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Full Research Project Final Report

- This report must be a stand-alone report, *i.e.*, must be complete in and of itself. Scientific articles or other publications cannot be substituted for the report.
- One electronic copy and one signed original copy are to be forwarded to the lead funding agency on or before the due date as per the investment agreement.
- A detailed, signed income and expenditure statement incurred during the entire funding period of the project must be submitted along with this report. Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage; and overhead (if applicable).
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Section A: Project overview

1. Project number: 2010C014R
2. Project title: Studies on the genetic and molecular basis for clubroot resistance in canola
3. Research team leader: Stephen Strelkov
4. Research team leader's organisation: University of Alberta
5. Project start date (yyyy/mm/dd): 2010/10/01
6. Project completion date (yyyy/mm/dd): 2015/11/19
7. Project final report date (yyyy/mm/dd): 2015/09/28

Section B: Non-technical summary (max 1 page)

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

Clubroot, caused by *Plasmodiophora brassicae*, is a destructive soilborne disease of crucifers. The disease is now prevalent on canola (*Brassica napus*) in central Alberta and appears to be spreading to other regions of the Prairies. The aim of this project was to improve knowledge of the genetic and molecular basis for clubroot resistance (CR) in canola, in order to facilitate the development of new resistant cultivars and other innovative clubroot management strategies.

Phenotypic evaluation of about 200 doubled haploid lines from two crosses between a clubroot susceptible and two clubroot resistant lines indicated that a major gene is involved in the control of clubroot resistance in these two populations. These lines have spring growth characteristics and will be an important germplasm for breeding programs in Canada. A genomic region of the chromosome A3 carrying resistance to the predominant pathotype 3 of *P. brassicae* housed 12 markers linked to the resistance introgressed from the European winter canola 'Mendel.' These markers will be useful in breeding programs, allowing the pyramiding of multiple CR genes for durable clubroot resistance. Additional studies with a population stemming from another cross between a susceptible and a resistant parent identified a resistance locus that is linked to the clubroot resistance gene *CRa*; the resistance in this *B. napus* population is controlled either by the combined effect of this new CR gene and *CRa* (with the effect of the former stronger than the effect of the latter) or by the new CR gene alone. The project also clearly demonstrated the capacity of local *P. brassicae* populations to adapt to the selection pressure imposed by the planting of clubroot resistant canola genotypes. Under greenhouse conditions, genetic resistance was significantly eroded in as few as two cycles of exposure to the same single-spore isolate or population of the pathogen, highlighting the need for careful resistance stewardship, especially where clubroot is an issue. The sowing of a resistant cultivar also was found to decrease inoculum potential, but the presence of some susceptible plants within a resistant crop reduced this effect. The roles of the primary and secondary zoospores of *P. brassicae* in host/non-host resistance and clubroot pathogenesis also were investigated, and it was found that secondary zoospores produced on a nonhost (ryegrass) are able to infect a host (canola). Moreover, primary zoospores were observed to cause secondary infection when the host already was under primary infection, suggesting that *P. brassicae* uses primary infection to overcome the basal resistance of the plant to cortical infection. A transcriptomic analysis of clubroot resistant and susceptible *B. napus* genotypes indicated a 4- to 5-fold turnover at the transcriptome level in the susceptible line relative to the resistant line as a consequence of *P. brassicae* infection. The data also revealed stronger control over alterations in normal metabolic processes in the resistant plants. Analysis of expressed sequence tags derived from a compatible *P. brassicae*-canola interaction identified 407 and 32 genes of canola and *P. brassicae* origin, respectively, which were subjected to gene ontology and *in silico* analysis. A subset of these genes was further examined by real-time PCR in order evaluate changes in gene expression associated with pathogenesis or host resistance. Collectively, the research conducted in this project has generated information and tools that will benefit clubroot resistance breeding activities, help guide the development of recommendations for proper resistance stewardship and, in the long term, enable rational approaches to clubroot management through an improved understanding of clubroot pathogenesis and the host response.

Section C: Project details

1. Project team (max ½ page)

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

The composition of the research team did not change over the course of the project. Dr. Strelkov served as team leader, coordinating team activities, as well as directing various components of the research related to clubroot pathology, histology and pathotype shifts and structure. Dr. Strelkov also provided most of the pathogen material used in all components of the research,

along with expertise and advice on working with the pathogen. As a canola breeder, Dr. Rahman led much of the work aimed at identifying major clubroot resistance genes, as well as the development of molecular markers to facilitate marker assisted selection. Dr. Hwang provided support with respect to activities dealing directly with the pathogen, and led the study on the effect of cropping clubroot resistant canola on pathogen populations in the soil. Working with Dr. Hwang, Dr. Feng conducted the studies to understand the function of differentially expressed host and pathogen genes. In a complementary study, Dr. Selvaraj carried out a transcriptome level analysis of host populations segregating for resistance to the clubroot pathogen. The research team enjoyed a good working relationship throughout the project.

2. Background (max 1 page)

Describe the project background and include the related scientific and development work that has been completed to date by your team and/or others.

Clubroot (*Plasmodiophora brassicae*) is a highly destructive disease of crucifers. The disease is now prevalent on canola (*Brassica napus*) in central Alberta and appears to be spreading into southern regions of the province, as well as into Saskatchewan and Manitoba (Cao et al. 2009; Strelkov and Hwang 2014). Yield losses of 30 to 100% have been observed in severely infected canola crops (Tewari et al. 2005; Strelkov et al. 2007), while oil content and 1000-seed weight were reduced an average of 4.7 to 6.1% and 13 to 26%, respectively (Pageau et al. 2006).

In Canada, the emergence of clubroot as a canola disease has led to large-scale screening of *Brassica* germplasm for clubroot resistance by both public institutions and private industry; these efforts have led to the release of clubroot resistant (CR) canola hybrids by several companies. The resistance in these hybrids, however, is most likely based on single CR-genes, and single gene-based resistance will eventually become eroded or breakdown altogether. Indeed, the fairly complex race or pathotype structure of *P. brassicae* in Alberta suggests that regional pathogen populations could adapt rapidly in response to the selection pressure imposed by the cropping of CR canola, resulting in the loss of the effectiveness of the resistance trait. Therefore, as part of a proactive strategy for durable clubroot resistance, it will be important to: (1) pyramid or stack resistance genes in canola cultivars and (2) rotate resistance genes in clubroot infested fields. It also will be important to understand the molecular basis for resistance and, conversely, for virulence in the pathogen, in order to enable a rational approach to resistance breeding.

This project was aimed at enabling the implementation of such clubroot management strategies, leading to the identification of multiple, independent CR-genes. Establishing the relationship between genes can eliminate duplication of efforts on the same target genes. Knowledge of the relationship between CR-genes also can help to guide crop rotations, allowing farmers to alternate canola cultivars with different sources of resistance if these become available. Moreover, the identification of molecular markers for resistance allows for efficient gene stacking or pyramiding, which is not possible when plants are screened based solely on resistance phenotype. Similarly, information on the molecular mechanisms of clubroot resistance/susceptibility, particularly as it relates to the canola-*P. brassicae* interaction, is valuable for the identification of novel targets for resistance breeding efforts and for the development of rational strategies for clubroot control. Collectively, therefore, this research contributed not only to enhanced knowledge of the genetic and molecular basis for clubroot

resistance in canola, but also to facilitating the development of new and durable varieties of CR-resistant canola.

