PROJECT DETAILS

- **Title**: Canadian Canola Clubroot Cluster Pillar 4: Surveillance and pathotype monitoring
- **Funders:** Alberta Canola
- **Research program:** Canola Agronomic Research Program
- **Principal investigator:** Stephen (Steve) Strelkov and Sheau-Fang Hwang
- **Collaborators/additional investigators:** Rudolph Fredua-Agyeman, Michael (Mike) Harding, Mary-Ruth McDonald and Bruce Gossen
- **Year completed:** 2019

Final report

Clubroot is a soilborne disease of canola caused by the pathogen *Plasmodiophora brassicae*. The disease is difficult to manage, as *P. brassicae* produces long-lived resting spores that can survive in the soil for many years. While clubroot was first detected in just a few fields in central Alberta, it has spread relatively quickly over the past decade, and there are now several thousand confirmed field infestations. The most effective management strategy is the planting of clubroot resistant (CR) canola cultivars. Recently, however, new strains of the pathogen have emerged that can overcome this resistance. There are multiple new pathogen strains and they are becoming more common.

The aim of the research is to allow us to track and respond to changes in the clubroot pathogen via a team approach that includes four specific objectives:
1) continued clubroot surveillance and detection of new strains overcoming resistance, including the isolation and characterization of single-spore isolates and field populations;
2) refinement of the current clubroot differential set;
3) examination of the genetic and virulence relationships between pathotypes and their interactions; and
4) development of molecular tests to distinguish between pathotypes.

Clubroot surveillance and characterization of single-spore isolates and field populations:
Surveillance in Alberta. Clubroot was found in 79 of the 543 canola crops visited in 2018 (Table 1). The identification of the first records of the disease in Birch Hills, Greenview and Northern Sunrise suggest that clubroot is spreading in the Peace Country of northwestern Alberta (Fig. 1) following its initial detection in Big Lakes in 2017. Similarly, the first cases of clubroot in Rocky View indicate further spread in southern Alberta, although dissemination in that region appears to be occurring more slowly. Limited or no surveillance had been conducted in the City of Edmonton in recent years, and as such it was targeted as part of the Alberta-wide survey in 2018. The identification of 30 new cases of clubroot in rural areas of Edmonton (Table 1) reflects how widespread the disease has become there. This is consistent with the increasing prevalence of clubroot throughout much of central Alberta (Table 1; Fig. 1).

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In most cases, clubroot severity was mild (Index of Disease, ID < 10%; 59 crops) or moderate (ID = 10-60%; 15 crops), although five crops were found to be heavily infested (ID > 60%). Two of the most heavily infested crops represented clubroot resistant (CR) canola cultivars, and were among many fields found in 2018 with potential resistance issues. This is consistent with a trend of increasing numbers of fields where first generation clubroot resistance has been broken or eroded (Strelkov et al. 2016, 2018). Pathogen populations from each of the potential cases of resistance loss were subjected to further evaluation under greenhouse conditions (see below). The appearance of new pathotypes capable of overcoming resistance is one of the most important threats facing canola production in clubroot-infested regions of the Prairies, and should continue to be monitored.

In addition to the 79 new records of clubroot detected in the Alberta-wide survey, another 221 cases of the disease were found in independent inspections conducted by municipal personnel (Table 1). Collectively, clubroot surveillance activities identified 300 new clubroot infestations in Alberta in 2018, for a grand total of 3044 documented field infestations since 2003. These are distributed across 40 counties and municipal districts, plus Edmonton, Medicine Hat and the Town of Stettler (Fig. 1).

