

**AgriInnovation Program Stream B****2017-18 Annual Performance Report**

Name of Recipient: Canola Council of Canada	
Project Title: Agri Innovation Program Stream B	
Project Number: AIP-P353	Period Covered by Report: 2017-04-01 to 2018-03-31
Activity #: 4 – 2015.14 Name of Activity: Characterization of new strains of the clubroot pathogen in Alberta	Principal Investigator: Dr. Sheau-Fang Hwang, AAF Dt. Stephen Strelkov, University of Alberta

**1. Performance Measures.** See Annex A for an explanation of each measure.

Innovation Items	Results Achieved	Provide a description (2-3 paragraphs) for each item produced and describe its importance to the target group or sector. Explain any variance between results achieved and targets. Use plain language.
# of Intellectual property items flowing from the project	N/A	
# of new/improved products	N/A	
# of new/improved processes or systems	0	
# of new/improved practices	1	We have developed the Canadian Clubroot Differential (CCD) Set. This represents an improved system for the identification of new virulence profiles of <i>Plasmodiophora brassicae</i> and their classification into pathotypes. The CCD Set has a greater differentiating capacity than the systems that were previously in use, and has allowed the identification of many new pathotypes that would have otherwise gone undetected. Since this system also includes the differentials of Williams and Somé et al., it allows users to obtain pathotype designations according to those systems as well. As such, the CCD Set is an important new tool that can be applied directly on the ground by the sector, including agronomists, breeders and researchers.
# of new varieties	N/A	
# of new/improved genetic materials	N/A	
# of new/ improved gene sequences	N/A	
# of improved knowledge	1	We have obtained knowledge regarding the occurrence and distribution of novel pathotypes of <i>P. brassicae</i> that are able to overcome clubroot resistance in Canola.



Information Items	Results Achieved	Provide the complete citation for each item. Please see Annex A for examples.
# of peer reviewed publications	3	<ul style="list-style-type: none"><li>Holtz, M., Hwang, S.F., and Strelkov, S.E. 2018. Genotyping of <i>Plasmodiophora brassicae</i> reveals the presence of distinct populations. <i>BMC Genomics</i>, 19: 254 (<a href="https://doi.org/10.1186/s12864-018-4658-1">https://doi.org/10.1186/s12864-018-4658-1</a>)</li><li>Strelkov, S.E., Hwang, S.F., Manolii, V.P., Cao, T., Fredua-Agyeman, R., Harding, M.W., Peng, G., Gossen, B.D., McDonald, M.R., and Feindel, D. 2018. Virulence and pathotype classification of <i>Plasmodiophora brassicae</i> populations collected from clubroot resistant canola (<i>Brassica napus</i>) in Canada. <i>Can. J. Plant Pathol.</i>, Accepted 25 Mar. 2018 (<a href="https://www.tandfonline.com/doi/abs/10.1080/07060661.2018.1459851">https://www.tandfonline.com/doi/abs/10.1080/07060661.2018.1459851</a>)</li><li>Zhou, Q., Hwang, S.F., Strelkov, S.E., Fredua-Agyeman, R., and Manolii, V.P. 2018. A molecular marker for the specific detection of new pathotype 5-like strains of <i>Plasmodiophora brassicae</i> in canola. <i>Plant Pathol.</i>, Accepted 6 April 2018 (<a href="https://onlinelibrary.wiley.com/doi/10.1111/ppa.12868">https://onlinelibrary.wiley.com/doi/10.1111/ppa.12868</a>)</li></ul>
# of information items	2	<ul style="list-style-type: none"><li>Holtz, M.D., Hwang, S.F., Zantinge, J., and Strelkov, S.E. 2017. Use of genotype-by-sequencing to characterize populations of <i>Plasmodiophora brassicae</i>. <i>Can. J. Plant Pathol.</i> 39(1): 98-99.</li><li>Strelkov, S.E., Hwang, S.F., Manolii, V.P., and Harding, M.W. 2017. The changing face of clubroot of canola (<i>Brassica napus</i> L.) in Canada. Program and Abstracts, 2017 International Clubroot Workshop, April 24-28, 2017, Wuhan and Jingmen, China. p. 7.</li></ul>
# of media reports	2	<ul style="list-style-type: none"><li>Anonymous. 2017. Digging deeper into the clubroot-canola interaction. <i>Canola Digest: Science Edition</i> 2017. p. 9. Available from: <a href="http://www.saskcanola.com/quadrant/media/files/news/pdfs/2017/CanolaDigestScienceEdition2017.pdf">http://www.saskcanola.com/quadrant/media/files/news/pdfs/2017/CanolaDigestScienceEdition2017.pdf</a></li><li>Duckworth, B. 2017. Clubroot spreads as new pathogens develop. <i>The Western Producer</i>, July 20<sup>th</sup>, 2017. [Information from one of my recent presentations was discussed, and the work of one of my graduate students was featured prominently in this article (as was a photo of the student)]. Available from: <a href="http://www.producer.com/2017/07/clubroot-spreads-as-new-pathogens-develop/">http://www.producer.com/2017/07/clubroot-spreads-as-new-pathogens-develop/</a></li></ul>
# of information events	4	<ul style="list-style-type: none"><li>Strelkov, S.E. 2018. Clubroot in oilseed rape: The Canadian experience. JKI/UFOP-Workshop "Clubroot Disease in Oilseed Rape – Status Quo and Research Demand", Jan. 15-16, 2018, Berlin, Germany.</li></ul>



