Pathogen profile

***Plasmodiophora brassicae*: a review of an emerging pathogen of the Canadian canola (*Brassica napus*) crop**SHEAU-FANG HWANG^{1,*}, STEPHEN E. STRELKOV², JIE FENG¹, BRUCE D. GOSSEN³ AND RON J. HOWARD⁴¹Crop Diversification Centre North, Alberta Agriculture and Rural Development, Edmonton, AB, Canada, T5Y 6H3²Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, T6G 2P5³Agriculture and Agri-Food Canada Research Centre, Saskatoon, SK, Canada, S7N 0X2⁴Crop Diversification Centre South, Alberta Agriculture and Rural Development, Brooks, AB, Canada, T1R 1E6**SUMMARY**

Plasmodiophora brassicae causes clubroot disease in cruciferous plants, and is an emerging threat to Canadian canola (*Brassica napus*) production. This review focuses on recent studies into the pathogenic diversity of *P. brassicae* populations, mechanisms of pathogenesis and resistance, and the development of diagnostic tests for pathogen detection and quantification.

Taxonomy: *Plasmodiophora brassicae* is a soil-borne, obligate parasite within the class Phytomyxea (plasmodiophorids) of the protist supergroup Rhizaria.

Disease symptoms: Clubroot development is characterized by the formation of club-shaped galls on the roots of affected plants. Above-ground symptoms include wilting, stunting, yellowing and premature senescence.

Disease cycle: *Plasmodiophora brassicae* first infects the root hairs, producing motile zoospores that invade the cortical tissue. Secondary plasmodia form within the root cortex and, by triggering the expression of genes involved in the production of auxins, cytokinins and other plant growth regulators, divert a substantial proportion of plant resources into hypertrophic growth of the root tissues, resulting in the formation of galls. The secondary plasmodia are cleaved into millions of resting spores and the root galls quickly disintegrate, releasing long-lived resting spores into the soil. A serine protease, PRO1, has been shown to trigger resting spore germination.

Physiological specialization: Physiological specialization occurs in populations of *P. brassicae*, and various host differential sets, consisting of different collections of *Brassica* genotypes, are used to distinguish among pathotypes of the parasite.

Detection and quantification: As *P. brassicae* cannot be cultured, bioassays with bait plants were traditionally used to detect the pathogen in the soil. More recent innovations for the detection and quantification of *P. brassicae* include the use of antibodies, quantitative polymerase chain reaction (qPCR) and

qPCR in conjunction with signature fatty acid analysis, all of which are more sensitive than bioassays.

Resistance in canola: Clubroot-resistant canola hybrids, recently introduced into the Canadian market, represent an important new tool for clubroot management in this crop. Genetic resistance must be carefully managed, however, as it has been quickly overcome in other regions. At least three resistance genes and one or two quantitative trait loci are involved in conferring resistance to *P. brassicae*. Root hair infection still occurs in resistant cultivars, but secondary plasmodia often remain immature and unable to produce resting spores. Fewer cell wall breakages occur in resistant hosts, and spread of the plasmodium through cortical tissue is restricted. More information on the genetics of clubroot resistance in canola is needed to ensure more effective resistance stewardship.

Useful websites: <http://www.canolacouncil.org/clubroot/resources.aspx>, http://tu-dresden.de/die_tu_dresden/fakultaeten/fakultaet_mathematik_und_naturwissenschaften/fachrichtung_biologie/botanik/pflanzenphysiologie/clubroot, <http://www.ohio.edu/people/braselto/plasmos/>

INTRODUCTION

The obligate parasite *Plasmodiophora brassicae* Woronin causes clubroot, an economically important disease of plants in the Brassicaceae family. Clubroot is known to occur in more than 60 countries and results in a 10%–15% reduction in yields on a global scale (Dixon, 2009). In Canada, the impact of clubroot historically has been greatest on cruciferous vegetables, with the most significant outbreaks of the disease restricted to regions of the country in which vegetable production predominates, including British Columbia, Ontario and Quebec (Howard *et al.*, 2010). The disease was not identified on the vast canola (*Brassica napus* L.) crops of the Canadian prairies until 2003, when 12 clubroot-infested commercial fields (less than 800 ha) were found in the central part of the province of Alberta (Tewari *et al.*, 2005). Since

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that initial report, the number of fields with confirmed clubroot infestations has increased steadily, and, by 2010, more than 560 fields (over 35 000 ha) in Alberta had been identified as being infested with *P. brassicae* (Strelkov *et al.*, 2011). Although the outbreak remains largely confined to the central part of the province, some cases of the disease have also been reported in southern Alberta (Cao *et al.*, 2009). The presence of *P. brassicae* inoculum, but not the disease itself, was also confirmed recently in one field in the neighbouring province of Saskatchewan (Dokken-Bouchard *et al.*, 2010). As such, *P. brassicae* poses an emerging threat to the 4.7-million-hectare canola industry in the Canadian prairie region.

