ARGO
Agriculture Research Grant Organizer

2019F076R:

Due Date

05/31/2022

Project Overview

Project number:	2019F076R
Project title:	Strelkov - Clubroot inoculum management for sustainable canola production
Project start date:	
Project completion date:	
This is an interim report for the reporting period to	

Research Team

Principal Investigator:	
Name:	**Institution:**
Stephen Strelkov	University of Alberta
Research team members:	

Non-technical summary

Provide a summary of the project results which could be used by the funder(s) for communication to industry stakeholders (e.g., producers, processors, retailers, extension personnel, etc.) and/or the general public. This summary should give a brief background as to why the project was carried out, what were the principal outcomes and key messages, how these outcomes and key messages will advance the livestock and meat industry, how they will impact industry stakeholders and/or consumers, and the economic benefits for the industry.

The long-term objective of this project was to develop techniques to suppress clubroot inoculum in order to mitigate the impact of the disease. The work consisted of several related studies. To study the spatial patterns of clubroot inoculum density and their relationship to various soil properties, four clubroot-infested fields in central Alberta were sampled in over multiple years, and inoculum density, soil pH, and boron, calcium, and magnesium concentrations were quantified. While soil pH, boron and magnesium levels were found to have some effect on spore viability, within-field spread of clubroot inoculum was more strongly affected by the movement of soil by wind and through machinery used in farm operations. Clubroot occurred in a patchy distribution and the growth of the patches over time was related to initial inoculum density. This study highlighted the importance of aggressive treatment of clubroot at field entrances before it becomes well established. In another experiment, the effect of cultivar rotation on pathotype composition was evaluated. The continuous cultivation of a susceptible canola cultivar caused an increase in the predominance of pathotype 3H, while cultivation of a resistant cultivar or alternating between different resistant cultivars caused an increase in the predominance of the resistance-breaking pathotype 5X. When resistant canola cultivars were alternated, pathotype 3H was eliminated in favor of pathotype 5X. This study showed that resistance-breaking clubroot pathotypes are selected for in response to host resistance. The fungicide amisulbrom was assessed as a possible tool to supplement resistance or manage resistance-breaking pathotypes. The in-furrow or broadcast application of amisulbrom generally resulted in a decline in clubroot severity and gall weight with increasing application rates. Much lower levels of disease developed in a resistant cultivar, but the same general trend was observed. This study showed the potential of amisulbrom to reduce the impact of clubroot on canola. The efficacy of lime as a soil amendment was also evaluated as a clubroot management tool. Amendment with limestone reduced clubroot severity mainly where pathogen spore concentrations were low. In contrast, amendment with hydrated lime reduced clubroot across inoculum concentrations, but results varied with environmental conditions (rainfall) and timing of application. This study showed that hydrated lime was more effective than limestone, and that it can be an effective tool for managing clubroot when optimal timing and rainfall are achieved. Finally, genetic resistance, hydrated lime and weed control were compared in various combinations for clubroot management. While the application of hydrated lime and weed management both reduced clubroot spore populations in the soil, and lime reduced clubroot severity on susceptible hosts, the lowest levels of disease and highest yields were obtained with the resistant hosts. This study showed that while clubroot resistance remains the most effective way of reducing the impact of clubroot on canola, the application of hydrated lime may be a useful strategy to manage clubroot inoculum when used in combination with resistance.

Project details

Project team

a. Describe the contribution of each member of the R&D team to the functioning of the project.
b. Describe any changes to the team which occurred over the course of the project.

The research team was led by Dr. Stephen Strelkov, a professor and plant pathologist at the University of Alberta who has monitored and characterized clubroot in Alberta since it was first detected. Dr. Strelkov's expertise was complemented by the involvement of Dr. S.F. Hwang in the work. Dr. Hwang is a world-recognized expert in clubroot disease and its management, and contributed to the planning and running of the field experiments. Three graduate students were also involved in various components of the research as part of their Master's or Ph.D. programs, including Andrea Botero-Ramirez, Brittany Hennig and Keisha Hollman. A research associate (Dr. T. Cao) contributed to the experiment on the effect of cultivar rotation on pathotype composition, while technical staff, a postdoctoral fellow and summer students assisted with the field plot and greenhouse experiments, as well as with sample processing, throughout the project.

Abbreviations

Define ALL abbreviations used.

CCD = Canadian Clubroot Differential
CDC-N = Crop Diversification Centre - North
CR = Clubroot-resistant
Ct = Threshold cycle
ID = index of disease
qPCR = quantitative polymerase chain reaciton

Background

a. Review the project background and update as needed.
b. State the related scientific and development work that has been completed to date by your team and/or others.

Clubroot (Plasmodiophora brassicae) is an important disease of canola that causes the formation of large root galls on susceptible hosts, leading to yield and quality losses. Clubroot of canola was first identified in Alberta in 2003 and was confirmed in over 3300 fields by 2021 (Strelkov et al., In Press). Spore populations increase rapidly under short canola rotations. Since this crop gives the highest rate of return among available options, it is grown at every opportunity.