3. Objectives and deliverables (max 1 page)

State what the original objective(s) and expected deliverable(s) of the project were. Also describe any modifications to the objective(s) and deliverable(s) which occurred over the course of the project.

The project objectives included:

a) Identification of major resistance genes and development of molecular markers
b) Clarification of behavior of these genes in different genetic backgrounds
c) Evaluation of the feasibility of resistance gene pyramiding and rotation
d) Identification and investigation of the biological function of host and pathogen genes differentially expressed during the infection process

The expected deliverables were:

a) Identification of major resistance genes and knowledge of their effectiveness against the predominant pathotypes of *P. brassicae* in western Canada
b) Knowledge of relationship between different sources of resistance to enable rotation of resistance genes in infested fields
c) Improved understanding of basis for resistance and influence of different resistance sources on pathogen soil populations
d) Molecular markers to facilitate marker assisted selection and utilization of resistance genes
e) Understanding of the functionality of a subset of differentially expressed host and pathogen genes, which could serve as rational targets for resistance breeding and fungicide development

4. Research design and methodology (max 4 pages)

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

a) Identification of major resistance genes and resistance to predominant pathotypes of P. brassicae *in western Canada*

Doubled haploid (DH) population development. Two clubroot resistant spring canola lines, 1CA0591.323 and 1CA0591.263, derived from spring canola × winter canola cv. Mendel crosses (Rahman et al. 2011), were crossed with a clubroot susceptible spring canola line A07-26NR. The microspore culture technique, as described by Kebede et al. (2010), was applied on a single plant basis to the F_1 progeny of the A07-26NR × 1CA0591.323 and A07-26NR × 1CA0591.263 crosses for the development of doubled haploid (DH) lines. DH lines developed from these two crosses will be referred to as Popl#1330 and Popl#1333, respectively. This population was evaluated with the *P. brassicae* single-spore isolate SACAN-ss1 (Xue et al. 2008), which is

classified as pathotype 3 on the differentials of Williams (1966) and represents the predominant strain of the clubroot pathogen in Alberta.

Another DH population (n=121) was developed by crossing a clubroot resistant genotype '12-3' and a susceptible genotype '12-1'. Genotype 12-3 is a spring inbred line and inherited its resistance from the European Clubroot Differential (ECD) 04 (Diederichsen & Sacristan 1996). The genotype 12-1, which is highly susceptible to clubroot, also is a spring inbred line. The crossing between the two parents was made in 2012 and the DH population was developed by microspore culture (Maluszynski et al. 2003). These populations were evaluated with five single-spore isolates of *P. brassicae*, corresponding to pathotypes 2, 3, 5, 6 or 8 (Xue et al., 2008) as defined on the differentials of Williams (1966). These represent all pathotypes of *P. brassicae* that have been reported from Canada (Strelkov & Hwang, 2014).

b) Relationship between different sources of resistance to enable rotation of resistance genes in infested fields

Durability of resistance in different host genotypes. Although the inoculation of different host cultivars with pathotype mixtures of *P. brassicae* has been shown to shift the composition of the mixture to the more virulent pathotypes on that host (Jones et al. 1982; Fahling et al. 2003), the effect of clubroot resistance on specific populations and single-spore isolates of *P. brassicae* is untested. To this end, a population (SACAN03-1) and single-spore-derived isolate (SACAN-ss1) of the pathogen, collected in Alberta and previously characterized for virulence and pathotype designation (pathotype = 3) (Strelkov et al. 2006; Xue et al. 2008), were used in a pathogen cycling experiment. Both the population and single-spore isolate were used to inoculate seven different host genotypes exhibiting a range of resistance phenotypes (resistant, moderately resistant, and susceptible) in five successive cycles of inoculation, resting spore extraction, and re-inoculation. The objectives of this study were two-fold: (i) to evaluate the change in virulence of the population and single-spore isolate following five successive infection cycles on the same host cultivars; and (ii) to assess the potential durability of resistance in the current clubroot resistant canola cultivars. A total of seven *Brassica* genotypes were included in the study, namely ECD 02, ECD 04, ECD 05, ECD 15, a canola breeding line (08N823R), and susceptible and resistant commercial hybrids currently on the market (designated CV-S and CV-R, respectively, in this report).

Cross-infectivity of cycled *P. brassicae* isolates and populations. In the second phase of the experiments, the virulence of the cycled isolates and populations described above was tested on other clubroot-resistant canola genotypes. Briefly, four clubroot-resistant canola hybrids available on the Canadian market were inoculated with four populations and three single-spore isolates of *P. brassicae* that had been subjected to five cycles of repeated inoculation, spore extraction, and re-inoculation on the resistant commercial hybrid CV-R, 08N823R, ECD 05 and ECD 15.

c) Improved understanding of the basis for resistance and influence of different resistance sources on pathogen soil populations

RNA sequencing for transcriptome analysis. This component of the work was aimed at obtaining an in-depth understanding of genes that are expressed differentially in resistant and

susceptible canola genotypes during challenge with pathotype 3 of *P. brassicae*. Briefly, 12 resistant and 12 susceptible DH lines of Popl#1333 (produced from the F_1 plants of the cross A07-26NR × 1CA0591.263) were inoculated with *P. brassicae*. Plants were grown in a randomized complete block design and each experiment was carried out in triplicate. Plant tissues (leaves, stems, flower buds, flowers, siliques and roots) were collected at 10 days, 22 days and 42 days post-inoculation (dpi) from individual resistant and susceptible plants as well as the parents. Total RNA was extracted from individual resistant and susceptible lines and sequenced at the National Research Council (NRC), Saskatoon, SK.

RNA analysis of segregating populations. Total RNA was isolated from the resistant and susceptible parents as well as pools of resistant and susceptible DH lines. RNAseq data were analyzed using CLCBio software to get a comprehensive list of differentially expressed genes, which were mapped to the *B. rapa* genome (v.1.2). Data were compared at the primary and secondary infection stages.

Histological and morphological comparisons: host vs. non-host resistance. Although primary infection by *P. brassicae* occurs in many plant species, secondary infection only continues to completion in susceptible hosts. In order to improve understanding of clubroot pathogenesis, secondary zoospores collected from infected root hairs of canola and perennial ryegrass (*Lolium perenne*) were inoculated onto healthy roots of both plant species. The treatments consisted of all possible combinations of the two plant species and the two sources of inoculum. A method for the production and purification of secondary zoospores had to be developed so as to be able to carry out this work. Briefly, the roots of seedlings inoculated with resting spores of *P. brassicae* were dug out from the potting mix 7 days after inoculation and washed with tap water. The foliage was cut off at the soil level, and the roots were rinsed again multiple times with water and incubated with gentle agitation for 20 h in distilled water. The zoospore suspension was centrifuged and 10 samples of the concentrated suspension were examined microscopically to confirm the absence of resting spores. The original suspension was then adjusted to 1×10^4 spores/ml and used immediately for inoculation. Clubroot assessments were made at 5 and 35 days after inoculation (dai). At 5 dai, the roots of the seedlings were examined under a microscope for (1) root hairs with primary infection and the total number of root hairs, (2) the presence or absence of secondary infection, and (3) the total number of secondary plasmodia. At 35 dai, all of the seedlings were harvested and clubroot symptoms and root infection were investigated either visually or microscopically. The experiments were repeated.

