resistant cultivars. Unfortunately, the widespread cultivation of resistant hosts has resulted in the emergence of new *P. brassicae* pathotypes that are able to overcome this resistance, highlighting the need for an integrated approach to clubroot control. The application of lime to increase soil pH represents a potential tool to complement resistance, since clubroot favors acidic soils. In this project, we addressed two questions: (1) Does the application of lime have varying effects on different *P. brassicae* isolates? and (2) Can liming inadvertently select for pH-insensitive strains of the pathogen?

Methods: To answer the first question, a clubroot-susceptible canola hybrid was grown under greenhouse conditions in a soil mixture with (pH 7.5) or without (pH 6.0) liming, and inoculated at high and low spore concentrations with each of nine field isolates representing various pathotypes of *P. brassicae*. To answer the second question, consecutive cycles of infection were carried out using four field isolates of the pathogen selected based on the results of the first study.

Results: At the higher spore concentration, the effect of lime application on clubroot disease severity index (DSI) was minimal. In contrast, at the lower spore concentration, the application of lime was effective in significantly reducing the DSI by an average of 27 points across all nine isolates. The extent of this reduction, however, was highly variable and depended on the isolate tested. The quantification of *P. brassicae* resting spores per plant indicated that liming also reduced spore production for all of the isolates; however, there was no significant correlation between the effect of liming on symptom development and resting spore production. Over three cycles of infection in the absence of liming, DSI tended to increase in each cycle if it was not already at the maximum. On the other hand, DSI in the liming treatment generally decreased over the cycles. In most cases, the DSI continued to decrease until it reached values that were comparable to those of a resistant variety. However, for one isolate, an increase in DSI was detected between the second and third cycles, including an increase in DSI from 22 to 81 points for one of the replicates. Trends similar to those observed for DSI were found with respect to resting spore production per plant over the multiple cycles of infection.

Conclusions: The results of this project suggest four main conclusions. Firstly, liming may not be sufficient, at least on its own, to provide satisfactory management of clubroot in soils that are highly infested with *P. brassicae*. Secondly, isolates of the pathogen show differential sensitivity to liming, which will affect the efficacy of this strategy depending on the sensitivity of the pathogen population in a specific field. Thirdly, the greatest impact of liming will occur after repeated exposure of *P. brassicae* populations to this treatment, as clubroot severity and resting spore production appear to decline for most isolates across multiple cycles of infection under limed conditions. Fourthly, while repeated liming may result in improved control over time, this

strategy also runs the risk of selecting for pH-insensitive strains of the clubroot pathogen. While liming may serve as a tool to complement host resistance, its potential utility is likely to be affected by field-specific conditions that need to be considered before growers invest in this approach.

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

Clubroot, caused by *Plasmodiophora brassicae*, is a major threat to the Canadian canola industry (Strelkov and Hwang, 2014). Disease development results in the formation of galls on the roots of susceptible plants, which limit plant water and nutrient uptake and can stunt aboveground growth and yields. Close to a billion *P. brassicae* resting spores can be produced from one infected canola plant. These spores serve as pathogen inoculum and can persist in the soil for more than 15 years (Hwang et al., 2014). Since their introduction in 2009-2010, clubroot-resistant canola cultivars have become the first line of defense against this disease (Strelkov and Hwang, 2014). Unfortunately, the widespread cultivation of resistant hosts has resulted in the emergence of new *P. brassicae* pathotypes that are able to overcome this resistance (Strelkov et al., 2018). Moreover, studies have shown significant genetic and virulence diversity in Canadian populations of *P. brassicae* (Strelkov et al., 2018; Holtz et al., 2018). This diversity can make breeding for clubroot resistance challenging, particularly given the limited number of highly effective resistance genes. As such, clubroot resistance should be used wisely and as part of a more integrated clubroot management plan (Hwang et al., 2014).