The first clubroot-resistant (CR) canola cultivars for the Canadian market possessed strong resistance to the pathotypes of P. brassicae known in Canada at that time (Strelkov et al. 2016, 2018). They allowed continued production of high-yielding crops in infested fields and became the most important clubroot management tool. However, resistance breakdown was confirmed in commercial fields in 2013 (Strelkov et al. 2016) and increased quickly with repeated cultivation of CR cultivars (Strelkov et al. 2018). The entire zone infested by clubroot can be considered at risk of resistance breakdown. Unfortunately, other disease control measures are often too impractical or expensive for use in canola crops, and are complicated by the longevity of the resting spores (Wallenhammar 1996). Susceptible crops and cruciferous weeds can potential multiply spore loads, and their control may help to reduce inoculum, or at least prevent further increases, in clubroot-infested fields.

Fungicide treatments have rarely been effective enough to justify their commercial use, often giving mixed results under field conditions. However, a new product from Japan (amisulbrom) has shown potential to control similar soilborne pathogens. Lime application has been used to increase soil pH, making conditions less favorable for clubroot development. The formulation of the lime products, however, has a major impact on their efficacy in field soils (Hwang et al., 2011). Soil liming by the application of calcium-rich compost has been reported to be effective in reducing clubroot disease severity in cruciferous crops (Myers and Campbell, 1985). Suppression of clubroot infection in Chinese cabbage at neutral pH was suggested to be related to the inhibition of resting spore germination (Gossen et al. 2013). Several studies suggested that high concentrations of calcium and magnesium may provide adequate control of clubroot even at pH <7.2 (Myers and Campbell, 1985; Campbell et al., 1985).

Limited work has been done to study the effects of raising the soil pH on other important micronutrients necessary for optimal growth and reproduction of canola, or on the potential residual effects of lime treatment on the following year's crop. There are various forms of lime. Some are relatively slow-acting, while hydrated lime and quicklime rapidly raise soil pH. Finely ground forms are more fast-acting than coarser forms. However, lime application has not been well studied under western Canadian conditions, since most soils are considered to have adequate calcium content for agricultural activities. This project was designed to develop methods of reducing spore buildup in Alberta soils, and thereby improve the managemetn of clubroot of canola.

Objectives

a. Review the original objective(s).
b. Indicate any modifications to the objective(s) that occurred over the course of the project.

Long-term objective: To develop techniques to reduce and suppress clubroot inoculum in fields in order to mitigate the impact of clubroot infection

Short term objectives: The objectives of this project stemmed from questions posed by the Clubroot Steering Committee, a diverse advisory group of stakeholders in the canola industry, and included the following:
A) Investigate the spatial patterns of P. brassicae spore density and their temporal variation in clubroot-infested fields and evaluate the effects of soil pH, boron, calcium and magnesium concentration on inoculum density
B) Assess whether rotation of CR cultivars in soils with low clubroot populations prevents establishment of clubroot or affects the composition of the clubroot spore populations
C) Evaluate the effect of weeds and best management practices such as liming on resting spore populations
D) Determine the efficacy of amisulbrom as a promising fungicide against clubroot
E) Measure the efficacy of liming and residual liming as a curative and preventative measure, and determine the most effective formulation of lime against clubroot

Research design and methodology

In summary, describe the project design, methodology, laboratory and statistical analysis used to carry out the project. Please provide sufficient detail to determine the experimental and statistical validity of the work and give reference to relevant literature where appropriate. For ease of evaluation, please structure this section according to the objectives cited above.

A. Spatial and temporal patterns of P. brassicae spore density in clubroot-infested fields and the effects of soil pH, boron, calcium and magnesium concentration on inoculum density:

Soil was sampled four clubroot-infested fields located in Sturgeon and Westlock counties in central Alberta over two years (with gap year in between). In the first sampling year, each field was sampled extensively in a regular grid pattern with approximately 500 g of soil collected at each node of the grids. All sampling locations were georeferenced and the geo-coordinates were recorded. In the second sampling year, sampling was intensified around the field entrances and points that had tested positive for P. brassicae in the first year. Three subsamples were taken from each homogenized soil sample,

including 0.25 g for DNA extraction, 10 g for pH measurement and 200 g for nutrient quantification.

Soil pH was measured using a commercial pH meter. Quantification of soil nutrients was conducted by Exova Canada Inc. Calcium and magnesium were extracted by the ammonium acetate method, while boron was extracted via the hot water method. The nutrients were quantified by inductively coupled plasma optical emission spectrometry.

Genomic DNA was extracted from 0.25 g of each soil sample. Subsequently, the DNA was diluted to a concentration of 2 ng/µL for conventional PCR or diluted 10-fold for quantitative PCR (qPCR) analysis. Conventional PCR was conducted with the primers TC1F and TC1R. Positive controls included 10 ng of P. brassicae DNA as a template, while 5 µL of nuclease-free water was substituted in place of the template in the negative controls. Amplicons were resolved on 2% agarose gels stained with 1X SYBR Safe. All samples that tested positive for the presence of P. brassicae DNA, along with all adjacent samples from the field (regardless of conventional PCR result), were evaluated further by qPCR analysis to quantify the P. brassicae inoculum level in the soil samples in a StepOnePlus Real Time PCR System. Estimation of the number of resting spores per sample was completed by comparison with a standard curve generated with DNA extracted from known quantities of resting spores. After each qPCR run, a melting point analysis to identify the amplified product was conducted.