Zoospores and primary and secondary infection. In an effort to further elucidate the mechanisms associated with clubroot disease development and resistance, primary and secondary infections were investigated on roots from plants inoculated with resting spores (primary zoospores) and secondary zoospores of *P. brassicae*. Tissue samples were evaluated with a Zeiss AXIO microscope (Carl Zeiss). Five fields of view (using the 20× objective lens) were examined on each sample, and in each field of view, two sets of data were collected: the percentage of root hairs with primary infection, and the total number of secondary plasmodia. Data from each time point were subjected to analysis of variance using the Microsoft Excel add-in DSAASTAT developed by Dr. Andrea Onofri at the University of Perugia, Italy. Differences between and among treatments were assessed using Fisher's LSD test.

Effect of cropping clubroot-resistant canola on *P. brassicae* soil populations. The hybrid canola cultivars 45H29 (clubroot-resistant) and 45H26 (clubroot-susceptible) were grown together to simulate various proportions of susceptible plants (volunteer canola or Brassica weeds) growing within a stand of a clubroot-resistant canola cultivar. Seed of the susceptible and resistant cultivars were mixed together in ratios of 1:0, 3:1, 1:1, 1:3 and 0:1, respectively, and then four rows of 25 seeds each were planted in each experimental unit under greenhouse conditions. Each experimental unit consisted of a plastic tub filled with soilless mix, which was inoculated with resting spores of *P. brassicae* single-spore isolate SACAN-ss1 (pathotype 3).

d) Molecular markers to facilitate marker assisted selection and utilization of resistance genes

Identification of SSR marker(s) linked to CR of 'Mendel'. Genomic DNA from the resistant parents (1CA0591.323 and 1CA0591.263) and susceptible parent (A07-26NR), as well as two bulks of DNA from the resistant and susceptible DH lines were used for bulk segregant analysis (Michelmore et al. 1991). Each of the resistant or susceptible DNA bulks was constituted from equal amounts of DNA from 12 DH lines. The genomic DNA of the parents and the bulks was screened with 370 (212 A genome and 158 C genome) simple sequence repeat (SSR) markers obtained from Agriculture and Agri-Food Canada (AAFC) through a material transfer agreement, and 73 published markers reported to be linked to the nine published CR loci. Thus, a total of 443 markers were screened by bulk segregant analysis. The 73 published markers comprised 11 sequence characterized amplified region (SCAR) markers (Matsumoto et al. 1998, 2012; Hayashida et al. 2008; Ueno et al. 2012), six SCAR or cleaved amplified polymorphic sequences (CAPS) markers (Piao et al. 2004), 40 SSR markers (Zhang et al. 2014; Kato et al. 2012, 2013; Suwabe et al. 2003, 2006), seven insertion/deletion (INDEL) markers (Kato et al. 2012, 2013), two SCAR markers (Matsumoto et al. 2012), and four STS markers (Hirai et al. 2004; Saito et al. 2006; Sakamoto et al. 2008) linked to different CR genes in the A genome of *B. rapa*. Lastly, a RAPD marker identified to be linked to clubroot resistance in the European *B. napus* cv. 'Mendel' also was tested (Diederichsen et al. 2006). To identify the markers linked to the CR gene(s), the polymorphic markers were screened on the parents as well as five resistant and five susceptible DH lines of each population. Markers from this test found to be associated with CR were tested on the remainder of the DH lines.

In addition, primer pairs for 15 markers linked to specific CR genes (*CRa, CRb, CRc, Crr1, Crr2, Crr3*) were synthesized and screened between the parents and between the two bulks of the third DH population (derived from the cross: resistant genotype '12-3' × susceptible genotype '12-1'). The sequences of the primers, as well as detailed information on the corresponding markers, were as reported by Hirai et al. (2004), Piao et al. (2004), Saito et al. (2006), Suwabe et al. (2006), Hayashida et al. (2008), Sakamoto et al. (2008), Ueno et al. (2012) and Gao et al. (2014). Primers specific to the *CRa* gene were designed based on the sequence of *CRa* (GenBank accession number AB751516) (Ueno et al. 2012). The nucleotide binding site (NBS) and leucine rich repeat (LRR) domains of *CRa* were defined based on Ueno et al. (2012).

e) Understanding of the functionality of a subset of differentially expressed host and pathogen genes

Suppression subtractive hybridization. suppression subtractive hybridization (SSH) and expressed sequence tag (EST) analysis were used to investigate gene expression during the early stages of colonization of canola roots by *P. brassicae*. Total RNA was extracted from 100 mg ground powder of root segments collected from healthy or inoculated plants at 7 dai using a NucleoSpin RNA II Kit (Clontech Laboratories). Two micrograms of the obtained total RNA was used to generate double stranded cDNA with a SMARTer PCR cDNA Synthesis Kit (Clontech Laboratories). SSH was performed by using a PCR-Select cDNA Subtraction Kit (Clontech Laboratories), with the protocol being followed starting from the *RsaI* Digestion step. Using the double-stranded cDNA from infected roots as tester and those from healthy roots as driver, subtractive hybridization and suppression PCR were performed. The PCR product was separated on a gel and the band at 250–4000 bp was cut and used to construct the cDNA library.

Sequencing and EST processing. Sequencing PCRs were conducted in BigDye terminator reagent (ABI) following the manufacturer's instructions. Since the cDNAs had been cut by *RsaI* (and were thus expected to be short) and only oneshot sequencing was conducted for each clone, the term 'expressed sequence tag' (EST) is used hereafter to refer to all of the generated sequences. All DNA sequences were checked for quality and then analysed by VecScreen, which identified and removed vector and adaptor sequences. Manual searching and removing of the two nested PCR primer sequences, which were part of the adaptors and had been introduced into the ESTs before SSH, also was conducted to complement the VecScreen analysis. The cleaned sequences were analyzed with the CAP3 program with default parameters to obtain cluster contigs. Identification of EST origin (canola or *P. brassicae*) was performed by similarity searches against the NCBI databases.

Functional annotation by Blast2GO. Functional annotation was performed for all ESTs except those identified by PCR to be *P. brassicae*-originated by using Blast2GO version 2.2.3 (Conesa et al., 2005), following the standard procedure of BlastX for the unigenes dataset, followed by mapping and annotation. GO terms were summarized according to their molecular functions, biological processes and cellular components. Enzyme mapping of the annotated sequences was performed with Direct GO to Enzyme Mapping and used to query the Kyoto Encyclopedia of Genes and Genomes (KEGG) to define the KEGG orthologs.