		<ul style="list-style-type: none"><li>Strelkov, S.E. 2017. Clubroot surveillance and epidemiology: Staying ahead of an important canola issue. Canola Discovery Forum, Dec. 6, 2017, Saskatoon, SK.</li><li>Strelkov, S.E. 2017. The Canadian Clubroot Differential. Clubroot Steering Committee Meeting, Oct. 25, 2017, Winnipeg, MB.</li><li>Strelkov, S., Ziesman, B., and Derksen, H. 2017. Tri-provincial clubroot update. Western Committee of Plant Disease – 42<sup>nd</sup> Annual Meeting, Oct. 26, 2017, Winnipeg, MB.</li></ul>
		<b>Provide the # of attendees</b>
# of individuals attending information events	375	100 - JKI/UFOP-Workshop, Jan. 15-16, 2018 175 - Canola Discovery Forum, Dec. 6, 2017 80 – Western Committee of Plant Disease, Oct. 26, 2017 20 - Clubroot Steering Committee Meeting, Oct. 25, 2017
		<b>Provide the # of attendees who intended to adopt new information or technology</b>
# of individuals attending information event who intend to adopt new innovation	250	This number included industry agronomists, government extension personnel, canola breeders and scientists
		<b>Provide the name, degree completed and date of completion</b>
# of persons who completed a M.Sc. or Ph.D. during project	0	

## 2. Executive Summary

The Executive summary contains two parts: Key highlights of activities and scientific results and Success story. Information may be used for internal and external communication purposes. Write for a general audience using plain language. Do not include sensitive or confidential information

<b>Key Highlights</b> - This section describes the key activities and final scientific results of an activity/project in such a way that readers can rapidly become acquainted with a large body of material without having to read it all. Include a brief statement of the problem(s), background information, concise analysis and main conclusions. Suggested length – maximum 1 page.
Clubroot, caused by <i>Plasmodiophora brassicae</i> , is an important soilborne disease of canola and other crucifers. In Canadian canola cropping systems, the disease is managed primarily by the planting of clubroot resistant (CR) canola varieties. Unfortunately, new strains of <i>P. brassicae</i> have emerged recently in Alberta that can overcome the resistance in CR canola. The aim of this project was to identify, characterize, and better understand strains of the clubroot pathogen that are able to overcome clubroot resistance.
Populations of <i>P. brassicae</i> representing 151 fields in Alberta were obtained from galled roots of clubroot resistant (CR) canola plants collected in 2014–2017 and characterized for virulence on seven CR canola cultivars. One-hundred and one of these populations could overcome resistance in at least one CR cultivar and were evaluated further by inoculation on 13 Brassica hosts termed



the Canadian Clubroot Differential (CCD) Set. The CCD Set included the differentials of Williams and Somé et al., selected hosts of the European Clubroot Differential Set, and the *B. napus* cultivars 'Brutor', 'Mendel', 'Westar' and '45H29'. Each unique virulence pattern on the CCD Set represented a distinct pathotype and was identified with a letter. Five reference isolates, obtained prior to the introduction of CR canola, also were assessed. A total of 17 pathotypes were detected using the CCD Set, compared with five pathotypes using the system of Williams and two with the system of Somé et al., suggesting that the CCD Set has a greater differentiating capacity. Pathotype A, a variant of pathotype 3 (as per Williams) which is able to overcome the resistance in CR *B. napus*, was predominant. The original pathotype 3, which is avirulent on CR canola, was classified as CCD pathotype H.