Information regarding the prevailing wind direction was obtained from the Alberta Climate Information Service. The data were collected from weather stations surrounding the sampled fields. Spatial autocorrelation in the P. brassicae inoculum density was evaluated. Experimental semi-variograms for each field in both sampling years were generated. The presence or absence of anisotropic patterns was determined by examination of the semi-variograms at N, NNE, NE, ENE, E, ESE, SE and SSE. Afterwards, a spherical model was fitted to each sample semi-variogram.

A Bayesian hierarchical spatial approach was used to model the relationship between P. brassicae inoculum density and soil pH, and concentration of boron, calcium, and magnesium in the soil to identify the possible effect of each covariate on the presence/absence of the pathogen and, when it was present, on the number of P. brassicae resting spores. Prediction surfaces of the mean of the posterior distribution of P. brassicae inoculum density were mapped to test the effect of maximum inoculum density and rotation scheme on clubroot patch diameter.

B. Effect of canola cultivar rotation on Plasmodiophora brassicae pathotype composition:

Two P. brassicae field isolates representing pathotypes 3 and 5X were inoculated into 6 kg of sieved black chernozemic soil with no history of clubroot and placed in each of nine polyethylene tubs. The tubs were placed on a bench in the greenhouse and allowed to air dry. The average infestation level was 2 ×

10^7 resting spores/g soil. Clumps were homogenized, passed through a 2-mm sieve and mixed evenly with two volumes of Sungro Professional Growing Mix.

The CR canola cultivars '45H29', '6056CR', '1960', '9558C', the clubroot susceptible cultivar ''45H26', and Chinese cabbage 'Granaat' were planted into four rows in each of the tubs, which were placed in large polyethylene trays and bottom-watered until full saturation of the soil was achieved. Three weeks after seeding, the seedlings were thinned to 60 - 70 plants/tub. The cultivar rotation treatments included: T1, continuous cropping of the same susceptible canola cultivar ('45H26'); T2, continuous cropping of the same resistant canola cultivar ('45H29'); and T3, alternating resistant canola cultivars in the rotation. Each of the three rotation treatments was replicated three times and one polyethylene tub was regarded as one experimental unit. The plants were uprooted gently with a spatula 6-7 weeks after seeding, and the roots were washed in water. Clubroot severity on the roots of each plant was rated on a 0 to 3 scale.

About 120 g of soil was collected prior to the start and at the end of each rotation cycle. Genomic DNA was extracted from 250 mg of the soil. The quantitative polymerase chain reaction (qPCR) primers P5XF3, P5XR3 and minor groove binding (MGB) probe P5XP3 were used to measure the amount of pathotype 5X DNA in each soil sample in a qPCR assay (Zhou et al. 2018). The correlation equation generated from the known amount of DNA and threshold values (Ct) was used to calculate the amount of pathotype 5X DNA present in each unknown sample on the same plate. The DNA calculated according to each standard curve was normalized on a per gram soil basis, which was subsequently used for mean comparisons of the treatments.

Resting spores of P. brassicae were recovered from the soil mix following the final cycle of canola by growing the universally susceptible Chinese cabbage 'Granaat' as a bait crop. The roots of the bait plants were collected after 6-weeks, and the recovered isolates were pathotyped on the differentials described above. The CR oilseed rape 'Mendel' also was included in the differentials, to identify isolates that could overcome resistance. Differential hosts were considered resistant if the mean ID of the four replicates was < 50% and the 95% confidence interval did not overlap 50%. A total of 90 single root galls, representing 10 single root galls from each of the nine combinations of rotation treatment and replication were pathotyped.

C. Effects of hydrated lime, weed management and the deployment of CR genetics on clubroot severity, incidence, and yield:

Replicated field trials were conducted in 2018 and 2019 to study the effect of various combinations of clubroot management strategies (resistance, soil liming and weed control) on disease severity, yield, and P. brassicae spore density. The pre-treatment pH of the soil in 2018 was 5.3, while in 2019, the soil pH values were 5.23 and 5.48, at the two sites. Hydrated lime was applied to the soil to adjust the pH to a target of 7.2, to a depth of 10 cm. Due to a lack of moisture in 2019 at the time of seeding, 30 L of water

(3.3 mm rain equivalent) was applied per plot immediately following the lime application.

In 2018, a CR canola cultivar '45H29' and a CS cultivar '45H31' were seeded. The development of clubroot galls was observed on the resistant variety during the growing season. Pathotyping of some of these galls indicated a virulence shift in this nursery from pathotype 3H to 3D, as defined on the Canadian Clubroot Differential set, the latter of which can overcome the resistance in '45H29'. Therefore, in 2019, '45H29' was replaced with '45CM39', a canola cultivar with 'second generation' resistance effective against pathotypes 2B, 3A, 3D and 5X.

Plots that required weed control were hand-weeded every two weeks after the canola emerged until the end of July. Hand-weeding ensured that no galls developed on any susceptible weeds, which could have affected the P. brassicae spore densities. Both susceptible and non-susceptible weeds were counted in non-weeded plots, but the proportion of susceptible weeds was strongly correlated with overall weed density, and model results using overall weed density or proportion of susceptible weeds as continuous variables were not substantially different from models with weed presence/absence, so we excluded this from further analysis. No herbicides were applied in either year at any site.