The application of lime to increase soil pH represents a potential tool to complement clubroot resistance, since *P. brassicae* generally is favored by acidic soils. Indeed, lime has been used for clubroot management in cruciferous vegetables for over a century, and holds potential for management of the disease in canola. However, pH amendments do not always produce consistent results (Donald and Porter, 2009), as shown by a number of studies carried out with canola (Hwang et al., 2011; Gossen et al., 2014; Fox et al., 2022). While further research into the efficacy of lime is now underway, little is known regarding the pathogen response to soil pH, and in particular, whether or not there could be variability in the pH sensitivity and/or pH optima among *P. brassicae* populations. Since soil liming in canola production systems would require considerable effort and expense (Hwang et al., 2011), it is important to understand all of the key factors that may impact the success of this strategy as a clubroot management tool. In addition to environmental factors such as soil moisture and temperature, a long overlooked but potentially important factor that could also affect the efficacy of pH amendments is the pathogen itself. Although infection and symptom development caused by *P. brassicae* are favored by acidic soils, it is possible that strains that can perform well in neutral or slightly basic soils also exist, particularly considering the vast diversity in this pathogen. While the acidic soils found in central Alberta are considered a key reason why clubroot has become so established in this region, cases of clubroot have been reported even in fields with a soil pH as high as 7.6 (Strelkov et al., 2007). In controlled-environment experiments, moderately severe clubroot could even develop at a pH of 8.0 (Gossen et al., 2013). The reason(s) for this apparent insensitivity to higher pH in some pathogen populations has not been examined. If the optimal soil pH for infection and clubroot development can vary among *P. brassicae* populations, then the pH sensitivity or insensitivity of specific strains could alter the efficacy of liming as a management tool. Therefore, understanding strain-specific responses to pH will have practical consequences: farmers may consider liming as an option only if pH amendments can control the strains present in their fields. If a particular strain is more tolerant to high pH, they may be better off focusing on other strategies to manage clubroot.

In this project, we addressed two questions: (1) Does the application of lime have varying effects on different *P. brassicae* isolates? and (2) Can liming inadvertently select for pH-insensitive strains of the pathogen? To answer the first question, a clubroot-susceptible canola hybrid was grown under greenhouse conditions in a soil mix with (pH 7.5) or without (pH 6.0) liming, and inoculated at high and low spore concentrations with each of nine field isolates representing various pathotypes of *P. brassicae*. To answer the second question, consecutive cycles of infection were performed under conditions similar to study 1, but using four field isolates selected based on the results of the first study.

4a. Methods – Include approaches, experimental design, methodology, materials, sites, etc.

4b. Major changes from original plan should be cited and the reason(s) for the change should be specified.

(1) Does the application of lime have varying effects on different *P. brassicae* isolates?

Isolate-specific responses to lime application were evaluated in greenhouse experiments. Briefly, nine field isolates of *P. brassicae* were tested, each representing a different pathotype as defined on the Canadian Clubroot Differential (CCD) set (Strelkov et al., 2018). These included the predominant pathotypes 3A, 3D and 3H, pathotype 5G (represented by an isolate recovered from a high pH field in southern Alberta), and pathotypes 5I, 5X (the first resistance-breaking pathotype from canola), 6B, 6M and 8E. The pH sensitivity of the isolates was tested simultaneously at pH 7.5, by adding 1.2 g of hydrated lime ($Ca(OH)_2$) per 100g of dry soil mixture (Sunshine Mix #4/LA4), and at pH 6.0, corresponding to the pH of the soil mix without the addition of hydrated lime. The quantity of lime required to increase the pH of the soil mixture to 7.5 was first determined by testing seven concentrations of hydrated lime (from 0 to 8 g per 100 g of dry soil mix). Two days after the addition of lime, the soil mixture was inoculated at each of two resting spore densities (5×10^4 and 5×10^5 spores/g of dry soil mix) and homogenized by mixing. One week later, nine seeds of the clubroot-susceptible canola hybrid '45H35' were sown into each (20 cm-diam.) pot, with four replicates (pots) per isolate × liming condition included in the experiment. Six weeks after sowing, the plants were harvested to determine the clubroot disease severity index (DSI). Finally, the galls were ground to quantify the resting spores produced per plant.

(2) Can liming inadvertently select for pH-insensitive strains of the pathogen?

Galled root material generated in study 1 was used at the starting inoculum for evaluating the effect of repeated exposure of an isolate to pH 6.0 and 7.5 on its pH sensitivity. The inoculation conducted in study 1 served as the first exposure of each isolate to the different lime treatments, with the root galls harvested and exposed twice more to the same conditions (for a total of three consecutive cycles). Based on the results of study 1, one inoculum concentration (5×10^4 spores/g of dry soil) and four isolates were selected for the additional cycles of infection. These included the two isolates with the lowest difference in DSI (pathotypes 3H and 5I) between the liming conditions (limed/not limed), as well as the two with the greatest difference in DSI (pathotypes 6B and 6M).