Gene expression analysis by real-time PCR. Total RNA was extracted from galls, and uninfected and infected roots collected at 7 dai, and used to generate first strand cDNA with an iScript cDNA Synthesis Kit (Bio-Rad Canada). Based on the obtained cDNA, real-time PCR was conducted to confirm the differential expression of 10 selected *P. brassicae* genes and seven canola genes. Primers were designed based on the EST sequences using the Primer3 server. Real-time PCR was run using an intercalating dye-based method (SYBR Green). The comparative CT method was used to analyze and present the data according to Schmittgen & Livak (2008). The canola elongation factor 1α alpha gene (FJ529180) and the *P. brassicae* actin 1 gene (AAR88383) were used for normalization.

5. Results, discussion and conclusions (max 8 pages)

Present the project results and discuss their implications. Discuss any variance between expected targets and those achieved. Highlight the innovative, unique nature of the new knowledge generated. Describe implications of this knowledge for the advancement of agricultural science. For ease of evaluation, please structure this section according to the objectives cited above.
NB: Tables, graphs, manuscripts, etc., may be included as appendices to this report.

a) Identification of major resistance genes and resistance to predominant pathotypes of P. brassicae in western Canada

Production of DH lines. In the case of the cross A07-26NR x 1CA0591.323, a total of 679 seedlings were obtained from 4,180 embryos that were transferred to solid medium. Out of the 679 seedlings transferred to soil, 121 DH lines were produced, giving a success rate of 17.8% for chromosome doubling. With respect to the production of DH lines from the second cross, A07-26NR x 1CA0591.263, a total of 751 seedlings were obtained from 4,657 embryos, and these were transferred to solid medium. Out of the 751 seedlings transferred to soil, 105 DH lines were produced, giving a success rate of 14.0% for chromosome doubling

Evaluation of DH lines for resistance to *P. brassicae* single-spore isolate SACAN-ss1 (pathotype 3). The phenotypic evaluation of resistance to *P. brassicae* pathotype 3 in the DH Popl#1330 was carried out with 101 of the 121 DH lines. This is because 20 DH lines did not produce sufficient seeds for the clubroot resistance test. Among 101 DH lines, 58 were found to be resistant while 43 were susceptible. These DH lines were produced from three F_1 plants; therefore, a test of homogeneity of the DH lines produced from these F_1 plants was done for 1:1 segregation for resistance and susceptibility. Homogeneity Chi-square value was well within the accepted limit (homogeneity $\chi^2 = 1.6655$, $df = 2$, $P = 0.4348$) and thus pooling data of these DH lines was justified. A chi-square test for goodness of fit for the DH lines produced from each F_1 plant, as well as the pooled data, did not deviate significantly from the expected 1:1 segregation ratio for resistance and susceptibility.

In the case of the DH Popl#1333, phenotypic evaluation for resistance to *P. brassicae* pathotype 3 was carried out with 97 out of the 105 lines. This is because 8 DH lines did not produce sufficient seeds for phenotypic screening. Out of 97 lines, 54 were found to be resistant while 43 were susceptible. The DH lines of Popl#1333 were produced from six F_1 plants. The homogeneity chi-square test (homogeneity $\chi^2 = 4.1945$, $df = 5$, $P = 0.5217$) suggested that pooling data of the DH lines produced from these F_1 plants was justified. A chi-square test for goodness of fit for the DH lines produced from different F_1 plants, as well as the pooled data, did not deviate significantly from the expected 1:1 segregation ratio for resistance and susceptibility. This suggests that resistance to *P. brassicae* pathotype 3 in Popl#1330 and Popl#1333 is most likely under monogenic control.

Resistance to single-spore isolates representing pathotypes 2, 3, 5, 6 and 8. The reaction of parents 12-3 and 12-1 was evaluated against five pathotypes (2, 3, 5, 6 and 8) of *P. brassicae*. The parent 12-3 was highly resistant to all pathotypes, developing mean indices of disease (IDs) < 5% in response to inoculation with any of the SSI, whereas the parent 12-1 was highly susceptible to all pathotypes (IDs > 95%). All DH lines and the two parents also were tested

against a single-spore isolate (SACAN-ss1) representing *P. brassicae* pathotype 3. Among the 121 DH lines, 61 were resistant and 60 were susceptible, indicating a 1:1 ratio for segregation of a single gene ($\chi^2 = 0.0083$ and $P = 0.9275$).

b) Relationship between different sources of resistance to enable rotation of resistance genes in infested fields

Durability of resistance in different host genotypes. Various clubroot resistant canola hybrids, produced and sold by different companies, are available on the Canadian market. Collectively, these hybrids represent the most important clubroot management tool available. In many other host/pathogen systems, however, the deployment of resistant cultivars has resulted in the selection of increased virulence in the pathogen population, potentially reducing the durability and stability of resistance. Although the inoculation of different host cultivars with pathotype mixtures of *P. brassicae* has been shown to shift the composition of the mixture to the more virulent pathotypes on that host (Jones et al. 1982; Fahling et al. 2003), the effect of clubroot resistance on specific populations and single-spore isolates of *P. brassicae* is untested. To this end, a population (SACAN03-1) and single-spore-derived isolate (SACAN-ss1) of the pathogen, collected in Alberta and previously characterized for virulence and pathotype designation (pathotype = 3) (Strelkov et al. 2006; Xue et al. 2008), were used in a pathogen cycling experiment. Both the population and single-spore isolate were used to inoculate seven different host genotypes exhibiting a range of resistances (resistant, moderately resistant, and susceptible) in five successive cycles of inoculation, resting spore extraction, and re-inoculation. The objectives of this study were two-fold: (i) to evaluate the change in virulence of the population and single-spore isolate following five successive infection cycles on the same host cultivars; and (ii) to assess the potential durability of resistance in the current clubroot resistant canola cultivars. A total of seven *Brassica* genotypes were included in the study, namely the European Clubroot Differential (ECD) 02, ECD 04, ECD 05, ECD 15, a canola breeding line (08N823R), and susceptible and resistant commercial hybrids currently on the market (designated CV-S and CV-R, respectively, in this report).

The mean ID on both the resistant canola hybrid and the moderately resistant line, 08N823R, increased significantly after the first cycle of inoculation with the single-spore isolate or population of *P. brassicae*. Following this initial shift, however, the ID remained relatively constant. One potential explanation for this phenomenon, at least in the case of CV-R, may be the presence of both major and minor genes for clubroot resistance, previously documented in the *Brassicas*. The major gene resistance may have eroded following the first inoculation cycle, resulting in an increase in ID in the following cycle. The lack of any further changes in ID may be a result of several minor resistance genes conferring partial resistance to *P. brassicae*. A similar pattern was observed for the moderately resistant variety 08N823R. However, given the initial intermediate ID value observed on 08N823R, it is likely that a source of resistance different from that in CV-R was involved.

Cross-infectivity of cycled *P. brassicae* isolates and populations. With only three exceptions, all clubroot-resistant canola genotypes exhibited a similar pattern of either complete resistance (ID = 0%) or an ID value that was not significantly different from zero when inoculated with four populations and three single-spore isolates of *P. brassicae* that had been subjected to five

cycles of repeated inoculation, spore extraction, and re-inoculation on the resistant commercial hybrid CV-R, 08N823R, ECD 05 and ECD 15. The canola genotypes CV-A (ID = 8.6% ± 2.9%), CV-C (ID = 1.9% ± 7.7%) and CV-D (ID = 11.1% ± 9.5%) inoculated with the *P. brassicae* population cycled on CV-R exhibited indices of disease greater than and significantly different from 0.0%, although the extent of erosion of resistance was less than on CV-R itself.