In addition to testing of the virulence phenotypes, restriction site-associated DNA sequencing (RADseq) was used to examine the genetic diversity within *P. brassicae* single-spore and field isolates collected from across Canada. The isolates included individuals that were either capable or incapable of causing disease on clubroot resistant canola cultivars. Population analysis indicated that most isolates belonged to one of two distinct populations, corresponding with the ability of isolates to cause disease on resistant cultivars. Finally, a molecular marker also was developed to identify pathotype 5X, which was the first of the resistance-breaking pathotypes of *P. brassicae* to be identified in Canada. A 127-bp product was amplified selectively from all new pathotype 5-like strains following optimized PCR analysis. A TaqMan probe-based quantitative assay also was developed.

This study has improved our understanding of the new strains of *P. brassicae* that have emerged in western Canada in recent years, and has resulted in the development of improved practices for their identification and management. It is clear that an integrated approach, combining other tools in addition to genetic resistance, will be needed for sustainable clubroot control.

**Success Story** - A success story presents a significant result or an important milestone achieved. It is intended to showcases achievements in applied research. Focus on research results, successful technology transfer, potential for pre-commercialization, and/or potential impact. A Success Story is not a progress report for each activity (suggested length 2 – 3 paragraphs).

The development of the Canadian Clubroot Differential (CCD) Set represents a significant milestone in this project and in Canadian clubroot research. The CCD Set is an improved system for the identification and classification of new strains of *P. brassicae*. The CCD Set has a greater differentiating capacity than the systems that were previously in use, and has allowed the identification of many new pathotypes of *P. brassicae* that would have otherwise gone undetected. This system also includes the differential hosts of Williams and Somé et al., allowing users to obtain pathotype designations according to those systems as well. The CCD Set is an important new tool for agronomists, breeders and researchers.

### **3. Objectives/Outcomes (technical language is acceptable for this section)**

Provide a brief summary that includes introduction, objectives, approach/methodology,



deliverables/outputs, results and discussion, and any Ph.D or Master students recruited to work on the project.

## Introduction

Clubroot, caused by *Plasmodiophora brassicae*, was first identified in a dozen canola fields in western Canada in 2003. Since then, more than 2700 clubroot-infested fields have been confirmed. While the outbreak is concentrated mainly in central Alberta, clubroot has continued to spread throughout the province, with isolated cases of the disease also found in Saskatchewan, Manitoba and North Dakota.

The clubroot pathogen rapidly multiplies in infected plants and persists in the soil for up to 20 years. Therefore, spore populations can increase rapidly, and yield losses in heavily infested fields can approach 100%.

Clubroot-resistant canola cultivars first became available to Canadian producers in 2009-10. These cultivars confer a high degree of resistance to the predominant strains of *P. brassicae* (including the highly virulent pathotype 3), and quickly became the most effective and important clubroot management tool for farmers. The availability of clubroot-resistant canola has enabled continued production of this crop in clubroot-infested fields.

Unfortunately, a strain capable overcoming resistance to *P. brassicae* in canola was identified in central Alberta in 2013. This new strain, referred to as pathotype 5X, appears to be highly virulent to all canola cultivars currently on the market. The identification of this new strain, together with preliminary reports of resistance loss or erosion in additional fields of clubroot resistant (CR) canola in 2014, led to the launch of this project in 2015.

## Objectives

The primary aim of this project was to mitigate the risk posed to the agricultural industry by the emergence of new strains of *P. brassicae* able to overcome the resistance in "clubroot resistant" canola. Specifically, this project would: (1) monitor spread of this novel clubroot strain through surveys, (2) assess the potential of resistance-defeating pathotypes to reappear in further outbreaks, (3) characterize the pathotypes of *P. brassicae* present where resistance had broken down, (4) multiply inoculum of resistance-defeating pathotype(s) for screening in containers and or securely contained field facilities, and (5) search for molecular markers for novel clubroot pathotypes.