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

(1) Does the application of lime have varying effects on different *P. brassicae* isolates?

This study consisted of two trials performed with two different inoculum concentrations (5×10^4 and 5×10^5 resting spores per g of soil mix). In a soil mixture inoculated with 5×10^5 resting spores/g soil mix, the clubroot DSI was equal to or very close to 100 in the absence of lime for all nine isolates (**Figure 1**). At this higher spore concentration, the effect of lime application on DSI was minimal; the greatest reduction in DSI (observed with isolate 3D) did not exceed 7 (observed with isolate 3D). These results suggest that above a certain concentration of inoculum (5×10^5 resting spores/g soil mix in this study), the application of lime does not efficiently limit the development of clubroot symptoms on a susceptible cultivar.

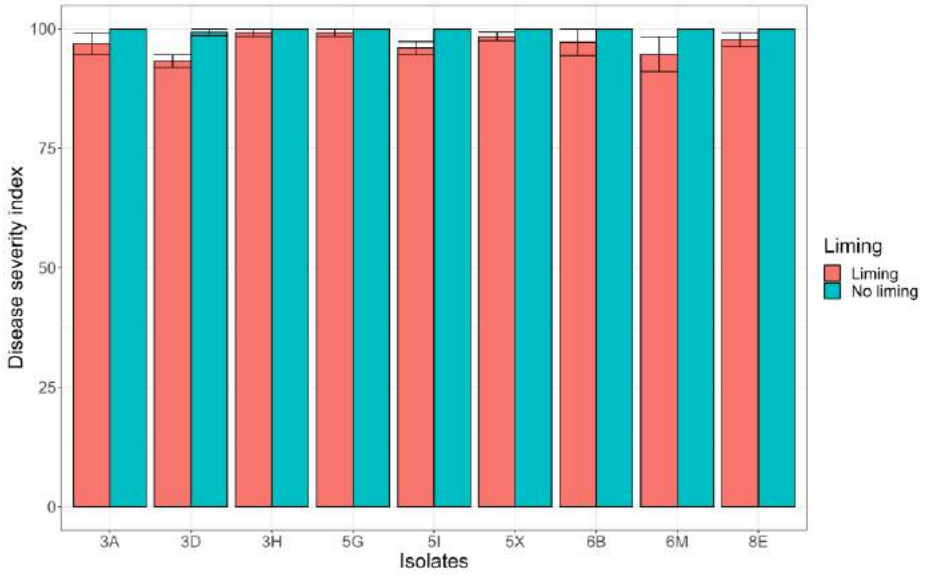

Fig. 1. Effect of liming on the clubroot disease severity index (DSI) on a susceptible canola hybrid grown in a soil mix inoculated with each of nine isolates of *Plasmodiophora brassicae* at a concentration of 5×10^5 resting spores per g of soil. In the liming treatment, hydrated lime was applied to a target pH of 7.5, while non-treated (no liming) soil mix had a pH of 6.0. Bars represent the standard error of the mean.

In the second trial, which was inoculated with 5×10^4 resting spores/g soil mix, the DSI was still very high (78-100) for all of the tested isolates in the absence of liming (**Figure 2**). However, in contrast with the results obtained at the higher inoculum concentration, the application of lime to soil mixture inoculated with the lower spore density (5×10^4 resting spores/g soil mix) was effective in significantly reducing the DSI. In the limed treatments, DSI was reduced by an average of 27 points across all nine isolates. The extent of this reduction, however, was highly variable and depended on the isolate tested. While the clubroot severity caused by some isolates barely declined, for others a very large reduction in DSI was observed. The isolates most sensitive to the liming treatment included pathotypes 6B, 6M and 8E, for which the DSI declined by 38, 54 and 42 points, respectively. Collectively, these results suggest that the effect of liming is not only dependent on the *P. brassicae* resting spore concentration, but also on the specific genotype of those resting spores.

