PROJECT DETAILS

- **Title**: Canadian Canola Clubroot Cluster Pillar 1: Integrated disease management
- **Funders:** Agriculture and Agri-Food Canada, Canola Council of Canada, Alberta Canola, SaskCanola and Manitoba Canola Growers
- **Research program:** Canadian Agricultural Partnership
- **Principal investigator:** Dr. Sheau-Fang Hwang & Dr. Stephen Strelkov
- **Collaborators/additional investigators:** Rudolph Fredua-Agyeman, Bruce Gossen and Mary Ruth McDonald
- **Year completed:** 2023

Final report

Introduction

Canola, one of the most important crops in the prairie region, contributes over $25 billion to the Canadian economy each year. By 2016, clubroot infestations were confirmed in 2443 canola fields in Alberta, up from just 12 fields when surveys started in 2003. Clubroot-resistant hybrids were released in response to the rapid spread of the disease and dramatically reduced the incidence and severity of clubroot. However, in 2013, clubroot reappeared in a field planted to a resistant hybrid variety and since then has been identified in 64 fields planted to these hybrids. This indicates that the utility of genetic resistance as a clubroot management tool is at risk. The entire zone infested by clubroot is susceptible to resistance breakdown and every commercially available clubroot-resistant genotype of canola is susceptible to the novel clubroot strains. Resistance breakdown also jeopardizes years of research and development that the seed industry has invested in breeding for clubroot resistance in canola.

It is becoming clear that the deployment of resistant canola varieties must be combined with other clubroot management strategies. Preliminary studies have shown that the soil environment plays a major role in clubroot infection and that soil amendments and treatments have some potential to mitigate clubroot. Preventative measures such as soil nutritional modification also hold some promise and will be explored. Furthermore, the effects of soil properties (such as structure, organic matter, and ionic content) on clubroot development will be investigated. The effectiveness of clubroot mitigation strategies can be more fully evaluated using models that relate crop yield losses to disease severity, since these provide a measure of the disease impact that is more relevant to farmers from a practical and economic perspective. Finally, changes in pathotype composition resulting from the rotation of resistant canola varieties in various combinations will also be assessed, in order to understand how resistance sources are impacting strains of the pathogen. The goal of this project is to develop management practices to reduce clubroot spore populations and prevent their buildup in at-risk areas.

Objectives:

These objectives focus on the development and validation of clubroot best management practices, where no single source of resistance is effective against all pathotypes of the clubroot pathogen.

1. Characterization of soils (soil properties) and pathotypes in clusters where resistance has been defeated

2. Field pre-treatment and amendment techniques, including reassessment of IPM techniques using qPCR; liming under varying spore concentrations; and treating field entrances with lime prior to clubroot introduction

3. Quantifying yield loss in relation to disease severity

4. Effect of cultivar rotation on clubroot pathotype structure

5. Disease nursery and resistance screening against novel clubroot pathotypes

Methodology

Objective 1. Characterization of soils (soil properties) and pathotypes in clusters where resistance breakdown has occurred (Hwang and Strelkov)

Soil processing and horizontal distribution of clubroot spores from patches of infestation with new strains – 600-g soil samples were collected in two fields (Westlock area and Sturgeon Valley) in Alberta, Canada. In each field, 100 samples were collected following a systematic sampling grid where georeferenced collection points were 80 m apart over a quarter-section of land. The samples were used to construct epidemiological variograms over each field (Fischer and Getis 2010; Oliver and Webster 2015).

Each sample was divided in two sub-samples. One, consisting of 100 g of soil, was used to quantify the P. brassicae inoculum density quantitative PCR analysis, while remaining 500 g was used to measure the soil pH, boron and calcium content. For PCR analysis, total DNA was extracted using the Powersoil DNA isolation kit (MoBio Laboratories Inc); the extracted DNA was quantified in a Nanodrop 200c spectrophotometer (Thermo Fisher Scientific Inc.), and the P. brassicae inoculum density was quantified using quantitative PCR as described Rennie et al. (2011) with the DR1R and DR1F primer set. Soil pH, boron and calcium concentrations were measured following the protocols described by Tabori (2015).

Correlation analysis was conducted to evaluate the correlation between the soil pH, boron and calcium content in soil, the management practices applied to each field and the inoculum density. Correlation among the measured variables and the inoculum density was assessed by the Spearman's correlation analysis (Hauke and Kossowski 2011). This analysis was done using the software SAS 9.4®

The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

Identification of major pathotypes present - Susceptible canola was seeded into each homogenized soil sample and grown until galls develop. The galls were used to make a spore suspension and tested against the Canadian Clubroot Differential (CCD) set (developed by Strelkov & Hwang) to determine the pathotype composition at each site.