## Approach/Methodology

### 1. Monitoring for clubroot resistance breakdown in clubroot-infested fields where CR canola has been repeatedly grown

- Surveys took place in late summer/early fall, after galls had developed and the crop harvested in order to facilitate field access. Surveillance activities were focused on field entrances where infected plants are typically found (Cao et al. 2009).
- Additional surveys also were carried out on known clubroot 'hotspots' where resistant canola cultivars were grown. The areas near known instances of resistance breakdown



also were monitored closely.

- Populations of *P. brassicae* were recovered from diseased plant samples as per our well-established protocols. These were multiplied *in vivo* for further analysis.
- Suspect samples were analyzed for resistance-defeating traits by re-inoculating onto: 1) the same resistant cultivar and onto 2) available commercial resistant cultivars. Pathogen collections made in 2014 and kept in storage were also included in this analysis, along with the collections made in 2015-2017.
- Pathogen resting spores that were multiplied *in vivo* were suspended in water. The roots of the canola plants were dipped into the spore suspension and the seedlings were then transplanted into potting medium and grown in a greenhouse for 6-8 wk, or until clubroot galls could be evaluated. Gall mass, clubroot severity and plant growth parameters were recorded.

## 2. Pathotype designation for newly isolated strains causing resistance breakdown

- If field populations were capable of causing significant amounts of clubroot disease on a suite of CR canola cultivars, they were evaluated for pathotype designations on the newly developed Canadian Clubroot Differential (CCD) Set. Selected single-spore isolations also were made and will serve as a valuable resource for future work.

## 3. Resistance screening to novel pathotypes

- The disease reaction to the resistance-defeating clubroot strains was compared among commercial cultivars and lines using the procedures in (2) above.
- Inoculum of resistance-defeating pathotypes was multiplied *in vivo* at the CDC North facility. The inoculum was used to screen new canola cultivars at CDC North. In addition, this inoculum was used to create a secure pathotype 5X field nursery at CDC North. Development of a “mixed nursery” (consisting of a mixture of novel, resistance overcoming pathotypes) is also underway.

## 4. Search for molecular markers for novel clubroot pathotypes

- Restriction site-associated DNA sequencing (RADseq) was used to examine the genetic diversity within *P. brassicae* single-spore and field isolates collected from across Canada. The isolates included individuals that were either capable or incapable of causing disease on clubroot resistant canola cultivars.
- In addition, in order to expedite the identification of new pathotype 5-like strains of *P. brassicae* that can overcome host resistance (pathotype 5X), three primer sets were developed based on the 18S-ITS region of the pathogen.

## Results/Discussion

### 1. Monitoring for clubroot resistance breakdown in clubroot-infested fields where CR canola has been repeatedly grown

The virulence of 151 *P. brassicae* populations (clubbed roots) was tested on a suite of CR canola cultivars, representing one club from each field in which clubroot was found on a CR canola crop from 2014 to 2017. Under greenhouse conditions, 101 of these populations were able to overcome resistance on at least one CR host, including 15 populations collected in 2014, 24



populations collected in 2015, 22 populations collected in 2016, and 40 populations collected in 2017. The remaining populations were avirulent on the CR hosts evaluated ( $ID < 20\%$ ) and, with the exception of population F41-14 collected in 2014, were not analyzed further. Most of the populations capable of overcoming resistance originated from fields in central Alberta, where the clubroot outbreak is most severe, although isolated cases were found as far south as Newell County and as far north as Athabasca County. As expected, all of the populations were highly virulent on the universal suspect, ECD 05.

Twelve of the 16 populations characterized from the 2014 collections were virulent on all seven of the CR canola cultivars tested, with IDs ranging from a mean ( $\pm SE$ ) of  $41.7\% \pm 10.0\%$  to  $100\% \pm 0\%$  on the various hosts. In contrast, two populations (CDCN#4 and CDCN#6) were virulent on most, but not all, CR hosts: CDCN#4 was virulent on '1960', '6056CR', '74-54RR', 'D3152' and 'L135C', but not on '9558C' ( $ID = 16.4\% \pm 4.5\%$ ) or '45H29' ( $ID = 34.7\% \pm 3.5\%$ ), while CDCN#6 was virulent on all hosts except '9558C' ( $ID = 9.7\% \pm 2.4\%$ ). Another population (CDCN#2) was virulent on only one of the CR hosts ('L135C'), on which it caused an ID of  $47.4\% \pm 3.8\%$ . This was regarded as a susceptible reaction since the 95% confidence interval overlapped a threshold ID of 50%, but the ID obtained was lower than that induced by the other virulent *P. brassicae* populations on the same host. Population F41-14 did not cause a susceptible reaction on any of the CR canola cultivars tested, inducing a highest ID of  $42.4\% \pm 2.6\%$  (on '45H29'). Nonetheless, F41-14 was included in a subsequent assessment of pathotype designation, since in a preliminary evaluation, a subsample of this population appeared to be virulent on '45H29'.