Shoot height, shoot weight, and clubroot symptoms were evaluated on 10 plants per plot. Each root was rated on a 0 to 3 scale, and the data were converted to an index of disease. The harvested seeds were cleaned and dried before they were weighed to obtain yield estimates. In the spring of 2020, 10 soil samples per plot were collected from the 2019 plot areas and subjected to quantitative PCR analysis of soil inoculum density.

D. Efficacy of amisulbrom as a potential fungicide against clubroot:

Amisulbrom (20% soluble concentrate) was provided by Gowan Canada. Replicated field trials were conducted in 2018 and 2019 to evaluate the efficacy of amisulbrom at 0, 500, 1000, and 1500 g ai/ha on clubroot disease severity, plant growth and yield of canola cultivars '45H31' (clubroot susceptible) and 'CS 2000' (moderately resistant). Ten plants per plot were collected 8 weeks after seeding in each year of the study for clubroot disease assessment. Plant height for all sampled plants was also measured along with the aboveground biomass, root weight, and gall weight. The plots were harvested and seeds were dried, cleaned and weighed to determine yield. Greenhouse trials were conducted using the same rates of amisulbrom as in the field.

E) Efficacy of liming, residual liming and lime formulation to prevent and cure clubroot:

Field trials were conducted to study the effects of multiple rates of hydrated lime on clubroot disease severity, plant growth and yield. The trials were located at the Crop Diversification Centre-North (CDC-N),

Edmonton, in a clubroot nursery that is naturally infested with P. brassicae. In each experimental year, the trial was replicated within the nursery. Eight weeks after seeding and at harvest, 10 plants were pulled from each plot and assessed for clubroot symptom severity. Individual plant height, aboveground biomass and root weight were recorded for 10 plants per plot. The seeds were dried, cleaned and weighed to determine yield. Field trials were conducted at these sites to assess the residual effects of the hydrated lime on clubroot development in the following season. The clubroot susceptible canola cultivar '45H31' was planted in plots that had received low, moderate or high rates of lime the previous year, and was assessed for clubroot severity (index of disease, ID) eight weeks later.

The effects of multiple rates of hydrated lime and limestone on clubroot development were compared at various inoculum levels on two canola genotypes in greenhouse trials. A clubroot-susceptible cultivar '45H31' and a clubroot-resistant cultivar '9558C', both treated with Prosper FX, were grown in a potting medium at an initial soil pH of 5.3. The potting medium was inoculated with P. brassicae resting spores (pathotype 3H) at 1 × 10^3, 1 × 10^4, and 1 × 10^5 resting spores/g potting medium. Following soil inoculation, the potting medium was treated with 'Zero Grind' limestone (Graymont) or hydrated lime (Graymont) and mixed thoroughly by hand again, at rates equivalent to 4.7, 8.1, 11.4 or 14.8 t/ha of lime, to adjust the pH to 6.0, 6.5, 7.0 or 7.5, respectively. There were two control treatments for each inoculum concentration where the medium was inoculated but did not receive a lime treatment.

The canola genotypes were sown 2-cm-deep at a density of 16 seeds per pot filled with 2.14 L of potting medium; treatments were thinned to five plants per pot 10 days after planting. The pots were maintained in a greenhouse until harvest. Each treatment was replicated five times. The experiment was repeated. At harvest, the plants were dug out from the potting medium, and the roots were washed and scored for clubroot symptom severity. Plant height was recorded for each plant and averaged per replicate. Additionally, dry-weights were recorded after the harvested plants were dried in the greenhouse for 1 week.

Soil samples were collected two weeks before lime application in the field trials to determine the starting level of P. brassicae inoculum. Total genomic DNA was extracted from the soil samples following Cao et al. (2007). In the greenhouse trials, root samples were collected to quantify colonization by P. brassicae. Total genomic DNA was extracted from these root samples. All samples were subjected to conventional PCR analysis to determine the presence or absence of P. brassicae DNA using primers TC1F and TC1R. Those samples which tested positive for the presence of P. brassicae DNA were analyzed further by q-PCR, with resting spore concentrations estimated on a five-point standard curve generated with standards of 1 × 10^2, 1 × 10^3, 1 × 10^4, 1 × 10^5 and 1 × 10^6 resting spores/g soil or g root tissue. Pathogen levels in the field soil samples are reported as resting spores/g soil, while in the root tissue samples they are reported as resting spores/g root tissue.

Results, discussion and conclusions

Present the project results and discuss their implications. Discuss any variance between expected targets and those achieved. Highlight the innovative, unique nature of the new knowledge generated. Describe implications of this knowledge for the advancement of agricultural science. For ease of evaluation, please structure this section according to the objectives cited above.