Table 1. Distribution of *Plasmodiophora brassicae*-infested canola fields identified in Alberta in 2018

County or municipality	Number of fields assessed in provincial survey	Number of new cases of *P. brassicae*-infested fields	Additional new cases identified by county/municipal staff	Total new cases
Athabasca	4	4	7	11
Barrhead	3	3	8	11
Big Lakes	0	--	5	5
Birch Hills	26	1	0	1
Bonnyville	22	3	0	3
Calgary	1	0	--	0
Camrose	0	--	24	24
Cardston	13	0	--	0
Clearwater	2	2	3	5
Cypress	2	0	--	0
Edmonton	43	27	3	30
Flagstaff	39	1	0	1
Grande Prairie	8	0	--	0
Greenview	24	2	5*	7
Lacombe	6	0	1	1
Lac La Biche	0	--	3	3
Lac Ste. Anne	0	--	5	5
Lamont	26	5	20	25

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Leduc	1	1	43	44
Lesser Slave River	22	0	0	0
Lethbridge	10	0	--	0
Minburn	7	0	2	2
Mountain View	6	0	1	1
Northern Sunrise	24	1	0	1
Paintearth	20	0	0	0
Parkland	14	9	22	31
Provost	26	0	0	0
Red Deer	22	4	2	6
Rocky View	18	3	1	4
Saddle Hills	17	0	--	0
Smoky Lake	2	2	0	2
Special Area 2	1	0	--	0
Special Area 3	2	0	--	0
Special Area 4	4	0	--	0
Starland	7	0	--	0
Stettler	7	0	11	11
St. Paul	28	6	11	17
Strathcona	0	--	12	12
Sturgeon	0	--	15	15
Two Hills	0	--	15	15
Vermillion River	18	1	0	1
Vulcan	20	0	--	0
Wainwright	5	0	--	0
Warner	6	0	--	0
Westlock	4	2	0	2
Wheatland	6	0	--	0
Willow Creek	5	0	--	0
Woodlands	22	2	0	2
Yellowhead	0	--	2	2
TOTAL	543	79	221	300

*Identified in late 2017 but results not communicated in time for inclusion in 2017 survey report.

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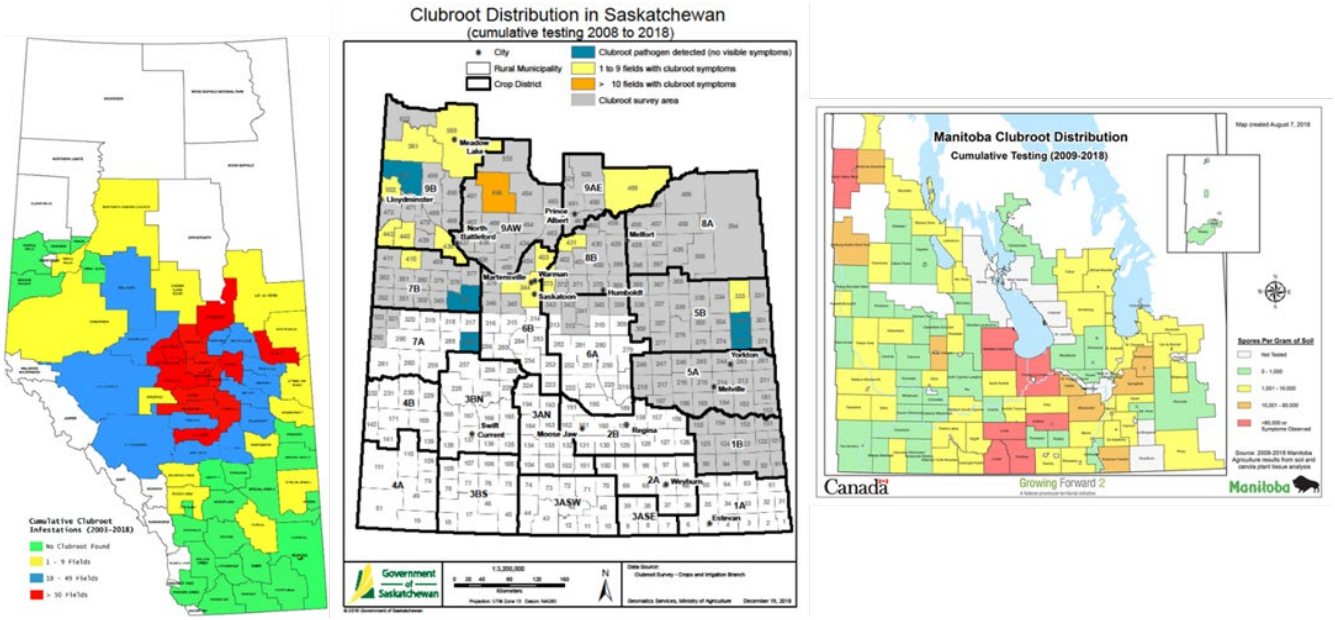

Figure 1. The occurrence of clubroot on canola in Alberta, Saskatchewan and Manitoba in 2018. The maps show the results of cumulative surveillance activities from 2003-2018 (Alberta), 2008-2018 (Saskatchewan) and 2009-2018 (Manitoba).