In the 2015 collections, 16 populations were virulent on all of the CR canola cultivars tested, with IDs ranging from  $43.8\% \pm 3.7\%$  to  $100\% \pm 0\%$ . Another population (F1-15) was virulent on all hosts except '9558C' ( $ID = 24.3\% \pm 4.2\%$ ), while population F6-15 was virulent on all hosts except '9558C' ( $ID = 29.0\% \pm 5.5\%$ ) and '74-54RR' ( $ID = 23.6\% \pm 7.0\%$ ). The population F12-15 was virulent on four of the CR canola cultivars ('1960', '45H29', 'D3152' and 'L135C') and avirulent on the other three. One population (F10-15) was virulent only on two CR hosts, namely '45H29' ( $ID = 57.8\% \pm 3.3\%$ ) and 'L135C' ( $ID = 53.5\% \pm 7.6\%$ ). Finally, four populations, including F7-15, F9-15, F20-15 and F26-15, were avirulent on all CR canola cultivars except '45H29', on which they induced IDs that ranged from  $49.4\% \pm 4.0\%$  to  $58.3\% \pm 5.7\%$ .

All 22 populations collected in 2016 were virulent on the entire suite of CR cultivars evaluated, with IDs ranging from  $51.4\% \pm 0.8\%$  to  $100\% \pm 0\%$ . These populations were generally more aggressive than the 2014 and 2015 collections, and produced the highest IDs on the suite of CR canola cultivars. The lowest ID, when averaged across all of the populations tested each year, was observed on '9558C', although these values were still  $> 50\%$  in 2014 ( $52.2\% \pm 6.1\%$ ), 2015 ( $62.8\% \pm 6.4\%$ ) and 2016 ( $70.5\% \pm 4.0\%$ ). When the populations were grouped into pathotypes, as classified below, it was clear that the CR canola cultivars were not effective differentials. These hosts were, in general, susceptible to the CCD pathotypes A, B, C, D, E, O and P. The response to pathotypes D and G was more variable, especially amongst the 2015 collections, with the range of ID values occasionally crossing the 50% threshold between a resistant and a susceptible reaction. Most of the CR cultivars were resistant to the pathogen populations classified as pathotypes H and I, an observation consistent with the finding that these shared pathotype designations with some of the 'old' *P. brassicae* strains. Interestingly, however, those populations classified as H and I collected



from fields planted to CR canola generally induced higher IDs than those recovered prior to the introduction of the CR trait, even if the ID values were still < 50%.

Amongst the 2017 collections, all 40 populations were virulent on the entire suite of CR canola genotypes. On most of these CR hosts, the pathogen populations induced IDs of >90%. The exception was '9558C', which developed IDs of  $30.4\% \pm 2.10\%$  to  $95.1\% \pm 0.88\%$  in response to inoculation.