Objective 2. Field pre-treatment and amendment techniques, including reassessment of Integrated clubroot management techniques; liming under varying spore concentrations; and treating field entrances with lime prior to clubroot introduction (Hwang, Strelkov, Gossen, and McDonald)

A. Evaluation of Lime Products as a Clubroot Management Tool in Canola (Hwang and Strelkov)

A1. Field study of various rates of hydrated lime - Field trials were conducted to study the effects of multiple rates of hydrated lime on clubroot disease severity, yield and various other plant growth parameters. The trials were located at the Crop Diversification Center North (CDC-N), Edmonton, Alberta, in a clubroot nursery (53 38' 48"N, 113 22' 33"W) that is naturally infested with P. brassicae. The soil in this nursery is a black Chernozemic loam (Soil Classification Working Group, 1998). In each experimental year, the trial was replicated within the nursery. Site 1 was located on the east side of the 6 ha nursery, and site 2 was located on the west side of the nursery, about 400 m from site 1. The plots in the second year of the study were placed adjacent to the previous year's plots, to avoid any possible residual effects of the lime treatment. Treatments were arranged in a randomized complete block design with four replicates. Each plot was 1.5 m × 6 m with a 0.5 m buffer between plots and a 2 m buffer between replicates. Both trial locations soil was prepared by cultivation with a rototiller.

Three rates of hydrated lime were spread manually as evenly as possible in their respective plots and a susceptible canola variety was seeded. Eight weeks after seeding and at harvest, 10 and 25 plants respectively, were pulled from each plot and assessed for clubroot symptom severity. Disease severity was assessed on a 0-3 scale. The incidence and severity ratings were used to calculate an index of disease (ID), ranging from 0 to 100%. Individual plant height, aboveground biomass and root weight were recorded for 10 plants per plot 8 weeks after seeding. The seeds were dried and cleaned before weighing and calculating yield.

A2.1. Greenhouse trials of various rates of hydrated lime - The effects of multiple rates of hydrated lime and limestone on clubroot development were compared at various inoculum levels on two canola genotypes. A clubroot-susceptible cultivar '45H31' (DuPont Pioneer) and a clubroot-resistant cultivar '9558C' (DuPont Pioneer), both treated with Prosper FX (Bayer Crop Science, Calgary, AB), were grown in a potting medium consisting of a mixture of 10% peat moss and 90% soilless mix (Sungro Professional Growing Mix, Sungro Horticulture, Seba Beach, AB) to create an initial soil pH of 5.3. Soil aliquots of 25 L were prepared and represented one treatment of inoculum, lime product and rate, for both cultivars.

The potting medium was inoculated with different concentrations of P. brassicae resting spores. The inoculum consisted of resting spores collected from root galls (stored at -20□C) caused by pathotype 3H (Strelkov et al. 2018). The same pathotype was used for both experiments, but the collection of galls used to create the

The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

inoculum came from two different previous greenhouse experiments. To extract the *P. brassicae* resting spores, galls were ground in a blender with water, and then filtered through six layers of cheesecloth (American Fiber & Finishing Inc., Albemarle, NC). A stock concentration of resting spores was estimated with a haemocytometer (VWR, Mississauga, Ontario) to target an inoculation of 1 × 106 resting spores g-1 medium for a 25 L aliquot of medium. Serial dilutions were performed with the stock concentration to target the three remaining final potting medium concentrations of 1 × 103, 1 × 104, and 1 × 105 resting spores g-1 potting medium.

The dilutions of resting spore suspensions were added to 25 L aliquots of potting medium and mixed thoroughly by hand. Following soil inoculation with *P. brassicae*, 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-1 of lime, to adjust the pH to 6.0, 6.5, 7.0 or 7.5, respectively. Rates were calculated as per Alberta Agriculture and Forestry guidelines (Government of Alberta, 2002) as described earlier for the field trials. Following the application of lime and inoculation with the corresponding concentration of *P. brassicae* resting spores, each 25 L aliquot of potting medium was mixed thoroughly with 1.8 L of water and left in the greenhouse for 1 week prior to being transferred into pots to prep for seeding.

The canola genotypes were sown 2 cm deep at a density of 16 seeds per 12 cm × 12 cm × 12 cm 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 at ca. 24°C with 30% relative humidity under natural light supplemented with artificial lighting (16 h day/8 h night). The pots were placed on water-filled trays for the first 2 weeks after seeding to ensure sufficient moisture for clubroot development, following which holes were poked in the trays and the plants were top-watered as needed with watering cans. Pots were fertilized with a 0.1% solution of 20:20:20 (N:P:K) once a week until harvest.