The significance of these tests has increased considerably since the 2013 and 2014 growing seasons, as several fields that had been sown to clubroot resistant cultivars developed higher levels of clubroot than expected in those years. Pathogen collections were made from these 'fields of concern' and evaluated under controlled conditions for increased virulence on the same cultivars from which they were recovered. In several cases, populations of *P. brassicae* from galls on symptomatic plants caused significantly increased levels of clubroot in the greenhouse, compared with a 'wild-type' population that had not been exposed to any resistance sources. As such, the quick erosion or defeat of clubroot resistance that was observed under greenhouse conditions through the pathogen cycling experiments seems to have accurately predicted what would happen under field conditions. The resistance, at least on the present set of clubroot resistant canola cultivars available, does not seem to be durable and will have to be managed carefully. Preliminary results with the field collected strains also indicate that they cause enhanced levels of disease on multiple clubroot resistant genotypes, and exhibit a greater amount of cross-infectivity than did the greenhouse generated strains, suggesting that rotation of clubroot resistant cultivars is not an adequate substitute for rotation with non-Brassica crops. Research is now underway under a separate project to monitor for and characterize these novel strains of *P. brassicae*.

c) Improved understanding of the basis for resistance and influence of different resistance sources on pathogen soil populations

Key features of the resistant and susceptible reactions. At the primary infection stage, there were 877 genes differentially expressed in an inoculated susceptible parent (10DSPI) compared with a control (10DSPU), whereas there were 555 genes differentially expressed in an inoculated, resistant parent compared with the same, un-inoculated parent (10DRPIvsRPU). At the secondary infection stage (i.e., 22DPI), around 250 genes were found to be unique to each data set (22DRPIvsRPU vs. 22DRPIvsSPI), suggesting possible roles for these genes in clubroot resistance. By contrast, 838 genes were present in both datasets (22SPIvsSPU and 22DRPIvsSPI), indicating possible roles in the compatible host-pathogen interaction or involvement in processes not specifically related to pathogenesis. A subset of genes (127-165) possibly playing key roles in clubroot resistance mechanisms has been identified at the transcriptome level.

At the primary infection stage (10DPI), 915 genes were up-regulated and 1,046 genes down-regulated in the inoculated susceptible parent. In the inoculated resistant parent, at the same stage, 984 genes up-regulated and 599 genes were down-regulated relative to the un-inoculated resistant plants. A total of 235 genes were found to be up-regulated, whereas 201 genes were down-regulated when the inoculated resistant parent was compared with the inoculated susceptible parent. The differential expression of these genes indicates that they might play a role in the clubroot resistance mechanism. At the secondary infection stage, even more differences

were found among the treatments. A total of 281 genes were up-regulated and 359 genes were down-regulated in the resistant parent. Collectively, these datasets suggest a 4-fold to 5-fold turnover at the transcriptome level in the susceptible parent as compared with the resistant parent as a result of *P. brassicae* infection. The data also indicate stronger control over alterations in normal metabolic processes in the resistant parent.

Cluster analysis of differentially expressed genes. Cluster analysis was performed using Mapman and Pageman software to obtain a functional categorization of the differentially expressed genes. Wilcoxon Benjamini Hochberg multiple testing corrections were applied and key clubroot resistance and susceptibility genes were classified based on function. Genes involved in phenylpropanoid and lignin biosynthesis were active at the early infection stage in the resistant parent, suggesting a role in the resistance response to *P. brassicae* infection. By contrast, genes involved in cellular biosynthesis, auxin metabolism and calcium signaling were up-regulated in the susceptible parent, a finding that is consistent with the hyperplasia and hypertrophy of the host tissues associated with a susceptible response to infection. At the secondary infection stage, the resistant parent continued to express genes related to the plant defense response (i.e., genes involved in phenylpropanoid synthesis and SA, JA and ethylene signaling).

Histological and morphological comparisons: host vs. non-host resistance. Secondary zoospores collected from infected root hairs of canola and ryegrass were inoculated onto healthy roots of both plant species. The treatments consisted of all possible combinations of the two plant species and the two sources of inoculum. At 5 dai, levels of root hair infection were similar and in a range of 50-68% on roots in all of the treatments. Secondary infection was also observed from all of the treatments, with approximately 50% on canola and 40% on ryegrass. The proportion of secondary infection and the number of secondary plasmodia were higher in canola inoculated with zoospores from canola than in ryegrass inoculated with zoospores from ryegrass, with the other combinations intermediate. At 35 dai, typical clubs developed on 14% of the canola plants inoculated with secondary zoospores from canola, and tiny clubs developed on 16% of the canola plants inoculated with zoospores from ryegrass. Secondary infection occurred in about one-third of ryegrass plants but no clubs developed, regardless of inoculum source. These results indicate that resistance to secondary infection in ryegrass is induced during primary infection. This is the first report that secondary zoospores produced on a nonhost can infect a host and reconfirms that secondary infection can occur in a nonhost.

Zoospores and primary and secondary infection. Resistance to *P. brassicae* in canola has been implicated to occur at various stages of clubroot pathogenesis, including suggestions that in some resistant canola cultivars, infection is inhibited as early as the root hair infection stage. However, it is difficult to draw general conclusions regarding the putative role of such resistance mechanisms without improved knowledge of the relationship and relative importance of the two infection stages (primary and secondary) of *P. brassicae*. Therefore, the early stages of infection of canola roots by the clubroot pathogen were investigated in a histological analysis.

Inoculation with 1×10^5 resting spores mL^{-1} resulted in primary (root hair) infection at 12 hours after inoculation (hai). Secondary (cortical) infection began to be observed at 72 hai. When inoculated onto plants at a concentration of 1×10^4 mL^{-1}, secondary zoospores produced primary

infections similar to those obtained with resting spores at a concentration of 1×10^5 mL^{-1}. Secondary zoospores caused secondary infections earlier than resting spores. When the plants were inoculated with 1×10^7 resting spores mL^{-1}, 2 days following challenge with 1×10^4 or 1×10^5 resting spores mL^{-1}, secondary infections were observed on the very next day, which was earlier than the secondary infections resulting from inoculation with 1×10^7 resting spores mL^{-1} alone, and more severe than those produced by inoculation with 1×10^4 or 1×10^5 resting spores mL^{-1} alone. Compared with the single inoculations, secondary infections on plants that had received both inoculations remained at higher levels throughout a 7-day time-course.

These data indicate that primary zoospores can directly cause secondary infection when the host is under primary infection, suggesting that primary and secondary zoospores may be essentially the same with respect to their ability to cause infection. Based on the present study, we can hypothesize that *P. brassicae* uses primary infection to overcome the basal resistance of the plant to cortical infection.