Surveillance in Saskatchewan. Surveys for clubroot in Saskatchewan were led by the Saskatchewan Ministry of Agriculture. Approximately 1800 fields were surveyed, with one field randomly selected within each township in the survey area. Thanks to the support provided via the current project, Dr. Victor Manolii, a senior technician with Dr. Strelkov's group at the University of Alberta, was able to participate in a clubroot surveillance training session for Ministry Crop Extension Specialists and Plant Health Officers hired by the Saskatchewan Association of Rural Municipalities. Plants were examined for visible symptoms of the disease (root galling) and soil was collected for DNA testing for the presence of *P. brassicae*. In total, 37 canola crops were found with visible clubroot symptoms (Fig. 1). A subset of 10 samples was sent to the University of Alberta (Dr. Strelkov) for pathotype characterization (see below). An additional 9 samples were sent for pathotyping to the University of Guelph/AAFC Saskatoon Research and Development Centre (Drs. McDonald and Gossen).

Surveillance in Manitoba. Provincial staff in Manitoba surveyed over 100 fields in 2018. Seventeen cases of clubroot were identified in 2018 (D. Froese, Manitoba Agriculture), bringing the cumulative total to 33 confirmed infestations since 2009. In addition, almost 300 fields (cumulative over several years) have tested positive for *P. brassicae* DNA by quantitative PCR analysis (Fig. 1). A subset of 6 clubroot samples collected in 2018 was sent to the University of Alberta for pathotype characterization, while 3 collections were sent to University of Guelph/AAFC Saskatoon.

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Characterization of single-spore and field isolates (populations). Methodologies for the recovery of single-spore isolates of *P. brassicae* were refined, with 39 single-spore isolates obtained and characterized for their virulence on a suite of CR canola cultivars and on the Canadian Clubroot Differential (CCD) Set (Strelkov et al. 2018). Many of the populations analyzed consisted of mixtures of virulent and avirulent single-spore isolates, underscoring the fact that field isolates can be heterogeneous. In total, 13 pathotypes were identified amongst the 39 single-spore isolates (Fig. 2). Since the single-spore isolates were derived from field populations collected in 2014, before the prevalence of pathotype A (= 3A) was clear, this pathotype was likely under-represented. The single-spore isolates as well as selected field isolates have been characterized further with molecular tools, as described later in this report.