In summary, 80 treatments were examined in each repetition of the greenhouse experiment. These included all combinations of the four tested inoculum concentrations (1 × 103, 1 × 104, 1 × 105 and 1 × 106 resting spores g-1 medium), five rates of each lime product (limestone or hydrated lime at rates of 0.0, 4.7, 8.1, 11.4 or 14.8 T ha-1 of lime) and two canola cultivars ('45H31' and '9558C'). There were two control treatments for each inoculum concentration (example: 1 × 103 resting spores g-1 medium + no limestone and 1 × 103 resting spores g-1 medium + no hydrated lime) where the medium was inoculated but did not receive a lime treatment. Each treatment was replicated five times. The entire 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 on a 0 to 3 scale. The individual severity ratings were used to calculate an ID for each replicate as described above for the field trials. 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.

A2.2. Quantification of Plasmodiophora brassicae DNA in root tissue

In the greenhouse trials, root samples were collected to quantify colonization by P. brassicae. Briefly, 10-day old seedlings were removed carefully from the potting medium and washed in standing water; the root system

was excised with a scalpel and stored in a small bag in a −20°C freezer until further processing. Total genomic DNA was extracted from these root samples with a NucleoSpin® Plant II DNA

Isolation Kit (Macherey-Nagel GmbH & Co. KG, Germany) as per the manufacturer's instructions. The

DNA samples were stored at 4°C if they were to be analyzed within 1 week, or stored at −20°C for longer

periods. All samples were subjected to conventional PCR analysis to determine the presence or absence of *P. brassicae* DNA using primers TC1F and TC1R as described by Cao et al. (2007). Those samples which

tested positive for the presence of P. brassicae DNA were analyzed further by quantitative PCR (q-PCR).

A3. Compare the effectiveness of the application of hydrated lime, weed management and the deployment of CR genetics on clubroot severity, incidence, and yield

Replicated field trials were conducted in 2018 (site 1) and 2019 (sites 2 and 3) 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 trials were located in a naturally infested clubroot nursery at the Crop Diversification Centre North (CDCN; 53◦380 N, 113◦210 W), Alberta Agriculture, Forestry and Rural Economic Development, Edmonton, Alberta. The soil at this site consists of a Black Chernozem. Each trial included four replicates arranged in a randomized complete block design. There were eight different combinations of the management strategies, creating a block-split-split plot design. Each plot had an area of 9 m2 (1.5 m × 6 m), with 4 rows per plot and a 2 m buffer between replicates. The pre-treatment pH of the soil at site 1 (2018) was 5.3, while at sites 2 and 3 (2019), the soil pH values were 5.23 and 5.48, respectively. The amount of lime needed to reach the target pH of 7.2 was calculated. Hydrated lime (Ca(OH)2, Graymont) was applied evenly, by hand, on May 28th, 2018 and 31 May 2019. Plots that required weed control were hand-weeded every two weeks after the canola emerged until the end of July (2018 and 2019). The soil samples were collected and stored at room temperature until used for quantitative PCR analysis of soil inoculum density.

B. Assess impact of Integrated Clubroot Management techniques under varying spore concentrations using qPCR in lab and field trials (Gossen and McDonald) – Study was conducted in "hot spots" where clubroot resistance had broken down in one commercial fields near Edmonton. Resting spore populations in the hot spots were monitored using qPCR analysis of soil samples collected from each plot using a standard soil corer (5 cores, 15-cm deep per plot). The initial studies were assess the interaction of treatments with lime, quick lime and a grass cover crop. Subsequent studies will include other promising IPM approaches as they are identified (possibilities include application of boron, solarization, fumigation where possible, etc.), and modifications to the initial treatments to optimize reduction of resting spore populations. In the final year of the study, the plot area will be broken, planted to canola, and clubroot levels will be assessed using standard rating techniques.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

Objective 3. Quantifying yield loss in relation to disease severity (Hwang and Strelkov)

This research aimed to evaluate the effect of clubroot on yield and yield-related parameters of three canola hybrids with different levels of resistance under field conditions in Alberta, Canada. Experiments were conducted in biosecure nurseries inoculated with P. brassicae pathotype 5X or a mix of pathotypes 5X and 3H at rates equivalent to 108, 106 and 104 resting spores per plant. The canola hybrid '45H31' is susceptible (S) both to pathotypes 5X and 3H, the hybrid 'CS2000' is partially resistant (PR) to 5X and resistant (R) to 3H, while the hybrid '45H29' is resistant to both pathotypes. Clubroot incidence and severity, along with seed weight, thousand-seed weight, productive branches, pods per branch and pods per plant, were measured 71 and 94 days after planting.