## 2. Pathotype designation for newly isolated strains causing resistance breakdown

The virulence pattern of each of the *P. brassicae* populations collected from 2014-2016 and able to overcome resistance on at least one of the CR canola cultivars was assessed on 13 Brassica hosts representing the CCD Set. All of the populations were virulent on the universally susceptible check, ECD 05, with IDs that ranged from  $86.8\% \pm 1.7\%$  to  $100\% \pm 0\%$ . Similarly, all of the populations were virulent on the *B. napus* cultivars 'Brutor' (IDs of  $54.4\% \pm 4.0\%$  to  $100\% \pm 0\%$ ) and 'Westar' ( $52.1\% \pm 1.3\%$  to  $100\% \pm 0\%$ ), and all but one (exception F10-15) were virulent on ECD 08. Most populations also were virulent on the differentials ECD 06 and ECD 09. The exceptions were CDCN#6, CDCS, F9-15, F10-15, F11-15 and F12-15, which were avirulent on both ECD 06 and ECD 09, and F381-16/C.C. and F395-16/C.C., which were avirulent only on ECD 06. The number of pathogen populations virulent on the rutabaga 'Laurentian' was high and appeared to increase over the years, with susceptibility to 12 of 16 populations in 2014, 21 of 24 populations in 2015, and all of the populations collected in 2016. Likewise, the cabbage ECD 13 was susceptible to 9 of 16 populations from 2014, 20 of 24 populations from 2015, and 21 of the 22 populations from 2016. In contrast, only one *P. brassicae* population, F183-14, was virulent on ECD 11, causing an ID of  $50.0\% \pm 2.0\%$ , while all others were avirulent (IDs ranging from  $0\% \pm 0\%$  to  $38.2\% \pm 3.1\%$ ). No population was virulent on the rutabaga ECD 10, which developed a highest ID of only  $35.4\% \pm 5.0\%$  after inoculation with F23-15. Similarly, ECD 02 was resistant to all of the populations tested, and in general developed the lowest IDs ( $0\% \pm 0\%$  to  $15.3\% \pm 1.4\%$ ) of any of the differentials. Neither of the two CR *B. napus* cultivars, 'Mendel' or '45H29', was resistant to all of the *P. brassicae* populations evaluated. Seven of 16 populations tested from 2014 and 15 of the 24 populations from 2015 were virulent on 'Mendel,' but this proportion increased to 21 of 22 from 2016. All but three pathogen populations collected in 2014 were virulent on '45H29', and this host was susceptible to all of the populations collected in 2015 and 2016.

Each unique virulence pattern on the hosts of the CCD Set was regarded as a distinct pathotype and assigned an identifying letter. In 2014, a total of nine distinct virulence patterns were observed. The most frequently identified was pathotype A, which represented 5 of 16 *P. brassicae* populations tested from that year. Populations classified as pathotype A are virulent on all of the differential hosts except ECD 02, ECD 10, and ECD 11. Two populations each were classified as pathotypes E, G, and H. It is worth noting that pathotype H was avirulent on both of the CR *B. napus* cultivars 'Mendel' and '45H29'. This indicates that it does not represent strains capable of overcoming existing clubroot resistance in the suite of CR cultivars, a fact confirmed by testing of the 'original' pathotypes (see section below). A single population (F183-14) was classified as pathotype B, which is distinguished from pathotype A by its virulence on ECD 11. One population each of pathotypes C, D, I, and P also was identified in 2014. Like pathotype H, pathotype I is



avirulent on 'Mendel' and '45H29', but is distinguished from H by its avirulence on ECD 13 and 'Laurentian'.

Pathotype A remained predominant among the *P. brassicae* populations collected in 2015 and 2016. Fifteen of the 24 collections from 2015 were classified as pathotype A. The second most frequently detected pathotype in 2015 was D, with 5 populations identified, followed by pathotypes G (2 populations), and J and K with one each. Of a total of five virulence patterns identified in 2015, three also had been found in 2014 (A, D and G), while two were novel (J and K). The vast majority of the populations collected in 2016 were classified as pathotype A (19 of 22 populations), with two populations classified as pathotype O and one as P. Pathotype O represented a new virulence pattern identified in 2016. The virulence patterns of the first *P. brassicae* populations found to be capable of overcoming clubroot resistance in canola, detected in 2013 (populations L-G1, L-G2, L-G3), were not observed amongst the collections made in 2014-2016. Therefore, these populations were given a distinct pathotype classification and designated as pathotype X, in keeping with common usage. Another unique virulence phenotype found in the 2013 collection (population D-G3) was designated as pathotype L.

The greatest number of pathotypes was identified in central Alberta, where pathotype A was most common. Populations capable of overcoming clubroot resistance, but identified further away from the main outbreak, tended be other pathotypes. For example, population CDCS collected from Newell County was classified as pathotype G, while population F175-14 from Athabasca County was classified as pathotype C. In a few instances, clusters of fields included distinct pathotypes, such as the populations representing pathotype O found in Westlock County.