The quantification of *P. brassicae* resting spores per plant indicated that liming reduced spore production for every isolate tested (**Figure 3**). In the limed treatments, the quantity of resting spores produced per plant declined by an average of 1.4×10^9 across all nine isolates. As with the DSI, the level of this reduction varied depending on the specific isolate, with greater declines observed for some vs. others. The isolates most sensitive to the liming treatment included pathotypes 3H and 6B, for which spore production declined by 2.9×10^9 and 1.9×10^9, respectively. Interestingly, no significant correlation ($R^2 = 0.09$) was found between the effect of liming on symptom development and resting spore production.

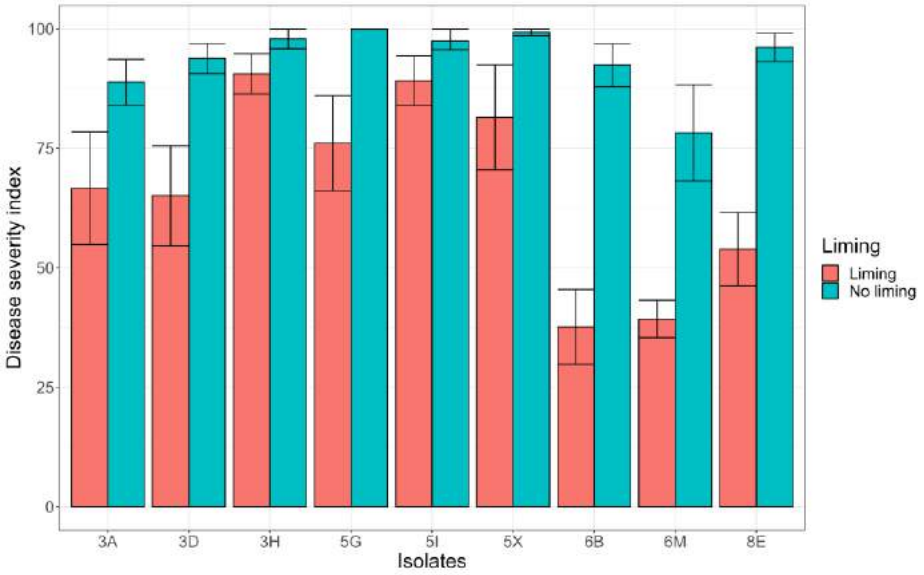

Fig. 2. Effect of liming on the clubroot disease severity index (DSI) on a susceptible canola hybrid grown in a soil mixture inoculated with each of nine isolates of *Plasmodiophora brassicae* at a concentration of 5×10^4 resting spores per g of soil. In the liming treatment, hydrated lime was applied to a target pH of 7.5, while non-treated soil mix (no liming) had a pH of 6.0. Bars represent the standard error of the mean.

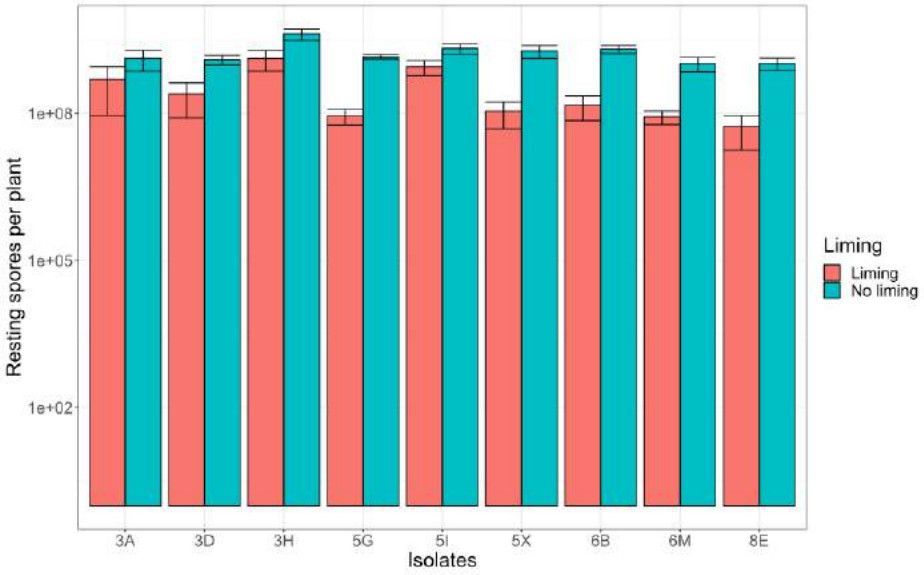

Fig. 3. Effect of liming on resting spores produced per plant in a susceptible canola hybrid grown in a soil mixture inoculated with each of nine isolates of *Plasmodiophora brassicae* at a concentration of 5×10^4 resting spores per g of soil. In the liming treatment, hydrated lime was applied to a target pH of 7.5, while non-treated soil mix (no liming) had a pH of 6.0. Bars represent the standard error of the mean. Note that the Y-axis represents a log 10 scale.