Objective 4. Effect of cultivar rotation on mixed pathotype population composition (Strelkov and Hwang)

Resting spores of P. brassicae were collected by homogenizing fresh root galls with a blender. Equal amounts of resting spores of the predominant pathotype 3 and the novel virulent pathotype '5x' of P. brassicae were mixed and inoculated into dried soil. The soil was sown with canola in three different crop rotations (treatments): T1) continuous 4 cropping of a susceptible canola cultivar (i.e., 45H26-45H26-45H26-45H26); T2) continuous 4 cropping of a resistant canola cultivar (i.e., 45H29-45H29-45H29-45H29); and T3) rotation of 4 different clubroot resistant canola cultivars with different resistance profiles (i.e., 45H29-6056CR- 1960-9558C). In cropping 5, the susceptible Chinese cabbage (B. rapa var. pekinensis) cv. 'Granaat' was used to recover P. brassicae resting spores from the soil mix. 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. An index of disease (ID) was calculated based on the disease severity rating data using the formula of Horiuchi and Hori (1980) as modified by Strelkov et al. (2006). Prior to planting of the next crop, fresh root galls from the previous crop were homogenized with a blender and integrated into the same soil mix, and soil samples were collected for qPCR analyses. At the end of each series of rotations, resting spores in the soil mix were recovered by growing the susceptible Chinese cabbage line ECD05 in six-week rotations. Single root galls on ECD05 were pathotyped using the Williams differential set plus B. napus cv. 'Mendel' at the end of rotation 4. The pathotype 5X DNA was measured with a quantitative PCR assay.

Objective 5. Disease nursery and resistance screening against novel clubroot pathotypes (Hwang, Strelkov and Fredua-Agyeman)

Spores of the key novel pathotype (P5X) were multiplied in planta at the CDC North facility and were incorporated into soil in securely fenced areas to establish disease nurseries at CDC North. The nursery was used as an ongoing resource to screen for new resistant lines of canola, and was used as a demonstration area for clubroot extension activities.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

Deliverables:
Objective 1.
• This project will determine the pathotype composition within clusters, characterize the movement of new pathotypes within a field or cluster, and identify commonalities and differences in soil types between clusters.

Objective 2.
• This study will help producers prevent the establishment of clubroot by application of soil amendments such as lime in critical parts of a field, investigate the role of soil properties on disease intensity, and provide recommendations to producers on managing hot small spots of clubroot in canola fields.

Objective 3.
• This will provide a practical and economically relevant understanding of the impact of clubroot severity on yields. Moreover, from a broader, industry/breeding perspective, this information will serve to more clearly delineate between resistant, moderately resistant and susceptible genotypes of canola based on the impact of clubroot on yields, helping to clarify what level of resistance is useful.

Objective 4.
• This study will determine the value of rotating existing clubroot resistant varieties in a field infested with a mixed population of clubroot spores.

Objective 5.
• Recommendations to producers on managing hot small spots of clubroot in canola fields
• 2018 International Clubroot Workshop

Results and Discussion: (2021-2022: Year 4)

Objective 1. Characterization of soils types (soil environment) and the movement of new pathotypes in clusters where resistance breakdown has occurred (Hwang and Strelkov)

Four clubroot-infested fields located in Sturgeon and Westlock counties in central Alberta, near the center of the clubroot outbreak, were selected for this study. Fields 1 and 2, in Sturgeon County, had an area of 37.6 ha and 37.2 ha, respectively; Fields 3 and 4, in Westlock County, had an area of 34.6 ha and 39.2 ha, respectively. The soil in Fields 1, 2 and 4 was an Eluviated Black Chernozem, while Field 3 in-cluded three different soil types, approximately 58.7% of the field was a Gray So-lodized Solonetz, 31.9% was an Eluviated Black Chernozem and the remaining 9.4% was an Orthic Humic Gleysol. The crop rotation from 2017 to 2019 included can-ola-wheat-peas (Field 1), wheat-canola-wheat (Field 2), canola-barley-canola (Field 3) and barley-canola-oats (Field 4). Each field was sampled extensively in a regular grid pattern (80 m × 80 m), with approximately 500 g of soil collected at each node of the grids. Soils were sampled to a depth of 15-cm using a small shovel and placed individually in paper bags. All sampling locations were georeferenced with a smartphone and the

The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

geocoordinates were recorded using the mobile application MapIt Spatial. Ninety-nine samples were collected from Field 1, 97 from Field 2 and 100 from each of Fields 3 and 4. After collection, soil samples were air-dried and stored at 4 °C until processing. All soil samples were ground and homogenized in a mortar with a pestle or in a commercial spice grinder WSG 60 (Waring commercial, Stamford, CT), which were washed with ethanol between samples. 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.