Effect of cropping clubroot-resistant canola on *P. brassicae* soil populations. Bioassays revealed greater clubroot severity and incidence, and reduced plant height, where 100% of a susceptible cultivar had been grown, as opposed to mixtures of resistant and susceptible canola genotypes. A higher proportion of susceptible plants within a resistant canola crop increased root hair and secondary infections. Regression analysis of root hair infection and the amount of *P. brassicae* DNA (as determined by quantitative PCR) revealed strong linear relationships between the two parameters. The linear relationships between root hair infection and *P. brassicae* DNA were stronger for the resistant cultivar than for the susceptible cultivar when regression analysis was conducted by cultivar over the sampling dates. The total resting spore count in the clubroot galls was greater after growth of a higher proportion of susceptible plants (in a mixture of resistant and susceptible plants). Root hair infection in a susceptible cultivar grown after a mixture of resistant and susceptible canola genotypes also rose in treatments with a greater proportion of susceptible plants. Rates of secondary infection below the epidermis and in the cortex, as estimated by the number of secondary plasmodia, also were greater in the treatments in which a higher proportion of susceptible plants were grown. In conclusion, the cropping of a resistant cultivar reduced clubroot severity, while the presence of susceptible volunteer canola increased inoculum potential. Quantitative PCR was a reliable tool for the quantification of root hair infection.

d) Molecular markers to facilitate marker assisted selection and utilization of resistance genes

Genotypic analysis of the SSR markers used in the bulk segregant analysis. The genotypic analysis of the 212 A genome and 158 C genome markers from AAFC showed that 32.5% and 32.3% of markers produced polymorphic bands, 43.9% and 47.5% markers produced monomorphic bands, whilst 23.6% and 20.2% markers failed to amplify genomic DNA, respectively, in the parents and the DH lines. None of the polymorphic markers from AAFC were found to be linked with the resistance derived from 'Mendel'. In the case of the 73 markers reported to be linked to the nine published CR loci, 38.4% produced polymorphic bands, 49.3% produced monomorphic bands, and 12.3% failed to amplify genomic DNA. Twelve of these 73 (16.4%) markers co-segregated with resistance to *P. brassicae* single spore isolate SACAN-ss1 (pathotype 3) in the two DH populations.

Association between CR of 'Mendel' and published markers. None of the markers reported by Suwabe et al. (2003, 2006), Hirai et al. (2004), Saito et al. (2006), Sakamoto et al. (2008) or Matsumoto et al. (2012) were associated with clubroot disease resistance in the two DH populations. The RAPD marker, reported by Diederichsen et al. (2006) to be linked to CR in 'Mendel', amplified genomic DNA of the parents and the DH lines, but gave monomorphic bands. Genotyping of the DH lines with the markers reported by Piao et al. (2004), Zhang et al. (2014), Matsumoto et al. (1998, 2012), Hayashida et al. (2008), Ueno et al. (2012) and Kato et al. (2012, 2013) revealed that some of the markers were linked to resistance in the DH lines. Among the 13 markers reported by Piao et al. (2004) and Zhang et al. (2014) and screened by the bulk segregant analysis, six detected polymorphism between the parents, five gave monomorphic bands while the remaining two failed to amplify genomic DNA. All the polymorphic markers tested on 200 lines of the two DH populations were not strongly associated with CR of 'Mendel'; recombination frequency between marker alleles and resistance was in the range of 10.5-21.5%.

Genotyping of the parents and the resistant and susceptible bulks with 11 markers reported by Matsumoto et al. (1998, 2012), Hayashida et al. (2008) and Ueno et al. (2012) revealed five markers to be polymorphic between the parents and bulks, three gave monomorphic bands while two failed to amplify genomic DNA. Among the polymorphic markers one marker was linked (recombination frequency of 1.5%) with the CR of 'Mendel' when genotyped on 200 DH lines of the two populations. However, recombination between marker alleles and phenotype for the other four polymorphic markers ranged from 8.0-25.5%.

In the case of the 37 DNA markers reported by Kato et al. (2012 and 2013) and screened by the bulk segregant analysis, 15 markers detected polymorphism between the parents, 17 gave monomorphic bands while five failed to amplify genomic DNA. About a quarter (26.7%) of the polymorphic markers tested on 200 lines of the two DH populations were not strongly associated with clubroot resistance; recombination frequency was in the range of 9.0-24.5%. The other 11 (73.3%) polymorphic markers co-segregated with CR in the DH populations. The recombination frequencies between marker alleles and resistance in the DH lines ranged from 1.5-2.0%.

Variation within the sequence of the *CRa* gene. Fifteen markers linked to the *CRa, CRb, CRc, Crr1, Crr2, Crr3* clubroot resistance loci were screened in the two parents and two bulks from the cross: resistant genotype '12-3' × susceptible genotype '12-1'. The marker BRMS096 linked to *Crr2* (Suwabe et al. 2003) on chromosome A1 (N1) showed polymorphism between the parents but not between the resistant and susceptible bulks. OPC11-2S linked to *Crr3* (Saito et al. 2006) on chromosome A3 (N3) also showed polymorphism between parents, but seven out of 16 individuals showed non-association between genotype and phenotype. GC1680 linked to *CRa* (Ueno et al. 2012) on chromosome A3 (N3) showed polymorphism between the parents and also between the resistant and the susceptible bulks, which was further confirmed on eight resistant and eight susceptible individuals selected from the resistant and susceptible bulks.

Three forward primers (FW3, FW4 and FW5) and three reverse primers (RV3, RV4 and RV6) derived from Ueno et al. (2012) and specific to *CRa* were used to amplify the sequence coding the NBS domain in the two parents, resistant genotype '12-3' and susceptible genotype '12-1'. Amplicons of the same size were obtained from the two parents; the amplicon also was the same

size as the corresponding fragments in the cloned *CRa* (GenBank Accession number: AB751522; Ueno et al., 2012). PCR analysis using the forward primer FW1 and either of the reverse primers RV1, RV2, RV3, RV4, RV5 and RV6 did not amplify any product from the susceptible parent, but amplified products of the predicted sizes from the resistant parent, suggesting that the upstream sequences of the *CRa* locus were different in the genotypes 12-3 and 12-1.

The primer pair FW2/RV2, specific to the LRR domain of *CRa*, did not amplify a band from either of the parents. Thus, two alternative forward primers (F6 and F7) were designed and used to amplify the LRR domain from the parents. Among the various primer combinations between the forward primers F6 and F7 and the reverse primers RV1, RV2, RV3, RV4, RV5 and RV6, the primer sets F6/RV5 and F7/RV5 amplified bands from line 12-3 but not from 12-1, whereas all the other primer combinations generated amplicons of the same size from both parents.

Six primer pairs (e1f/e1r, i1f/i1r, e2f/e2r, i2e3f/i2e3r, i3e4f/i3e4r and e4f/e4r) specific to *CRa* were used to amplify fragments from the two parents, and the resultant fragments were sequenced. The NBS sequence (amplified by primer pair e2f/e2r) from both parents was identical with that of the cloned *CRa* in GenBank (accession no. AB751516). The sequence amplified by primer pair i1f/i1r from line 12-1 did not contain a 16 kb fragment insertion, in contrast to the susceptible genotype Q5 (Ueno et al., 2012). The primer pair e4f/e4r, which flanked the LRR region, amplified a fragment from line 12-3 that was identical to the sequence in GenBank (accession no. AB751516). No band could be amplified from line 12-1 with the primer pair i3e4f/i3e4r or e4f/e4r.