A. Spatial and temporal patterns of P. brassicae spore density in clubroot-infested fields and the effects of soil pH, boron, calcium and magnesium concentration on inoculum density:

Between 53% and 99% of the soil samples tested negative for the presence of P. brassicae. The maximum inoculum density observed differed among fields, ranging from 1.7 × 10^3 to 3.2 × 10^7 resting spores/g soil. Variation in maximum inoculum density was within the same order of magnitude in Fields 1 and 2, but increased by two orders of magnitude in Fields 3 and 4 between the two sampling years. All fields had acidic soil, with mean pH values between 5.03 and 6.23. Within field variation was observed in all fields. Calcium, boron, and magnesium varied among the fields. Mean calcium concentration ranged between 3853 and 4648 mg/kg, mean boron concentration ranged between 1.52 and 2.34 mg/kg and mean magnesium concentration ranged between 319.7 and 756.8 mg/kg. Within field variation was also observed.

Soil samples that tested positive for the presence of P. brassicae were located mostly within 10 m of the field edges and/or adjacent to the entrance. The low number of positive samples impeded the adjustment of any model to evaluate the relationship between the density of P. brassicae inoculum and soil properties. Initial patch diameters ranged between 40 m and 346.1 m and increased to a range of 77.7 m to 634.9 m, with average patch growth of 221.3 m. There was a positive correlation between maximum inoculum density and the patch diameter ($p = 0.015$), but no significant effect from the number of years when canola was grown. The patchiness of the P. brassicae inoculum was confirmed with the semi-variograms, and a higher spatial autocorrelation was observed in the second vs. first sampling year, caused mainly by an increase in the number of positive samples and larger patches. Patch growth indicates within-field pathogen dispersal. The direction of spread suggests that wind dispersal over short distances could expand resting P. brassicae infestations within an infested field or between immediately adjacent fields. Farming operations conducted with large equipment such as tractors and seeders could also contribute to within-field spread.

The mean calcium, boron and magnesium levels in the sampled fields were generally consistent with soils from the Canadian prairies, but soil pH, boron, calcium, and magnesium concentrations were not found to have an important effect on the inoculum density of P. brassicae. These results do not necessarily indicate that none of the soil chemical properties affect pathogen inoculum density, but

rather suggest that other underlying spatial processes have a greater influence on spatial patterns. Only weak negative correlations were found between soil pH and clubroot severity on canola in surveys of P. brassicae-infested fields in Alberta (Cao et al. 2007; Gossen et al. 2013).

B. Effect of canola (Brassica napus) cultivar rotation on Plasmodiophora brassicae pathotype composition:

At the end of the first cycle of each rotation, IDs of 91.5%, 78.0%, and 69.1% were observed in T1, T2 and T3 (continuous susceptible, continuous resistant and alternating resistant cultivars), respectively. All three IDs were significantly different from each other. At the end of cycle 2, the IDs were 93.4%, 92.2% and 91.3% in T1, T2, and T3, respectively. At the end of cycle 3, the IDs were 97.6%, 97.9% and 98.2% in T1, T2, and T3, respectively. The IDs at the end of cycles 2 and 3 showed no significant differences between treatments. At the end of cycle 4, IDs of 99.7% and 98.4% were observed in T1 and T2, respectively, values that were significantly greater than the ID of 14.1% observed in T3. Following each of the rotations, IDs of 99.8%, 99.1% and 98.7% developed in T1, T2 and T3 in the Chinese cabbage 'Granaat'. None of these were significantly different from the other treatments.

The amount of pathotype 5X DNA quantified by qPCR analysis was regarded as a proxy for the size of the 5X population in the soil mix. In T1, this amount (on a per gram air-dried soil basis, mean ± 1 SE) fluctuated from 740 ng ± 180 ng at the beginning of cycle 1 to 650 ng ± 140 ng by the end of cycle 4, with no statistically significant differences at any time. In T2, the amount of pathotype 5X DNA also was fairly constant from the beginning of cycle 1 (590 ng ± 97 ng) to the end of cycle 3 (610 ng ± 89 ng), but increased significantly to 2000 ng ± 310 ng by the end of cycle 4 (Fig. 1). In the case of T3, the amount of pathotype 5X DNA ranged from 580 ng ± 140 ng to 840 ng ± 280 ng over most of the cycles, but peaked at 1100 ng ± 110 ng at the end of cycle 3. Across treatments, 5X DNA levels were highest for T3 at the end of cycle 3 and for T2 at the end of cycle 4. Pathotype 5X was recovered at a high frequency (66.7%) from the root galls of bait plants grown at the end of the rotations in T2 and T3. In contrast, the percentage of galls classified as 5X was significantly lower (6.7%) in T1. Conversely, while pathotype 3 was most commonly recovered (63.3%) in T1, it was rare in galls recovered from T2 and not found at all in galls from T3. In addition to the two pathotypes originally (5X and 3) used to inoculate the soil mix, several other pathotypes were recovered at lower frequencies in the three treatments.

These results show how rapidly, after just a few cycles, one pathotype can become predominant over another. In the absence of selection pressure, pathotype 3 was recovered most commonly at the end of the rotation, while when one or more CR canola cultivars were rotated, pathotype 5X became predominant, while pathotype 3 was rare or absent. In addition to the two 'initial' pathotypes 3 and 5X inoculated at the start of each rotation, various other pathotypes (5, 6 and 8) could be recovered at low frequencies at the end of the experiment. The identification of additional pathotypes is interesting and

may reflect the fact that field isolates (which can consist of pathotype mixtures) were used as the starting inoculum. Moreover, the possibility of additional diversity resulting from reproduction by the pathogen over the course of the experiment cannot be ruled out, since karyogamy and meiosis occur in the P. brassicae life-cycle.