Inoculum Density - The maximum inoculum density observed differed among fields, ranging from 1.7 × 103 resting spores g of soil in Field 3 to 3.2 × 107 resting spores g of soil in Field 4. These values are similar to inoculum densities previously observed in commercial fields in Alberta and Europe where canola or rapeseed, respectively, is grown. It has been reported that inoculum densities between 1 × 103 and 1 × 105 resting spores g of soil are sufficient to cause clubroot symptoms under field conditions, and that concentrations between 3 × 103 and 1.3 × 105 resting spores per g of soil caused yield losses in susceptible hosts.

All fields had acidic soil pH values between 5 and 6.2. Mean pH in Fields 1, 2, 3, and 4 was 5.34, 5.03, 5.49, and 5.83, respectively. Calcium, boron, and magnesium varied among fields. Mean calcium concentra-tion in Fields 1, 2, 3, and 4 was 4648, 4129, 3853, and 4247 mg kg-1, respectively. Mean boron concentration was 1.97 (Field 1), 2.22 (Field 2), 1.52 (Field 3), and 2.34 mg kg-1 (Field 4), respectively, while mean magnesium concentration was 756.8 (Field 1), 477.6 (Field 2), 374.1 (Field 3), and 319.7 mg kg-1 (Field 4), respectively. Within field variation was also observed.

Spatial Patterns - 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. Nonetheless, there was some variation in each field. The low number of positive samples also impeded the adjustment of any model to evaluate the relationship between the density of P. brassicae inoculum and soil properties. The patch diameter, measured by the spatial range, changed between years in all of the fields, with average patch growth of 221.3 m. Statistical analysis indicated a positive effect of the maximum inoculum density observed on the patch diameter ($p = 0.015$), but no significant effect from the number of years when canola was grown.

Models to evaluate the effect of soil properties on inoculum density did not show an important effect of pH, boron, calcium, or magnesium on P. brassicae inoculum density. Means of the posterior distribution of pH, boron and calcium were erratic in all fields in both years, while for magnesium it was negative in all fields for both years, suggesting that, although this nutrient is not a critical factor defining P. brassicae inoculum density, it may influence it to some extent.

The maximum P. brassicae inoculum density values are similar to inoculum densities previously observed in commercial fields in Alberta and Europe where canola or rapeseed, respectively, was grown. It has been reported that inoculum densities between 1 × 103 and 1 × 105 resting spores/g soil are sufficient to cause clubroot symptoms under field conditions, and that concentrations between 3 × 103 and 1.3 × 105 resting spores/g soil can cause yield losses in susceptible hosts. In the current study, P. brassicae resting spore numbers, as opposed to clubroot severity, were used to assess inoculum density and spatial patterns, since

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

spore numbers are not influenced by the resistance or growing conditions of the particular crop. Nonetheless, the infestation levels observed in some of the fields suggested that significant levels of clubroot would have developed on a susceptible canola crop.

Objective 2. Field pre-treatment and amendment techniques, including reassessment of Integrated clubroot management techniques; liming under varying spore concentrations; and treating field entrances with lime prior to clubroot introduction *(Hwang, Strelkov, Gossen, and McDonald)*

A. Efficacy of lime for clubroot management *(Hwang, Strelkov)*

A1. Field study of hydrated lime- At the time of seeding, soil samples were collected to confirm the change in pH. At the first site, the control blocks buffered down to a pH of 5.6. the lowest lime treatment changed the pH to 6.8, the medium rate of lime had a pH of 7.7 and high rate a pH of 7.8 at the time of seeding. At the highest application rate, the lime treatment reduced the clubroot disease severity index by 91% at 8 weeks after planting and by 71% at harvest time. Mean above-ground plant biomass increased by 58%. Mean plant height increased by 30.4%. At the highest application rate, lime treatment increased yield by 12.66%.

At the second site, the control buffered up to a pH of 5.5 at the time of seeding. The lowest rate of lime changed the pH to 7.0, medium lime rate to a pH of 7.6 and highest lime rate to a pH of 7.7 at the time of seeding. At the highest application rate, lime treatment reduced the clubroot disease severity index by 45% at 8 weeks after planting and 50% at harvest time. Mean above-ground plant biomass increased by 116%. Mean plant height increased by 50%. At the highest application rate, lime treatment increased yield by 343%

The liming of acidic soils shows promising results for the suppression of clubroot disease in Alberta soils. Observations in the field show increased above-ground biomass, decreased disease severity and incidence and no phytotoxic effects at any of the chosen rates on a susceptible canola cultivar in a heavily infested field. The magnitude of the effect of liming clubroot infested soils was shown in two trials with very different starting pH values. This magnitude may depend on initial soil inoculum concentration, initial soil pH, initial soil nutrients (i.e. calcium).