Association between *CRa* markers and clubroot resistance. The primer pair i3e4f/i3e4r was used to detect the polymorphism in the 121 DH individuals. Specific DNA fragments were amplified from 60 of the 61 resistant lines, and two of the 60 susceptible lines. In total, three recombinants were identified in the population. These data indicate that the CR gene under study is not necessarily the *CRa* gene, but rather is another gene linked to *CRa*.

e) Understanding of the functionality of a subset of differentially expressed host and pathogen genes

Analysis of expressed sequence tags derived from a compatible *P. brassicae*-canola interaction. To complement the RNA Seq work described above, suppression subtractive hybridization (SSH) and expressed sequence tag (EST) analyses were used to investigate gene expression during the early stages (7 DPI) of colonization of canola roots by *P. brassicae*. A cDNA library was constructed by SSH which consisted of 797 clones that represented 439 unigenes. Thirty-two of these genes were demonstrated to be of a *P. brassicae* origin, and of these, 24 had not been previously reported. The remaining 407 genes, which were of a canola origin, were subjected to gene ontology and *in silico* analyses, providing a profile of gene expression changes associated with *P. brassicae* infection. The differential expression of 10 and seven selected genes from *P. brassicae* and canola, respectively, was analyzed further by real-time PCR. The results were represented as the ratio between resting spores and *in planta* expression at 7 DPI for the *P. brassicae* genes, and between healthy and inoculated plants for canola genes. Among the 10 *P. brassicae* genes, seven were up-regulated and three were down-related at 7 DPI. One gene showed the most distinct up-regulation and, compared with the

housekeeping gene, was highly expressed both in resting spores and at the 7-DPI infection stages. For the seven canola genes, which were selected based on high sequence similarity to *Arabidopsis* genes previously reported to be differentially expressed during clubroot pathogenesis, five were up-regulated and two were down-regulated. The differential expression observed for these genes suggests that they are important in clubroot pathogenesis or host resistance.

6. Literature cited

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

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7. Benefits to the industry (max 1 page; respond to sections *a)* and *b)* separately)

a) Describe the impact of the project results on Alberta's agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).

The results achieved in this project have provided important information to Alberta's agriculture and food industry. From an immediate perspective, 12 molecular markers were identified that demonstrated robustness in marker assisted selection and will be useful in breeding programs, including in CR gene pyramiding for durable clubroot resistance, as well as in map-based cloning of the genes. In addition, a resistance locus was identified that is linked to *CRa*, with resistance to *P. brassicae* in one of the *B. napus* populations controlled by the combined effect of this new CR gene and *CRa* (with the effect of the former stronger than that of the latter), or controlled by the new CR gene alone. From a medium-term perspective, the information on the durability of resistance and cross-infectivity of *P. brassicae* strains highlighted the need for careful resistance stewardship, in particular longer rotations out of canola in fields where clubroot is an issue. Similarly, the study on the impact of resistant cultivars on soil inoculum levels demonstrated how even relatively small percentages of susceptible plants within a resistant crop can help to maintain inoculum levels, reducing the effectiveness of rotations. These results are being used to formulate recommendations for farmers. From a long-term perspective, significant insights were obtained on the role of primary and secondary infection on clubroot pathogenesis, host and non-host resistance, and molecular and histological changes associated with resistance and susceptibility to *P. brassicae*. This information will facilitate efforts to gain

a better understanding of the mechanisms of host resistance (and pathogen virulence), which will facilitate the rational development of novel and sustainable clubroot management strategies.

b) Quantify the potential economic impact of the project results (*e.g.*, cost-benefit analysis, potential size of market, improvement in efficiency, etc.).

The canola industry contributes an average of $15.4 billion per year to the Canadian economy, including $8.9 billion in farm cash receipts alone (Rempel et al. 2014, Can. J. Plant Pathol. 36 (S1):19-26). Clubroot represents a very significant threat to this industry, as a consequence of potential yield and quality losses (Pageau et al. 2006) and the costs associated with disease management, including forced rotations out of canola and efforts to exclude the pathogen from non-infested fields and regions. Hard numbers are lacking with respect to the total dollar value for losses associated with clubroot in Alberta, but working on the (conservative) assumption that approximately one-quarter of the traditional canola growing area in this province is at risk for the disease, and using a moderate estimate of 25% yield losses, then 25% of one-quarter of the provincial canola cash receipts could be lost, totaling about $44 million per year (M. Hartman, Alberta Agriculture and Forestry, personal communication). As such, research to understand and manage the disease, through genetic and pathology approaches, is an important investment in mitigating the impact of clubroot. The critical financial support received from the Funders for this project ($297,500 per year) would represent approximately a 150-fold return on investment. In addition, the project enabled an increase in provincial research capacity and expertise, including the training of numerous highly qualified personnel.

8. Contribution to training of highly qualified personnel (max ½ page)

Specify the number of highly qualified personnel (*e.g.*, students, post-doctoral fellows, technicians, research associates, etc.) who were involved in the project.

In addition to the deliverables achieved and information obtained, this project played a very important role in the training of 16 highly qualified personnel at the University of Alberta, Alberta Agriculture and Forestry, and the Plant Biotechnology Institute, National Research Council of Canada. These included 2 research associates, 2 post-doctoral fellows, 4 technicians, 4 graduate students, and 4 summer students. Personnel received training in the areas of genetics, host-pathogen interactions, plant breeding, plant pathology, agricultural biotechnology, experimental design and statistical analysis.

9. Knowledge transfer/technology transfer/commercialisation (max 1 page)

Describe how the project results were communicated to the scientific community, to industry stakeholders, and to the general public. Organise according to the following categories as applicable:

a) Scientific publications (*e.g.*, scientific journals); attach copies of any publications as an appendix to this final report.

- Rahman, H., Fredua-Agyeman, R., Kulkarni, M., and Selvaraj, G. xxxx. Studies on the genetic and molecular basis for clubroot resistance in spring canola introgressed from European winter canola cv. Mendel. Euphytica (submitted).