### 3. Resistance screening to novel pathotypes

The host reaction to the resistance-defeating novel strains of *P. brassicae* was compared among commercial cultivars and lines. Six representative novel strains of the pathogen and one pathotype common in western Canada since 2004 were inoculated onto two susceptible canola cultivars, one resistant line, and six CR cultivars. Although all cultivars/lines exhibited a susceptible response to inoculation with the new strains of *P. brassicae*, the severity of clubroot, root hair infection rates, and the amount of *P. brassicae* DNA present in each canola genotype varied depending on the strain. In addition, the effect of inoculum concentration on disease severity and gall formation was recorded for one of these new strains across one susceptible canola cultivar, a universally susceptible cabbage line, eight resistant canola cultivars, and two resistant canola lines. Although clubroot galls were observed at inoculum concentrations of  $1 \times 10^3$  spores/mL of soil, clear differentiation of susceptible and resistant reactions among canola cultivars/lines was not observed until inoculum concentration reached  $1 \times 10^5$  spores/mL. At spore concentrations of  $1 \times 10^6$  spores/mL and above, all cultivars/lines developed susceptible reactions, although there was some differentiation in the degree of reaction.

*P. brassicae* populations representing the key pathotypes have been made available to private and public breeders (subject to appropriate biosafety considerations) for screening purposes, in order to assist with the identification of effective resistance sources and the development of new CR canola products. Pathotypes 3A and 2B are of particular interest in the development of the next



generation of clubroot resistant cultivars, since 3A is the most common of the new strains and 2B can overcome resistance in one host (ECD 11, *B. oleracea* 'Badger Shipper') resistant to 3A. These new pathotypes are being used to complement screening with other, older strains, under greenhouse conditions. The first of the new pathotypes identified, '5X', also remains of interest and continues to be included in many resistance tests. This includes greenhouse "tub screening", in which plants are grown in tubs of naturally infested soil to more closely resemble the field situation. A pathotype 5X field nursery was established in a biosecure location at CDC-N and in 2017 underwent final validation (by planting a susceptible host genotype and monitoring disease development) to ensure it is ready for use by stakeholders in 2018. Work also is underway on another biosecure nursery which will include a mixture of the some of the most relevant new pathotypes including 3A and 2B.

#### 4. Search for molecular markers for novel clubroot pathotypes

Over 8750 variants were identified through RADseq. Population analysis indicated that most isolates belonged to one of two distinct populations, corresponding with the ability of isolates to cause disease on resistant cultivars. Within each population, there were low levels of genetic diversity. One thousand and fifty of the genetic variants that distinguished the two populations were nonsynonymous, altering the coding sequences of genes. The identification of two distinct populations of *P. brassicae* in Canada suggests multiple introductions of the pathogen into the country. The genetic variation found here will be important for future research and monitoring of the pathogen, including development and validation of additional markers specific for the new pathotypes.

In parallel work, a more targeted approach was taken to develop molecular markers specific to the resistance-breaking pathotype 5X. Three primer sets were developed based on the 18S-ITS region of the pathogen. With the primers P5XF3 and P5XR3, a 127-bp product was amplified from all new pathotype 5-like strains following optimized PCR analysis. A TaqMan probe-based quantitative assay also was developed. These protocols could be used to detect as little as  $\leq 500$  fg *P. brassicae* DNA, and as few as  $1 \times 10^4$ /mL pathogen resting spores; infection of host tissues could be detected as soon as 4 days after inoculation. The PCR and qPCR assays developed as part of this project represent useful tools for the rapid and reliable diagnosis and quantification of new pathotype 5-like strains of *P. brassicae*.

#### 5. Issues

- Describe any challenges or concerns faced during the project. How were they overcome or how do you plan to overcome?
- Describe any potential changes to the work plan and the budget. How were or how will they be managed?

The biggest challenge associated with this project was that it involved work with highly virulent strains of *P. brassicae* that are capable of overcoming the currently used sources of clubroot resistance. Therefore, all work had to be conducted under biosecure conditions. The principal investigator (PI) obtained biosafety certification from the Environmental Health and Safety Office



(EHSO) of the University of Alberta before initiating the research. The PI and EHSO coordinated with the Canadian Food Inspection Agency and the Pest Surveillance Branch of Alberta Agriculture and Forestry as needed on biosafety related issues. Work conducted by the collaborator at CDC-N was overseen by the Pest Surveillance Branch. The biosafety precautions did not, however, affect the work or the successful completion of the project, since these were incorporated into the original project design.

## **6. Lessons Learned:**

Describe the key lessons learned gained as a result of executing the project (e.g., a more efficient approach to performing a specific task for activity / project).

Experimental methods and protocols were streamlined in earlier years of the project. There were no further changes in 2017-2018.

## **7. Future Related Opportunities:**

Describe the next steps for the innovation items produced by the activity/project. Is additional research required? Is there potential for commercialization or adoption?