A2.1. Greenhouse study of hydrated lime - A greenhouse experiment was conducted to evaluate the effects of hydrated lime, limestone and different rates of each product on *P. brassicae* infection and clubroot severity (index of disease) in susceptible and moderately resistant canola cultivars grown in soils with varying inoculum levels. Eight weeks after inoculation, indices of disease of 92-100% and 9-13%, respectively, were observed in the susceptible and resistant controls (no lime) treatments. The index of disease decreased to 0% in both the susceptible and resistant cultivars following treatment with any of four tested rates of hydrated lime. In contrast, the application of limestone resulted in a modest decrease in clubroot severity and only at the two lowest inoculum levels evaluated.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

A2.2. Quantification of Plasmodiophora brassicae DNA in root tissue - Ten-day old root tissue was subjected to conventional and quantitative PCR analysis to detect and measure the amount of *P. brassicae* DNA under the various treatment regimes. When seedlings were exposed to a low level of inoculum, no *P. brassicae* DNA could be detected in the roots at any of the four rates of hydrated lime evaluated. When seedlings were exposed to a high level of inoculum, *P. brassicae* DNA could be detected at all but the highest rate of hydrated lime. There were no clear trends in pathogen DNA content in the roots following the application of limestone. The results highlight the need for careful selection of lime products and rates for clubroot management in canola.

A3. Compare the effectiveness of the application of hydrated lime, weed management and the deployment of CR genetics on clubroot severity, incidence, and yield

A3.1. Field Analysis - Replicated field trials were conducted a naturally infested clubroot nursery at the Crop Diversification Centre North (CDCN; 53°38' N, 113°21' W), Alberta Agriculture, Forestry and Rural Economic Development, Edmonton, Alberta. Each trial included four replicates arranged in a randomized complete block design. There were eight different combinations of the management strategies, creating a block-split-split plot design. The amount of lime needed to reach the target pH of 7.2 was calculated based on recommendations from the Government of Alberta. Hydrated lime (Ca(OH)2, Graymont) was applied evenly, by hand. In 2018, a CR canola cultivar '45H29' and a CS cultivar '45H31' were seeded at site 1. The development of clubroot galls was observed on the resistant variety during the growing season. In 2019, '45H29' was replaced with '45CM39' (Dupont Pioneer). Plots that required weed control were hand-weeded every two weeks after the canola emerged until the end of July (2018 and 2019). Hand weeding ensured that no galls developed on any susceptible weeds, which could have affected the P. brassicae spore densities. Shoot height, shoot weight, and clubroot symptoms were evaluated. The harvested seeds were cleaned and dried before they were weighed to obtain yield estimates. Ten soil samples per plot were collected and stored at room temperature until used for quantitative PCR analysis of soil inoculum density.

A3.2. Resting Spore Densities - 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.

A3.3. Clubroot Severity - 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.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

A3.4. Yield - 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 (all p = 0.0002), and the intereaction between culitvar 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.

A3.5. Growth Parameters - The effect of canola cultivar and the application of hydrated lime on gall weight was significant ($p < 0.0001$). The greatest difference was observed between treatments that included a CR cultivar and treatments that used a susceptible cultivar without the application of hydrated lime. Gall weight was lower when hydrated lime was applied to treatments using a susceptible 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, 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.

B. Assess impact of *Integrated Clubroot Management (IPM)* techniques under varying spore concentrations using qPCR in lab and field trials *(Gossen and McDonald)*

B1. Effects of perennial grasses and rotation on spore density - Growth room studies to assess the effect of dense seedings of perennial grasses and conventional rotation crops (wheat, barley, pea and soybean) on resting spores of *P. brassicae* were conducted at the University of Guelph. They showed that resting spore concentration declined more quickly in the presence of grass and wheat seedlings than in bare soil. Wheat was as good at reducing spores in soil as perennial ryegrass, but the effect of barley was less consistent, and soybean did not result in any measurable reduction relative to bare soil.

In the field study at Spruce Grove, the perennial ryegrass survived each winter until 2022 in excellent condition, with nearly 100% cover and about 30 cm height of residual grass. The residual forage was mowed off in late April each year to facilitate new growth. Each year, soil samples (5 cores, 15-cm depth) were collect from each

The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

plot for assessment of soil pH and resting spore concentration. additional lime (hydrated and zero-grind) was applied to each plot in the spring of 20219, based on the pH of samples collected in the previous summer, to bring the pH up to the target level of 7.5, and immediately incorporated using a tractor-mounted rototiller to 5-10 cm depth. Each year, the plots were hand-weeded and brassica species with clubs (weeds and volunteer canola) were removed.