(2) Can liming inadvertently select for pH-insensitive strains of the pathogen?

As noted above, four isolates were assessed for potential shifts in pH sensitivity following repeated exposure to a soil mixture limed to pH 6.0 or 7.5. These included isolates representing pathotypes 3H, 5I, 6B and 6M, which were selected based on the results of the first study; isolates 3H and 5I were chosen because of their apparent insensitivity to liming, while 6B and 6M were selected for their high sensitivity to the liming effect. Over three cycles of infection in the absence of liming, DSI tended to increase each cycle, when it was not already at the maximum (**Figure 4**). In contrast, DSI in the liming treatment generally decreased over the cycles. These

reductions in DSI were observed in all the replicates of the four isolates tested. From cycle 1 to 2, the DSI for 3H and 5I dropped from 90 and 89 to 38 and 19, respectively. Similarly, the DSI for 6B and 6M dropped from 37 and 39 to 19 and 10, respectively. In most cases, the DSI continued to decrease in cycle 3, declining to values in the clubroot-susceptible hybrid '45H35' that were comparable to those of a resistant variety. However, unlike in cycle 2, there was some evidence of a reduced effect of liming for isolate 6B (**Figure 4**) as indicated by an increase in the DSI. In fact, the DSI in one of the four replicates of isolate 6B increased from, 22 in cycle 2 to 81 in cycle 3. These results suggests that liming becomes more effective at reducing DSI when applied before each new crop, but also indicate the possibility for selection of liming-insensitive isolates, as observed for one of the 6B replicates.

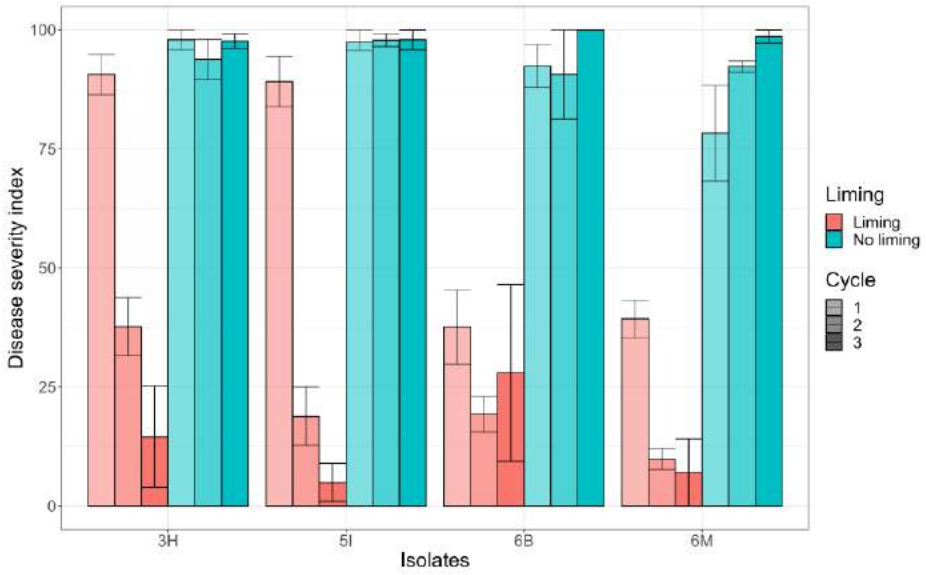

Fig. 4. Change in clubroot disease severity index on a susceptible canola hybrid following cultivation in a soil mixture inoculated (5×10^4 resting spores/g soil mix) with four isolates of *Plasmodiophora brassicae*, which were repeatedly exposed to liming (pH 7.5) or no liming (pH 6.0) conditions over three consecutive cycles. Bars represent the standard error of the mean.