C. Compare the effectiveness of the application of hydrated lime, weed management and the deployment of CR genetics on clubroot severity, incidence, and yield:

The effect of canola cultivar and hydrated lime on P. brassicae resting spore density was significant ($p < 0.0001$ and $p = 0.03$, respectively), and the three-way interaction between the cultivar, hydrated lime, and weed management was close to significance ($p = 0.0825$). Spore densities in the treatments that included the CR cultivar '45CM39' and the application of hydrated lime or weed management were all lower than the treatments that included the susceptible cultivar '45H31' without the application of hydrated lime. Hydrated lime plots had 48–80% lower resting spore densities, relative to untreated controls, in plots where the CS cultivar was grown.

The effect of canola cultivar and hydrated lime on clubroot disease severity was significant ($p < 0.0001$). Within the treatments that included the CR cultivar '45CM39', there was no difference in clubroot severity between treatments using no hydrated lime with and without weed management. Within the treatments using the susceptible cultivar '45H31', clubroot severity in plots with hydrated lime decreased by 34–36% relative to those without hydrated lime.

The effect of canola cultivar, the application of hydrated lime, and management of weeds, as well as the interaction between cultivar and the application of hydrated lime on yield, were all significant ($p = 0.0002$), and the interaction between cultivar and weed management was close to significance ($p = 0.07$). The treatments that included the CR cultivar '45CM39' with weed management showed the largest difference to the treatments that included the susceptible cultivar '45H31' without the application of hydrated lime. Yields in the treatments using the CR cultivar '45CM39' with unmanaged weeds were not significantly different from treatments that included the susceptible cultivar '45H31' with the application of hydrated lime. Hydrated lime increased seed yield by 70–98% in the CS canola cultivar.

Canola cultivar, the application of hydrated lime, and the management of weeds all had significant effects on shoot weight (all $p < 0.0001$). Treatments using a CR canola cultivar with the application of hydrated lime and/or managed weeds were similar, and had significantly greater shoot weights relative to all other treatments. In contrast, when the CR cultivar was grown in the absence of hydrated lime or weed management, shoot weight was not significantly different from treatments with a susceptible canola cultivar when either no hydrated lime was applied or the weeds were not managed.

The effects of canola cultivar, application of hydrated lime, and the interaction between canola cultivar

and lime on shoot height were significant ($p < 0.0001$, 0.013, and 0.0006, respectively), and the interaction between lime and weed management also approached significance ($p = 0.06$). There was no significant difference between treatments when using a CR cultivar, although they were mostly greater than treatments with a susceptible cultivar and no application of hydrated lime. Finally, the treatments that included the CR cultivar with no application of hydrated lime and managed weeds had significantly taller plants than all treatments with a susceptible canola cultivar.

Cropping of the CS cultivar resulted, on average, in 2.1 × 10^7 more resting spores per gram of soil relative to a CR cultivar in the spring following cultivation. Nonetheless, the spores that are produced on CR canola, while less in number, may be enriched for pathotypes able to overcome the resistance, and may contribute to virulence shifts. Lime could be a particularly useful tool to manage clubroot in patches where resistance-breaking pathotypes are predominant and resistance is no longer effective. It could also serve as a tool to supplement genetic resistance before it is lost or eroded. This suggests that combining genetic resistance with lime could slow increases in P. brassicae inoculum in the soil, and hence extend the durability of resistant varieties.

D. Evaluation of the efficacy of varying rates of amisulbrom in reducing clubroot severity:

In the first year of the field experiment, the effect of canola cultivar on clubroot disease severity was statistically significant ($p = 0.0001$), with the moderately resistant genotype ‘CS2000’ developing very little disease in any of the treatments including in the untreated control (ID = 2.5%), and the susceptible cultivar ‘45H31’ showing moderate levels of disease. Given the near absence of clubroot symptoms on ‘CS2000’, this cultivar was not included in the greenhouse trials. In the case of ‘45H31’, the application of amisulbrom resulted in numerical decreases in ID, from 30.0% in the untreated control to 15.0%, 13.3% and 10.8%, respectively, at the low (500 g ai/ha), mid (1000 g ai/ha) and high (1500 g ai/ha) rates, but these declines were not statistically significant ($P = 0.1909$).

In the second year, due to very high rainfall after seeding and throughout the season, part of the trial was submerged in water for multiple days. This flooding appeared to diminish clubroot development in the susceptible cultivar ‘45H31’, with IDs ranging from 3.3% to 6.7% across rates of amisulbrom, including the untreated control. In the case of the moderately resistant cultivar ‘CS2000’, the ID on the control was 20.8%, which was not significantly different ($p = 0.4752$) from the ID observed at any of the application rates. The effect of cultivar on ID was significant ($p = 0.0029$), although it was the opposite of what was expected based on resistance ratings.