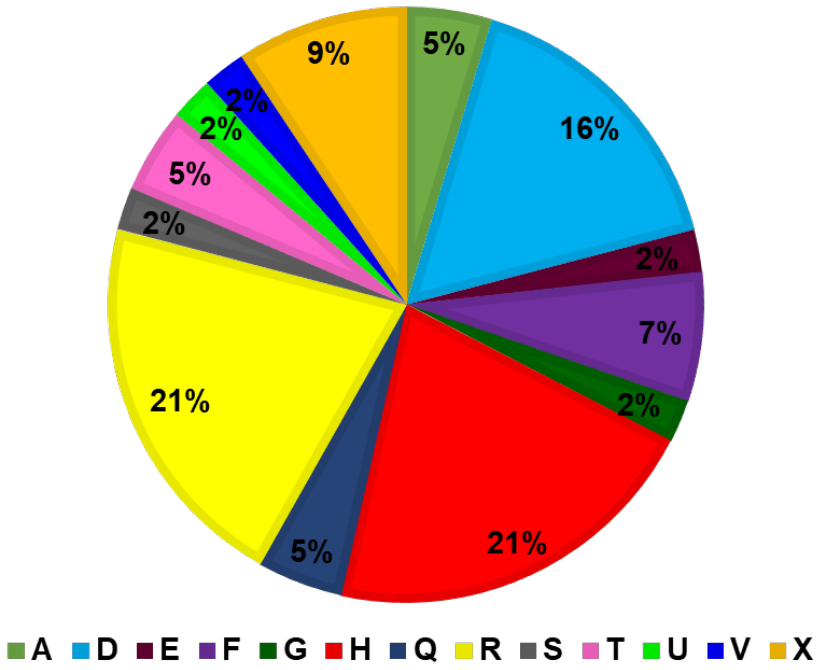

Figure 2. Tentative pathotype classification of single-spore isolates of *Plasmodiophora brassicae* obtained from field populations of the pathogen collected in Alberta in 2014. Isolates were classified into pathotypes based on their virulence on the Canadian Clubroot Differential Set.

In addition to the recovery and characterization of single-spore isolates of *P. brassicae*, pathogen field populations also were collected and tested for pathotype designation. For those collections from Alberta, the focus was on characterizing populations of *P. brassicae* recovered from CR canola, since new strains of the pathogen represent the greatest risk to genetic resistance. In contrast, all samples received from Saskatchewan and Manitoba were evaluated for pathotype. A total of 119 *P. brassicae* populations, each representing a different field, was tested at the University of Alberta from collections made in 2018, including 103 from

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Alberta, 10 from Saskatchewan and 6 from Manitoba. As in previous years, pathotype 3A seemed to be most common in Alberta, representing nearly half of the characterized field isolates (Fig. 3). Also as in recent years, pathotype 3D was the second most common (about a third of all samples), followed by 3H. The latter represents the 'original' pathotype 3, which is highly virulent on canola without a resistance trait, and which was prevalent throughout much of Alberta prior to the introduction of CR canola. Eighty seven of the 103 field isolates collected from Alberta in 2018 could overcome clubroot resistance, bringing the total number of confirmed fields with resistance issues to 191 in the province (Fig. 4).

Of the 10 samples from Saskatchewan tested at the University of Alberta, eight were pathotype 3H and two were 5L (neither pathotype overcomes first generation clubroot resistance) (Table 3). The six field isolates from Manitoba included two pathotype 3H, one pathotype 5L, and two classified as pathotype 2 on the system of Williams (1966) but representing a novel virulence pattern on the CCD Set (Table 3). These did not overcome the resistance in CR canola. Unfortunately, however, one isolate from Manitoba was classified as pathotype 3A and could overcome clubroot resistance. This is the first confirmed case of a clubroot resistance-breaking pathotype detected on canola outside of Alberta. Six of nine collections from Saskatchewan that were sent to the University of Guelph/AAFC also were evaluated and found to be pathotype 3 and avirulent on the CR canola '45H29'. One collection from Manitoba also was tested at the University of Guelph/AAFC and classified as pathotype 9 on Williams' differentials; it was unable to overcome the resistance in '45H29'.

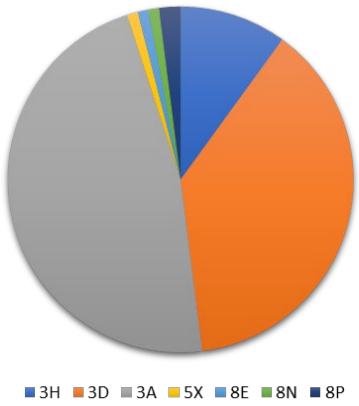

Figure 3. Pathotype designations of field isolates of *Plasmodiophora brassicae* collected from the roots of clubroot resistant canola in Alberta in 2018. Classifications are based on the Canadian Clubroot Differential Set (letters) and on the differentials of Williams (numbers). A total of 103 isolates were tested, with 47 classified as pathotype 3A, 38 as pathotype 3D, and 10 as pathotype 3H. Other pathotypes represented the remainder.