Trends similar to those observed with DSI were found with respect to resting spore production per plant over the multiple cycles of infection (**Figure 5**). In the non-limed treatments, the number of resting spores produced per plant remained consistent or increased in each cycle, likely reflecting the increases in DSI as these approached 100 points. In contrast, in the limed treatments, spore numbers generally decreased. For example, from cycle 1 to cycle 2, the resting spores per plant produced by isolates 3H and 5I declined from 1.3×10^9 and 9.0×10^8 to 7.9×10^7 and 8.9×10^7, respectively. Similarly, for isolates 6B and 6M, the spore numbers decreased from 1.5×10^8 and 8.6×10^7 to 2.8×10^7 and 4.3×10^7 resting spores per plant, respectively. These results suggest that, as with the DSI, liming becomes more effective at reducing resting spore production over multiple infection cycles when applied before each canola crop. However, the quantity of resting spores produced by all of the treatments remained greater than the initial amount of inoculum applied to the soil at the start of the experiment.

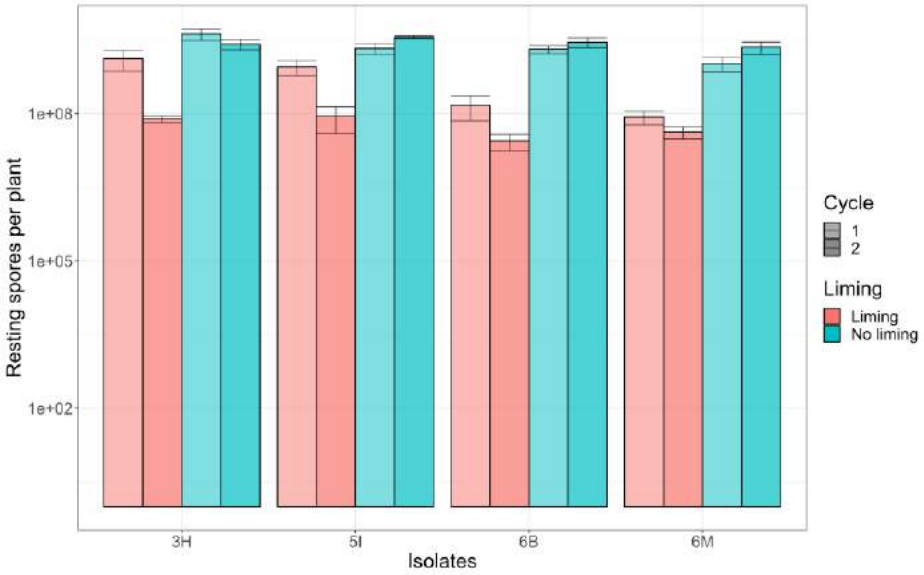

Fig. 5. Change in resting spores produced per plant in a susceptible canola hybrid following cultivation in a soil mixture inoculated (5×10^4 resting spores/g soil mix) with four isolates of *Plasmodiophora brassicae*, which were repeatedly exposed to liming (pH 7.5) or no liming (pH 6.0) conditions over three consecutive cycles. Bars represent the standard error of the mean. Note that the Y-axis represents a log 10 scale.

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.

This project has generated several important findings. Firstly, the *P. brassicae* inoculum density in the soil affects the efficacy of lime as a clubroot management tool. Greater reductions in DSI were observed when hydrated lime was applied to a soil mixture infested at a lower level. Secondly, the application of lime has varying effects on different *P. brassicae* isolates, which in turn also affects the efficacy of liming as a clubroot management tool. The reductions in DSI and resting spore production across the nine field isolates tested varied considerably, with some isolates much more sensitive to lime treatment than other isolates. Thirdly, repeated exposure to liming may result in the selection of pH-insensitive strains of *P. brassicae*, as was observed with one of the replicates of isolate 6B for which a large increase in DSI occurred after the third cycle of infection under higher pH conditions. Conversely, for most isolates, the opposite was true: liming became more effective at reducing DSI and resting spore production in soil containing resting spores generated under limed conditions.

The observation of an enhanced effect of lime for reducing clubroot after repeated exposure of the pathogen to limed conditions was unexpected, and could reflect various possibilities. These include a situation wherein a greater proportion of resting spores produced under liming are not viable, and hence do not contribute to clubroot development in subsequent cycles of infection. Another possibility is that the resting spores generated under liming are less virulent or efficient at infecting the host plant, and hence produce milder symptoms. The third possibility is that repeated exposure to the lime treatment results in an increased sensitivity to this condition in the pathogen. Additional experiments would be required to determine which of these processes is at play.