In the first year of the study, there was no significant difference among the amisulbrom treatments for shoot weight, root weight, gall weight, plant height, or yield in either cultivar. There was, however, a statistically significant interaction between ID and root weight ($p = 0.0002$) and ID and gall weight ($P =$

0.0001) for both cultivars. Although not significant at $p < 0.05$, there was a numerical trend of decreasing root gall weight with increasing rate of amisulbrom (from 37.3 g in the control to 5.5 g at the highest rate) in the susceptible cultivar. Similarly, in the second year, there were no statistically significant differences for shoot weight, root weight, gall weight, plant height or yield as a result of the amisulbrom treatments. There was, however, a statistically significant interaction between ID and gall weight ($p = 0.0001$) as well as ID and yield ($p = 0.0369$) for both cultivars.

E) Efficacy of liming, residual liming and lime formulation to prevent and cure clubroot:

In the first year of the field experiment, the high and moderate rates of lime reduced clubroot severity compared with the low rate and the control treatment at site 1. The average ID for the control treatment (pH 6.3) at site 1 was 47%, and the ID for the lowest rate of lime was 37.5%. The moderate and high rates of lime (8.0 and 11.4 t/ha) reduced ID to 6.7%, and 4%, respectively. The ID and lime rate were positively correlated ($R2 = 0.46$, $p = 0.0023$). As determined by q-PCR, the average P. brassicae resting spore concentration in the soil at site 1 was 3.3×10^3 spores/g soil (range of 1.65×10^3 to 5.05×10^3 spores/g soil). In contrast, in the second year of the study, there was no treatment effect observed at either site. Site 1 developed no visible symptoms of clubroot in three of the replicates and only very mild symptoms in the fourth replicate. The ID in the control treatment was 3% and IDs varied from 1% to 4% across the various lime treatments. Clubroot disease pressure was higher at site 2. The average ID in the control treatment was 61%, which was not significantly different from the 67-68% observed across the various lime treatments. The average P. brassicae resting spore concentration was estimated via q-PCR analysis and was 1.22×10^4 spores/g soil at site 1 and 8.55×10^5 spores/g soil at site 2.

In the first greenhouse trial, clubroot severity was significantly affected by the canola cultivar grown ($p < 2.2 \times 10^{-16}$), lime product applied ($p < 2.2 \times 10^{-16}$), inoculum concentration ($p= 1.56 \times 10^{-5}$), and their interactions (cultivar*inoculum ($p = 0.000275$), cultivar*lime product ($p = 5.06 \times 10^{-5}$), lime*inoculum ($p < 2.2 \times 10^{-16}$), cultivar*lime*inoculum ($p = 6.69 \times 10^{-8}$)). The susceptible cultivar ‘45H31’ developed an ID of 92% to 100% across all resting spore concentrations in the absence of lime amendments. The application of hydrated lime at any of the rates evaluated resulted in a decrease in ID to 0% across all spore concentrations, with the exception of the rate 8.1 t/ha at 1×10^6 spores/g medium, which developed an ID of 18%. On the resistant cultivar ‘9558C’, ID ranged from 9% to 13% in the absence of lime amendments; this declined to 0% across all resting spore concentrations when hydrated lime was applied at any of the rates examined. The reduction in ID was less pronounced with the application of limestone. On the susceptible canola ‘45H31’, significant reductions in ID were observed only at the lower resting spore concentrations (1×10^3 and 1×10^4 spores/g medium), and limestone treatment at any rate had no effect on ID at spore concentrations of 1×10^5 and 1×10^6 spores/g medium. Similar trends were observed with the canola cultivar ‘9558C’, except that ID values generally were lower. As was observed with ‘45H31’, the application of limestone at any rate did not

appear to have a consistent effect on ID on '9558C', relative to the control treatments, at 1 × 10^5 and 1 × 10^6 spores/g medium. When the greenhouse trial was repeated, the IDs in the control treatments were generally lower at the lower inoculum concentrations, but similar at the higher inoculum concentrations. Overall, the trends were consistent in both runs of the trial, and ID was significantly affected by cultivar ($p < 2.2 \times 10^{-16}$), lime product ($p < 2.2 \times 10^{-16}$), inoculum concentration ($p < 2.2 \times 10^{-16}$), and the interactions of cultivar*inoculum ($p = 0.00069$).

Collectively, the results of these trials indicated that lime holds potential as a tool for the management of clubroot on canola when optimal timing and rainfall are achieved. Hydrated lime was more effective than limestone and may be the preferred form to apply.

Tables, graphs, manuscripts, etc., may be included as appendices to this report.

Literature cited

Provide complete reference information for all literature cited throughout the report.

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magnesium, and their interactions. Phytopathol. 75, 670–673.
Peng, G., et al. 2015. A >2-year crop rotation reduces resting spores of Plasmodiophora brassicae in soil and the impact of clubroot on canola. Eur. J. Agron. 70: 78-84.
Rashid A., H. et al. 2013. Effects of root exudates variable pH and on resting spore germination and root-hair infection of canola by Plasmodiophora brassicae. Crop Prot. 48: 16-23.
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Strelkov, S.E., et al. 2018. Virulence and pathotype classification of Plasmodiophora brassicae populations collected from clubroot resistant canola Brassica napus in Canada. Can. J. Plant Pathol. 40: 284-298.
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Wallenhammar, A. C., et al. 2012. In-field distribution of Plasmodiophora brassicae measured using quantitative real-time PCR. Plant Pathol. 61:16-28.
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Zhou, Q., et al. 2018. A molecular marker for specific detection of new pathotype 5-like strains of Plasmodiophora brassicae in canola. Plant Pathol. 1582-1588.