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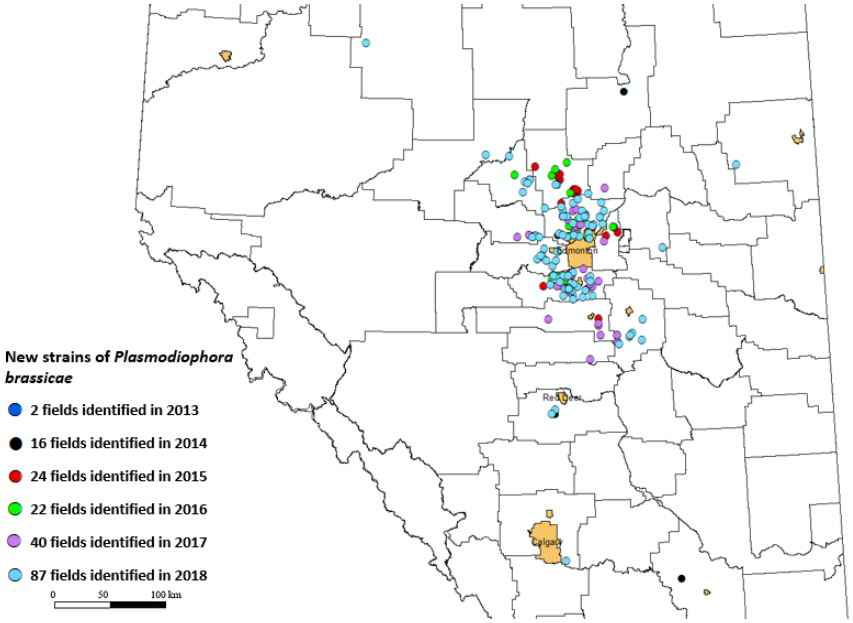

Figure 4. Distribution of fields in Alberta confirmed to be infested with pathotypes of *Plasmodiophora brassicae* capable of overcoming clubroot resistance in canola (2013-2018).

Table 1. Pathotype designations of field isolates of *Plasmodiophora brassicae* collected from canola root samples received from Saskatchewan and Manitoba in 2018. Classifications are based on the differentials of Somé et al. (1996), Williams (1966) and the Canadian Clubroot Differential (CCD) Set (Strelkov et al. 2018)

Sample code	Province	Somé et al.	Williams	CCD Set	Combined designation*
2018-DB-1	Saskatchewan	P2	3	H	3H
2018-DB-2	Saskatchewan	P2	3	H	3H
2018-DB-3	Saskatchewan	P2	3	H	3H
2018-DB-4	Saskatchewan	P2	3	H	3H
2018-DM-1B	Saskatchewan	P2	3	H	3H
2018-CN-2	Saskatchewan	P2	3	H	3H

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2018-CP-62	Saskatchewan	P2	3	H	3H
2018-CW-1	Saskatchewan	P3	5	L	5L
2018-LLH-3	Saskatchewan	P2	3	H	3H
2018-RA-56	Saskatchewan	P3	5	L	5L
MB-1-18	Manitoba	P3	2	ND**	--
MB-2-18***	Manitoba	P2	3	A	3A
MB-3-18	Manitoba	P3	8	ND	--
MB-4-18	Manitoba	P2	3	H	3H
MB-5-18	Manitoba	P2	3	H	3H
MB-6-18	Manitoba	P2	5	L	5L

*Combined designation refers to a combination of the Williams and CCD designations, as per common usage in Canada.
**ND, not designated; refers to a novel virulence pattern that has yet to be assigned a pathotype designation on the CCD Set.
***Sample MB2-18 from Manitoba was able to overcome clubroot resistance in canola as reflected by its pathotype 3A designation.