The pH and spore concentration for each plot was assessed, each year, and the results from the final year of the study (2022) were completed with funding from A24 and reported there. In summary, the resting spore concentration at this site declined very substantially over time. But none of the treatments had a substantial effect on the final spore concentration. The only treatment that had a major impact early in the study was perennial ryegrass, where DNA of P. brassicae was much higher than the control in the early years of the study. This was almost certainly due to the effect of root hair infection of perennial ryegrass, which increases the number of zoospores of P. brassicae in the soil but does not result in the production of new resting spores, and so can not contribute to an increase of resting spore concentration over time.

Thanks to the DL Seeds crew at Edmonton, who maintained the study site at Spruce Grove by mowing and removing foliage in the perennial ryegrass plots during the growing season and again in early spring after snow melt. The collected samples from the site were to Saskatoon for analysis. In October 2021, it was

B2. Analysis of the effect of various potential bait crops on resting spore concentration - Two unexpected observations: First, all of the control treatments fell in areas of the plot that had the lowest concentration of resting spores when the experiment was initiated. That made comparison with the control problematic throughout the remainder of the study. Second, the estimates of spore concentration in the grass plots increased dramatically in the perennial ryegrass plots after seeding, but fell back to a similar level to that in the other treatments by 2019 and stayed in the same range as the other treatments in 2021. The initial increase in the DNA of *P. brassicae* (used to estimate spore numbers in ddPCR) likely indicated that root hairs of the grass seedlings became infected with P. brassicae but weren't able to infect and increase in the absence of a susceptible host. However, the results of this field study do not support the results of a companion growth room study that showed that spores of *P. brassicae* were reduced more quickly under a plant canopy relative to bare soil.

Objective 3. *Quantifying yield loss in relation to disease severity (Hwang and Strelkov)*

Effect of *Plasmodiophora brassicae* inoculum density on canola

Trial 1 - This research aimed to evaluate the effect of *Plasmodiophora brassicae* inoculum levels on clubroot severity and yield in two canola hybrids with differing resistance to the disease. Experiments were conducted under field conditions in central Alberta. The susceptible canola '45H31' and the clubroot resistant (CR) canola '45H29' were grown in soil infested with pathotype 5X at rates equivalent to 107, 106, 105, 104 and 103 resting spores per gram of soil. Clubroot incidence and severity, along with yield parameters including

seed weight, productive branches and pods per plant, were measured at 79 and 122 days after planting. It was found that as *P. brassicae* inoculum density increased, so did the incidence and severity of clubroot in both the resistant and susceptible cultivars, although no statistically significant differences were observed at the lower inoculum densities (103 - 105 resting spores per plant). Yield was reduced by 31-51% in the susceptible cultivar. Regression analysis indicated that a second-grade polynomic equation best described the effect of inoculum density on yield in the susceptible hybrid.

Trial 2 - As *P. brassicae* inoculum density increased, so did clubroot incidence and severity. The number of branches and pods per plant in the R and PR hybrids declined as the density of pathotype 5X inoculum increased, while in the S hybrid both parameters remained similar across all inoculum densities. The highest DSIs developed on the susceptible '45H31', followed by the second-generation CR cultivar 'CS2000', then by the 1st generation CR '45H29'. In the mix of 5X and 3H, no differences in the number of branches or pods per plant were observed for any inoculum density. The number of pods per branch was reduced in all hybrids as 5X inoculum density increased, but a reduction was observed only in the S hybrid when plants were grown in the mix of 5X and 3H. Yield declined in all three hybrids with increasing inoculum density of 5X or the 5X and 3H mixture, although greater yields were obtained with the R hybrid. The yield of the PR hybrid was comparable to that of the R hybrid when grown in the 5X and 3H pathotype mix. In both the field and greenhouse experiments, the yield, pods per plant and 1000-grain weight decreased as the DSI increased. Yield was affected by the DSI and canola cultivar in the greenhouse and field, but not by pathotype. An increment of 1% in the DSI resulted in a decrease of 0.48 % in the PPP and of 0.34% in 1000-grain weight; under field conditions, these percentages were reduced to 0.27% and 0.23%, respectively. While the yield varied in each cultivar, the rate at which yield was reduced (0.49% under field conditions and 0.26% under greenhouse conditions) for each 1% increase in DSI was consistent for the CR cultivars as well as for the susceptible genotype. Yield reductions caused by *P. brassicae* are affected by inoculum density and specific host-pathotype interactions, reflecting the impact of host resistance on clubroot development.