In summary, four main conclusions and recommendations can be drawn from these findings:

(1) Liming may not be sufficient, at least on its own, to provide satisfactory management of clubroot in soils that are highly infested with *P. brassicae*

(2) Isolates of *P. brassicae* show differential sensitivity to liming, which will affect the efficacy of this strategy depending on the sensitivity of the pathogen population in a specific field

(3) The greatest impact of liming will occur after repeated exposure of pathogen populations to this treatment, as clubroot severity and resting spore production appear to decline for most isolates across multiple cycles of infection under limed conditions

(4) While repeated liming may result in improved control over time, this strategy also runs the risk of selecting for pH-insensitive strains of the clubroot pathogen

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

Presentations and Posters

Roth, M.N., Manolii, V.P., Hwang, S.F., and Strelkov, S.E. The effect of pH on clubroot development. Oral Presentation. The 42nd Annual Meeting of the Plant Pathology Society of Alberta, Nov. 4-5, 2021, Virtual Meeting.

Roth, M., Manolii, V.P., Hwang, S.F., and Strelkov, S.E. 2021. Evaluation of the pH sensitivity of Plasmodiophora brassicae strains. Oral Presentation. Tri-Society Virtual Conference, July 5-9, 2021. Abstract citation: Abstract No. 109 in Tri-Society Flipbook, p. 60; https://trisocieties2021.ca/

Roth, M.N., Hwang, S.F., Manolii, V.P., Strelkov, S.E. Influence of agricultural lime and pH on the clubroot pathogen: Identifying pH-insensitive strains. Poster Presentation. 19th Annual Bentley Lecture in Sustainable Agriculture, Feb. 24, 2021, Edmonton, Alberta.

Roth, M.N., Hwang, S.F., Manolii, V.P., Strelkov, S.E. Liming and the clubroot pathogen. Poster Presentation. Farm Tech 2020, Jan. 28-30, 2020, Edmonton, Alberta.

Strelkov, S.E., Manolii, V.P., Hollman, K., Harding, M., and Hwang, S.F. 2021. Clubroot: A soilborne challenge to canola production. CPS Virtual Workshop – Advances in the Understanding and Management of Soil-Borne Pathogens of Canadian Crops. Canadian Phytopathological Society, September 17, 2021.

Graduate Student Dissertations

Roth, M.N. 2022. Evaluation of *Plasmodiophora brassicae* for the occurrence of pH insensitive strains. M.Sc. Thesis, University of Alberta, Edmonton, Canada. 97 pp.

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

The CARP program and each of the individual funders (Alberta Canola, SaskCanola and the Manitoba Canola Growers Association) were acknowledged for their financial support in all presentations, posters and dissertations stemming from this project. In oral presentations, this included listing the funders and their logos in an acknowledgements slide at the end of each talk and verbally thanking them for their support. In poster presentations, this included listing the funders, their logos and the project no. in the acknowledgements. In the graduate student dissertation, the funders and project no. are indicated in the "Preface" section, and they are also thanked under the "Acknowledgements" section. As the project is just ending, we are still in the process of writing an article for publication in a peer-reviewed journal; the funders and project number will be indicated in this paper as well, once it is complete.

9. Literature Cited

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Fox NM, Hwang SF, Manolii VP, Turnbull G, Strelkov SE. 2022. Evaluation of lime products for clubroot (*Plasmodiophora brassicae*) management in canola (*Brassica napus*) cropping systems. *Canadian Journal of Plant Pathology* 44, 21-38.

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Strelkov SE, Hwang S-F, Manolii VP, Cao T, Fredua-Agyeman R, Harding MW, Peng G, Gossen BD, Mcdonald MR, Feindel D. 2018. Virulence and pathotype classification of *Plasmodiophora brassicae* populations collected from clubroot resistant canola (*Brassica napus*) in Canada. *Canadian Journal of Plant Pathology* 40, 284–298.

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

Training of HQP

1 Postdoctoral Fellow

1 MSc Student

2 Summer Students

(Technical personnel also assisted with various aspects of the project)

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

n/a

12. Financial (to be provided to CCC)

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. Invoice

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 ☐ 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 ☐ No

Please send an electronic copy of this completed document to:

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