2) Refinement of the clubroot differential set:
Potential Brassica host differentials were screened with 22 *P. brassicae* field and single-spore isolates representing multiple pathotypes. Duncan test comparisons were conducted using the indices of disease (IDs) that developed on specific hosts following inoculation with the various isolates. Evaluation of 30 clubroot resistant Brassica accessions identified by Fredua-Agyeman et al. (2019) grouped the isolates into two classes: one consisted of the 'old' pathotype 6 (mean ID 30.1%), while the other consisted of the remaining pathotypes (mean ID 10.0-23.9%). In contrast, testing of 23 moderately resistant accessions identified by Fredua-Agyeman et al. (2019) permitted the grouping of isolates into at least four classes. One group with a mean ID range 50.6-59.2% included four of the 'new' *P. brassicae* pathotypes (Strelkov et al. 2018) and two 'old' pathotypes (classified as pathotypes 6 and 8 on Williams' system). The second group with a mean ID range 42.1-45.1% comprised three 'new' *P. brassicae* pathotypes (2B, 3A and 8E), while a third group with mean ID range 27.2-37.1% comprised pathotypes 3D, 3O, 5I, 5K, 5X and 8J (as defined on the CCD Set) and isolates representing two 'old' pathotypes (2, 3 and 5 as per Williams). The fourth group consisted of pathotype 8P. Use of all 53 resistant and moderately resistant accessions identified by Fredua-Agyeman et al. (2019) as differential hosts

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grouped the 22 *P. brassicae* isolates into five groups. Thus, increasing the number of differential hosts did not improve the differentiating capacity. Evaluation of 18 *B. napus* ssp. *napobrassica* accessions indicated that they could separate the 22 isolates into 7 classes, with an ID range of 27.3-93.4%. Collectively, the results obtained from the first year of work identified 19 Brassica accessions that had good 'differential capability' and potential as differential hosts. These included two *B. napus*, eight *B. rapa* and nine *B. nigra* accessions.

Unfortunately, all two *B. napus* and eight *B. rapa* accessions were winter-types, which required 12 weeks of vernalization to induce flowering. As a result, genetic crosses were made with the selected germplasm as males and spring-type *B. napus* (7 accessions), *B. rapa* (5 accessions) and *B. juncea* (two accessions) as females. About 5000 crosses were made, of which about 800 resulted in siliques with seeds. Seeds of the selected *B. nigra* showed segregation for clubroot resistance. Selfed seeds at the F2 generation have been produced with the goal of carrying out more selfing.

3) Examination of the genetic and virulence relationships between pathotypes and their interactions: Resting spores representing 45 isolates of *P. brassicae,* including the 39 single-spore isolates described earlier in this report plus an additional 6 field isolates, were selected for genomic analysis. Briefly, resting spores of each isolate were purified and high quality DNA extracted and prepared for sequencing by Admera Health (South Plainfield, NJ). High quality reads (Phred score > 30) were used to generate individual assemblies with SOAPdenovo. Reads also were mapped against a *P. brassicae* reference genome (https://www.ncbi.nlm.nih.gov/assembly/GCA_001049375.1/) to find single nucleotide polymorphisms (SNPs) and small indels. The variant files (.vcf) were used as input to build a binary matrix to construct a tree of relationships using PhyloSNP; this preliminary tree is illustrated in Fig. 5.