Objective 4. ***Effect of cultivar rotation on mixed pathotype population composition (Strelkov and Hwang)***

Effect of canola cultivar rotation on clubroot pathotype composition - New pathotypes of *P. brassicae* have emerged that are virulent on most CR canola cultivars. To understand the impact of cultivar rotation on pathotype composition, greenhouse experiments were conducted in which different canola cultivar rotations were grown in a soil mix containing equal amounts of pathotypes 5X and 3, which are virulent and avirulent, respectively, on CR canola. Plant materials included the CR canola cultivars '45H29', '6056CR', '1960', '9558C', the clubroot susceptible cultivar ''45H26', and the aforementioned Chinese cabbage 'Granaat'. About 160 seeds of a specific canola cultivar were planted in four rows in each of the nine polyethylene tubs, which were placed in large polyethylene trays (115 cm × 198 cm × 9.5 cm). The seeded tubs were bottom-watered until full saturation of the soil was achieved. Three weeks after seeding, the seedlings in each tub were thinned to 60 - 70 plants. 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

The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

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.

Effect of canola cultivar rotation on clubroot severity - At the end of the first cycle of each rotation, the highest ID (91.5%) was observed in T1 on the canola '45H26', followed by IDs of 78.0% and 69.1% on '45H29' in T2 and T3, respectively. All three IDs were significantly different from each other. At the end of cycle 2, the IDs were 93.4% on '45H26' in T1, 92.2% on '45H29' in T2, and 91.3% on '6056CR' in T3. At the end of cycle 3, the IDs were 97.6% on '45H26' (T1), 97.9% on '45H29' (T2) and 98.2% on '1960' (T3). 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 on '45H26' and '45H29' in T1 and T2, respectively, values that were significantly greater than the ID of 14.1% observed on the cultivar '9558C' in T3. Following each of the canola rotations, the Chinese cabbage 'Granaat' was grown as a bait crop in all of the treatments. The highest ID developed on the 'Granaat' plants in T1 (99.8%), while a significantly lower ID (98.7%) developed on 'Granaat' in T3; in T2, the bait plants developed an intermediate ID of 99.1%, which was not significantly different from the other two treatments.

Effect of canola cultivar rotation on amount of pathotype 5X DNA - 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 7.4 × 102 ng ± 1.8 × 102 ng at the beginning of cycle 1 to 6.5 × 102 ng ± 1.4 × 102 ng by the end of cyle 4, with no statitistically significant differences at any time. In T2, the amount of pathotype 5X DNA also was fairly constant from the beginning of cycle 1 (5.9 × 102 ng ± 9.7 × 101 ng) to the end of cycle 3 (6.1 × 102 ng ± 8.9 × 101 ng), but increased significantly to 2.0 × 103 ± 3.1 × 102 ng by the end of cycle 4. In the case of T3, the amount of pathotype 5X DNA ranged from 5.8 × 102 ng ± 1.4 × 102 ng to 8.4 × 102 ng ± 2.8 × 102 ng over most of the cycles, but peaked at 1.1 × 103 ng ± 1.1 × 102 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 (i.e., at the end of the canola rotational sequence).

Effect of canola cultivar rotation on pathotypes recovered - 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 included pathotypes 5 (6.7%) and 8 (23.3%) in T1, pathotypes 5 (6.7%), 6 (10%) and 8 (10%) in T2, and pathotypes 5 (16.7%), 6 (6.7%) and 8 (10%) in T3.

Objective 5*. Disease nursery and resistance screening against novel clubroot pathotypes (Hwang and Fredua-Agyeman)*

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

Two unique clubroot nurseries (pathotypes 3H and 5X) were established and maintained on land kindly provided by Alberta Agriculture and Forestry. Lines developed by seed companies for resistance to pathotype 3H and/or pathotypes 5X were assessed for their performance at these dedicated clubroot nurseries. Forty lines were included in all experiments at each nursery, with clubroot susceptible canola '45H31' included as a check.

In 2022, 11 lines were seeded into the 3H nursery, and a new site with predominant 3A inoculum was used to assess these lines plus an additional 66 lines. Treatments were arranged in a RCBD with plots seeded on June 25 and June 5th, 2021, and June 8 and 9, 2022. Disease severity was assessed on Oct. 8, 2021 and August 23-30, 2022. A total of 25 plants per plot (for a total of 100 plants per genotype per site) were carefully dug out from the soil and assessed for disease severity on a 0 to 3 rating scale (Kuginuki et al., 1999; Xue et al., 2008), where: 0 = no galling, 1 = a few small galls (small galls on <1/3 of the roots), 2 = moderate galling (small to medium-sized galls on 1/3 - 2/3 of the roots), and 3 = severe galling (medium to large-sized galls on >2/3 of the roots). An index of disease (ID) was then calculated using the formula of Horiuchi and Hori (1980) as modified by Strelkov et al. (2006). The data for each entry was forwarded to the company of origin.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.