Continued monitoring will be required to track the continued shifts in *P. brassicae* populations and mitigate the negative impacts these shifts could have on clubroot management. The tools developed as part of this project should be very helpful for this purpose. The CCD Set will serve as an effective method to identify novel pathotypes and quickly determine their ability to overcome certain key sources of resistance. The genomic information obtained and analyses conducted provide an excellent resource for quickly identifying potential molecular markers, and for comparing genetic similarities or differences between different populations of the pathogen. The molecular marker for pathotype 5X will facilitate screening of larger numbers of samples for the presence of this pathotype, and together with the genomic information could serve as the basis for development of additional markers for new pathotypes identified in this study and in the future.

**Annex A**

<b>Innovation Items</b>	
<b>Performance Measures</b>	<b>Description</b>
# of Intellectual property items flowing from the project	These include: declaration of invention, patent application, patents, trademarks, copyrights, trade secrets, signed license agreements, and royalties generated. This does not include IP for plant varieties; those should be reported under “# of new varieties” below.
# of new/improved products	New products could include: a new commercial product, bacterial strain, cartographic product, cell culture, analysis certificate, computer software, database, enzyme, equipment/instrument, fertilizer, hormone, methodology, model, monoclonal antibody, pest control product, polyclonal antibody, standard reference-chemical, standard reference-biological, standard reference-plant, etc.
# of new/improved processes or systems	This is the set of operations performed by equipment in which variables are monitored or controlled to produce an output. A combination of inter-related components or processes is arranged to perform a specific function and generate a given outcome.
# of new/improved practices	This is for a research that generated new knowledge that can be applied directly on the ground by the sector. This is mostly for new agronomic practices but can also cover new practices by processors.
# of new varieties	This includes registered varieties, cultivars, or breeds. This includes invention disclosure, protection and license for new plant varieties. For each new variety, please provide the registration number and the variety name.
# of new/improved genetic materials	This could include genetic map and gene probes. Include new varieties, cultivars or breeds in category “New varieties.”
# of new/ improved gene sequences	The discovery of order of bases of a DNA [segment] making up a gene.
# of improved knowledge	This category is for reporting results following completion of the final year of the activity, or results against an activity’s improved knowledge target. It is intended for results that do not fit in any of the above categories.
<b>Information Items</b>	
<b>Performance Measures</b>	<b>Description</b>
# of peer reviewed publications	<p>These are published items such as: research papers published in scientific journals, books, book chapters, review articles, conference proceedings, research notes, or other that receive peer-review. Items that are not yet published (ex. manuscripts in development or review) should not be reported.</p> <p>For each reported item, please provide the following: author(s), year of publication, article title, title of journal, volume (issue), and page number(s).</p> <p>If the item is a book or a book chapter, add name of publisher.</p> <p>If the item is an article for conference proceedings, add title of published proceedings, location, and year/month/day.</p>
# of information items	<p>Information items include: posters, abstracts, pieces in publications such as trade journals, articles in industry magazines or press, industrial reports (confidential or not), technical bulletins, brochures, guides, flyers, newsletters, or other technical transfer publications. If an item is published in a medium whose audience is the general public, it should be reported in the # of media reports category below.</p> <p>For each reported item, please provide the following: author(s), article title, title of magazine/trade publication etc., page number(s), type of information item such as poster or abstract or guide etc., and year/month/day.</p>



# of media reports	<p>Examples include articles or interviews about project results in media such as newspapers, TV, radio, and the Internet (announcements about project funding are excluded). (These are items prepared by a third party, usually with input by the project). If an item is published in an industry journal, newspaper, or magazine, it should be reported in the # of information items category above.</p> <p>For each reported item, please provide the following: author(s), article title, name of interviewee(s), source of reports (TV or radio interview etc.), and year/month/day.</p>
# of information events	<p>These are events such as a scientific meeting, symposium, conference, industry meeting, or field day where a project participant has been invited to present a talk or presentation directly related to the activity.</p> <p>For each reported item, please provide the following: name of presenter, title of presentation, name of the event, location, and year/month/day.</p>
# of individuals attending information events	Please provide the number of attendees per event.
# of individuals attending information event who intend to adopt new innovation	Please provide the number of attendees intending to adopt the new innovation per event.
# of persons who completed a MSc or PhD during project	Only students who completed their MSc or PhD in the last year should be included in this category. For each reported graduate, please provide the following: the name of the student, degree completed and date of completion.