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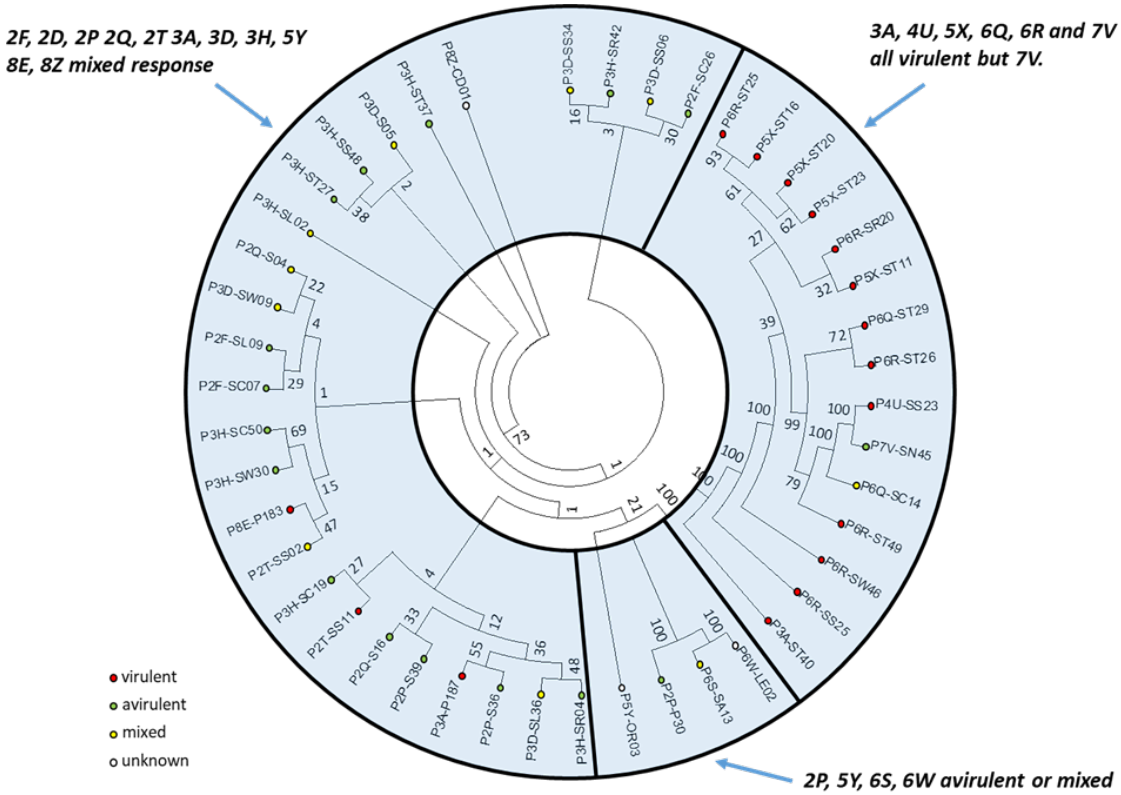

Figure 5. The circular dendrogram shows the relationship between 45 *Plasmodiophora brassicae* field and single-spore isolates. The dendrogram represents a bootstrap consensus tree built using PhyloSNP with 100 bootstrap replications. The colour-coded dots indicate whether an isolate is virulent (red), avirulent (green) or gives a mixed reaction (yellow) on a suite of clubroot resistant (CR) canola cultivars. Those isolates that were not tested for virulence on CR canola are denoted as 'unknown' (white circles). Image credit: Stephen Strelkov.

4) Development of molecular tests to distinguish between pathotypes:
Reformatted vcf files were used as input for the Integrated Genomics Viewer (IGV) to visualize specific polymorphisms among the 45 *P. brassicae* genomes described above. An initial analysis of the variation among the 45 full genomes identified 131 potential polymorphisms that can be used for PCR-based discriminatory assays (Fig. 6). Primers based on Indel polymorphisms have been designed for three genomic regions and are currently being tested. In addition, an rhPCR assay for SNPs is being standardized. This assay is based on placing ribonucleotides in the positions corresponding to the polymorphic bases, which need to be nicked by a ribonuclease H before PCR can proceed. This makes the methodology highly specific and more accurate than standard PCR. When completed, these assays will be used to identify and quantify pathotypes from plant, soil and water samples.

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Figure 6. Detection of polymorphisms among sequenced genotypes of *Plasmodiophora brassicae* representing various pathotypes. The figure depicts single nucleotide polymorphisms (SNPs) and Indels (red lines) in each genotype. Detection of a SNP present only in virulent pathotypes (arrow) can be used to design rhPCR-specific primers that can distinguish between virulent and non-virulent clusters.

Significant Accomplishments

(1) Collected updated information on clubroot distribution and spread.
(2) Produced and characterized a large collection of single-spore isolates of *P. brassicae* (likely the largest collection of single-spore isolates of this pathogen in the world).
(3) Monitored the performance of CR canola cultivars, and collected and characterized field isolates of the pathogen for virulence phenotypes and pathotype designations across Alberta and the Prairies.
(4) Obtained and initiated analysis of genome sequence information for 39 single-spore and 6 field isolates of *P. brassicae.*
(5) Initiated development of molecular markers for the identification of *P. brassicae* pathotypes using the genome sequence information.

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