Benefits to the industry

a. Describe the impact of the project results on Alberta's agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).
b. Quantify the potential economic impact of the project results (e.g., cost-benefit analysis, potential size of market, improvement in efficiency, etc.).

a. Canola is a success story in Canadian agricultural innovation and has grown into an industry that contributes over $25 billion annually to the Canadian economy. To sustain this industry, potential threats to production such as clubroot must be addressed before they become unmanageable. From just 12 confirmed field infestations found near Edmonton in 2003, clubroot had spread to more than 3300 fields in Alberta by 2021. Once present, clubroot can contaminate the soil for many years in the form of resting spores, severely restricting the ability to grow canola and thereby reducing the value of the land on which the infestation occurs.

Clubroot disease is one of the most serious threats to the sustainability of this industry, because it persists in the soil, spreads readily on farm equipment and results in devastating crop losses. The most effective tool for management of clubroot and prevention of its further dispersal, while continuing canola cultivation, has been the cultivation of clubroot-resistant canola cultivars. However, resistance breakdown has the potential to eliminate the advantage of planting clubroot-resistant (CR) canola in affected regions, allowing the unmitigated spread of clubroot throughout the province and rendering large tracts of land unsuitable for canola cultivation. Severe clubroot has been found in over 350 fields planted to CR canola varieties. This indicates that the utility of genetic resistance as a clubroot management tool is at risk. It is becoming clear that the deployment of resistant canola varieties must be combined with other clubroot management strategies. Resistance breakdown also jeopardizes years of research and development that the seed industry has invested in breeding for this trait in canola.

b. Approximately one-quarter of the traditional canola growing area in Alberta is at risk for clubroot (Hartman, pers. comm.). Assuming 25% yield losses, then approximately 12% of the provincial canola cash receipts could be lost, equivalent to about $44 million per year in Alberta alone. The primary beneficiary of the proposed work will be the canola production industry. This project was designed to investigate methods of inoculum mitigation for sustainable canola production. The results obtained will help guide the integrated management clubroot as per the objectives outlined above. Prevention of even a 1% decline in yields for canola producers (a conservative estimate) will outweigh the cost of this project by a ratio of more than 150 to 1, and will also help to maintain Canada’s export reputation.

Performance Measures

Collaboration and partnerships

# of Industry partners: 1

# of Public partners: 1

# of international partners:	0

Training of Highly Qualified Personnel (HQP)

# of Undergraduate students trained:	0
# of graduate students trained:	4
# of postdoctoral fellows trained:	1
# of research associates trained:	1
# of technicians trained:	2
Others (Such as Visiting scientist):	0

Technology Transfer & Commercialization

# of Peer reviewed scientific publications:	4
# of scientific presentations, posters and abstracts:	6
# of industry communications:	5
# of patents and licences:	0

# of new innovations / products / practices: 1

Project resources

Statement of revenues and expenditures

In a separate document certified by the organisation's accountant or other senior executive officer, provide a detailed listing of all cash revenues to the project and expenditures of project cash funds. Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage (CDL); and overhead (if applicable).

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Total Files: 1

Provide a justification of project expenditures and discuss any major variance (i.e., ± 10%) from the budget approved by the funder(s).

Expenditures were consistent with the original budget and included personnel costs (Grad Student Salaries, Postdoctoral Fellow Salaries, Other Salaries incl. Research Assoc./Term Faculty Position as originally proposed) and materials and supplies. The only major variance was with respect to travel (originally budgeted at $1,000 but spent only $40.18). This variance reflected travel restrictions associated with the COVID-19 pandemic, with nearly all industry and scientific meetings either cancelled or moved to a virtual format.

Resources

Provide a list of all external cash and in-kind resources which were contributed to the project.

Sources	Amount	Percentage of total project cost
Funder(s)	$470,400	58.29%
Other government sources: Cash	$0	0.00%
Other government sources: In-kind	$210,000	26.02%
Industry Cash	$117,600	14.57%
Industry In-kind	$9,000	1.12%
Total project cost	$807,000	100.00%

External sources of funding for the entire project.

Clearly indicate any changes to confirmed sources of funding as well as any new sources of funding.

Government Sources

Name (no abbreviations unless stated in Section 3)	Amount cash	Amount in-kind
University of Alberta	$0.00	$210,000.00
	$0.00	$0.00
	$0.00	**$210,000.00**

Industry Sources

Name (no abbreviations unless stated in Section 3)	Amount cash	Amount in-kind
Alberta Canola	$117,600.00	$0.00
Sturgeon Valley Fertilizers	$0.00	$9,000.00
	$117,600.00	**$9,000.00**

Attachments

Attachments

Please attach any supplemental documents

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Total Files: 1

Does your agreement with RDAR include funds from the Canadian Agricultural Partnership (CAP) Program?

No