- Zhang, H., Feng, J., Hwang, S.F., Strelkov, S.E., Falak, I., Huang, X., and Sun, R. 2015. Mapping of clubroot (*Plasmodiophora brassicae*) resistance in canola (*Brassica napus*). Plant Pathol., In Press (Available Online), Doi: 10.1111/ppa.12422.
- Feng, J., Cao, T., Rennie, D.C., Strelkov, S.E., and Hwang, S.F. 2014. Host-parasite interactions in clubroot of crucifers. Can. J. Plant Pathol. 36(S1): 113-121.
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- Rahman, H., Peng, G., Yu, F., Falk, K.C., Kulkarni, M., and Selvaraj, G. 2014. Genetics and breeding for clubroot resistance in Canadian spring canola (*Brassica napus* L.). Can. J. Plant Pathol. 36(S1): 122-134.
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- Feng, J., Xiao, Q., Hwang, S.F., Strelkov, S.E., and Gossen, B.D. 2012. Infection of canola by secondary zoospores of *Plasmodiophora brassicae* produced on a nonhost. Eur. J. Plant Pathol. 132: 309-315.

b) Industry-oriented publications (*e.g.*, agribusiness trade press, popular press, etc.) attach copies of any publications as an appendix to this final report

- Dr. Strelkov and the members of the research team were regular contributors of information and data to industry websites, such as the Canola Council of Canada's *clubroot.ca*; information also was disseminated in industry newspapers and magazines, including *The Western Producer*, *Top Crop Manager* and the *Canola Digest*; research updates were provided to the Pathology Sub-Committee of the Western Canadian Canola/Rapeseed Recommending Committee.

c) Scientific presentations (*e.g.*, posters, talks, seminars, workshops, etc.)

- There were a total of 21 scientific presentations over the course of the project. These included: 4 conference proceedings, 6 abstracts, and 11 conference/workshop oral or poster presentations. The presenters included members of the research team, and/or students and other highly qualified personnel affiliated with the project. Presentations were made at regional, national and international meetings as appropriate.

d) Industry-oriented presentations (*e.g.*, posters, talks, seminars, workshops, etc.)

- A total of 11 industry-oriented presentations were made by the team leader, with additional talks given at various meetings by the members of the research team. The venues for these presentations included grower meetings, canola industry meetings, and broader agricultural industry meetings such as FarmTech. In addition, slides and other relevant information were widely distributed to other scientists, Canola Council agronomists, as well as provincial government and industry research personnel.

e) Media activities (*e.g.*, radio, television, internet, etc.)

Information was posted on the *clubroot.ca* website hosted by the Canola Council of Canada, which is billed as "your comprehensive source for clubroot information." In addition, research team members were occasionally interviewed for radio programs such as "Call of the Land", and for television programs such as "FARMGATE" (CTV), were they provided updates on findings and recommendations.

f) Any commercialisation activities or patents

No commercialization activities were undertaken; rather, the choice was made to make all information publicly available as described above.

N.B.: Any publications and/or presentations should acknowledge the contribution of each of the funders of the project.

Section D: Project resources

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

   A statement of award and expenditures will be prepared and forwarded by the Research Services Office of the University of Alberta.

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

2. **Resources:**

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

Total resources contributed to the project		
Source	**Amount**	**Percentage of total project cost**
Funders	$1,190,000	60.7%
Other government sources: Cash	$0	0%
Other government sources: In-kind	$700,000	35.7%
Industry: Cash	$63,635	3.2%
Industry: In-kind	$8,000	0.4%
Total Project Cost	**$1,961,635**	**100%**

External resources (additional rows may be added if necessary)		
Government sources		
Name (only approved abbreviations please)	Amount cash	Amount in-kind
University of Alberta	$0	$400,000
Alberta Agriculture and Forestry	$0	$200,000
AAFC	$0	$100,000
Industry sources		
Name (only approved abbreviations please)	Amount cash	Amount in-kind
DL Seeds	$2,875	$0
Dow	$6,785	$0
Cargill	$2,875	$0
Bayer	$8,875	$0
Monsanto	$6,475	$0
HyTech Prod.	$2,875	$0
Viterra (now Crop Production Services)	$12,875	$8,000
BASF	$20,000	$0

Section E: The next steps (max 2 pages)

Describe what further work if any needs to be done.

a) Is new research required to deal with issues and opportunities that the project raised or discovered but were not dealt with within the current project?
b) Is there related work that needs to be undertaken to continue advancement of the project technology or practice?
c) Did the project identify any new technology or practice that needs to be developed?
d) What suggestions do you have that increase commercial use of results by farmers and/or companies. These may be:
   1. commercial uptake.
   2. further research toward commercial use.
   3. extension and information disbursement.

a) The project clearly demonstrated the capacity of local *P. brassicae* populations to adapt to the selection pressure imposed by the cropping of clubroot resistant canola genotypes. Under greenhouse conditions, genetic resistance was significantly eroded in as little as two or three cycles of exposure to the same single-spore isolate or population of the pathogen. The significance of these results increased over the past two cropping seasons, as new, highly virulent

strains of *P. brassicae* that can overcome the resistance in most canola cultivars were identified in commercial fields. These findings highlighted the need for the development of strategies to manage and understand the evolving strains of *P. brassicae* on canola. This need has been met with the approval of project no. 2015C008R by ACIDF, which commenced in May of this year and will focus precisely on these issues. Additional funds were requested from the Canola Agronomic Research Program (CARP), which would represent matching support for related components of the research.

b) As noted above, related work is now being undertaken through project no. 2015C008R; similarly, findings with respect to the genetics and molecular basis for clubroot resistance have laid a foundation for some of the research activities under the Growing Forward 2 program (AAFC/CCC), which are building on opportunities that this project raised.

c) The project identified numerous new processes and practices. A key process was the ability to produce and purify secondary zoospores for inoculation purposes. This not only allowed dissection of the roles of primary vs. secondary zoospores in host/non-host resistance and clubroot pathogenesis as described in this report, but also will facilitate mechanistic studies into the genetic control of resistance against each of these stages by specific resistance genes and quantitative trait loci.

d) Open lines of communication with farmers and industry are critical to the uptake of the information provided in this report. This has been facilitated by stakeholders such as the Canola Council of Canada, which has been communicating information to growers and the canola industry in general. The research team also has been active in dissemination of results through various venues as indicated in the “Knowledge transfer/technology transfer/commercialisation”, and cooperates with numerous industry partners on clubroot-related issues, which further facilitates use of the results by farmers and companies.

Section F: Research Team Signatures and Employers' Approval

The team leader and an authorised representative from his/her organisation of employment MUST sign this form.

Research team members and an authorised representative from their organisation(s) of employment MUST also sign this form.

By signing as representatives of the research team leader's employing organisation and/or the research team member's(s') employing organisation(s), the undersigned hereby acknowledge submission of the information contained in this final report to the funder(s).

Team Leader's Organisation

Team Leader	
Name: **Stephen Strelkov**	**Title/Organisation:** **Professor/University of Alberta**
Signature: 	**Date:** **September 28, 2015**
Team Leader's Employer's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**

Research Team Members (add more lines as needed)

1. Team Member	
Name: **Habibur Rahman**	**Title/Organisation:** **Professor/University of Alberta**
Signature:	**Date:**
Team Member's Employer's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**

2. Team Member	
Name: **Sheau-Fang Hwang**	**Title/Organisation:** **Scientist/Alberta Agriculture and Forestry**
Signature:	**Date:**
Team Member's Employer's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**

3. Team Member	
Name: **Jie Feng**	**Title/Organisation:** **Scientist/Alberta Agriculture and Forestry**
Signature:	**Date:**
Team Member's Employer's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**
4. Team Member	
Name: **Gopalan Selvaraj**	**Title/Organisation:** **Scientist/National Research Council – Plant Biotechnology Institute**
Signature:	**Date:**
Team Member's Employer's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**