Given the potentially devastating impact of clubroot on affected crops, the appearance of this disease on canola has been a cause for concern. Yield losses of 80%–91% were reported in studies with canola grown on clubroot-infested fields in Quebec (Pageau *et al.*, 2006). Seed quality was also reduced significantly, with declines of 4.7%–6.1% in oil content and 13%–26% in 1000-seed weights. In Alberta, estimated yield losses in severely infected canola crops have ranged from 30% to 100% (Hwang *et al.*, 2010; Strelkov *et al.*, 2007), and have resulted in legislated control of *P. brassicae* under the Alberta Agricultural Pests Act. A coordinated research effort has been launched in Canada in order to develop a better understanding of the biology, dissemination and management of clubroot, especially in the canola production systems of the prairies. This review paper focuses on recent research activities, including studies into the pathogenic diversity of *P. brassicae* populations, mechanisms of pathogenesis and resistance, and the development of diagnostic tests for pathogen detection and quantification in the context of the recent outbreak of clubroot in western Canada.

DISEASE SYMPTOMS AND CAUSAL ORGANISM

Clubroot is characterized by the development of galls on infected roots (Fig. 1), which disrupt water and nutrient uptake in affected plants (Dixon, 2006), resulting in wilting, stunting and premature ripening of the above-ground organs (Fig. 2). When infection is severe, yield and quality losses in cruciferous crops can be significant. Each large gall contains millions of resting spores that can persist in the soil for up to 20 years (Wallenhammar, 1996).

Together with other plant pathogens of the genera *Polymyxa* and *Spongospora*, *Plasmodiophora* comprises the order Plasmodiophorida, which belongs to the class Phytomyxea (plasmodiophorids) (Cavalier-Smith and Chao, 2003). On the basis of phylogenetic analyses of the small subunit ribosomal RNA genes (Bulman *et al.*, 2001; Castlebury and Domier, 1998; Ward and Adams, 1998) and other protein-coding genes (Archibald and Keeling, 2004), the class Phytomyxea is currently considered to constitute part of the protist supergroup Rhizaria within the phylum Cercozoa and the Endomyxa (Neuhauser *et al.*, 2011). The

Fig. 1 A severe case of root galling as a result of *Plasmodiophora brassicae* infection in which the affected region extends above the soil line.

Fig. 2 A severe clubroot infection in canola at early flowering. Note the wilting, premature ripening and stunting of plants.

Plasmodiophorida possess a peculiar form of closed mitosis, known as cruciform nuclear division, in which a persistent nucleolus elongates perpendicular to the condensed metaphase chromosomes (Braselton *et al.*, 1975). Because they produce spores and

have been studied intensively by mycologists from the perspective of plant disease, the plasmodiophorids have historically been classified as fungi (Waterhouse, 1972).

The pathogen has a complex life cycle comprising three stages: survival in the soil as resting spores, root hair infection and, finally, cortical infection (Ayers, 1944; Ingram and Tommerup, 1972; Naiki and Dixon, 1987). As the life cycle of *P. brassicae* has been reviewed in detail recently (Kageyama and Asano, 2009), only an outline is presented here. Resting spores, which are the primary inoculum of the pathogen, are capable of surviving for up to 20 years, with a half-life of about 4 years (Wallenhammar, 1996). They germinate to release one oval-shaped or pyriform biflagellate motile spore (Ayers, 1944), known as a primary zoospore. These zoospores require a film of water in the soil to swim to and infect root hairs by penetration of the cell wall. Infection of root hairs, which initiates the primary infection stage, is followed by the formation of primary plasmodia within the root hair. The primary plasmodia cleave into zoosporangia, each containing 4–16 secondary zoospores, which are released into the soil. Primary infections do not cause macroscopic symptoms and are not responsible for significant yield and quality losses (Howard *et al.*, 2010). The secondary zoospores, which initiate the secondary infection stage, penetrate the root epidermis of the host and invade the cortical tissues of the main roots. Secondary zoospores cannot be visually differentiated from primary zoospores. Binucleate zoospores are sometimes found and are interpreted as having been formed by the zoosporangium. After the zoospores are released, the empty zoosporangia remain in the root hairs. Secondary infections lead to the development of secondary plasmodia within cells of the root cortex, resulting in the recognizable symptoms of the disease, i.e. club-shaped malformations of the roots (Kageyama and Asano, 2009). Each secondary plasmodium will eventually be cleaved into millions of resting spores within the root gall. As the root tissues disintegrate, the resting spores are released into the soil to complete the disease cycle. The pathogen usually completes one cycle per growing season in western Canada and cannot spread rapidly in the soil, as zoospore motility is limited.

HOST RANGE

All 330 genera and 3700 species of the family Brassicaceae are possible hosts of *P. brassicae*, but few studies on host range have been conducted outside the genera *Brassica*, *Raphanus* and *Arabidopsis* (Dixon, 2009). Commonly cultivated *Brassica* host species include all varieties of *B. oleracea* L. (Brussels' sprouts, cabbage, cauliflower, kale and kohlrabi), *B. rapa* L. (turnip, turnip rape and Chinese cabbage), *B. napus* L. (rutabaga or swede turnip, oil seed rape, mustard and canola), and all crops derived from *B. carinata* A. Braun, *B. nigra* L. and *B. juncea* (L.) Czern. and *Raphanus sativus* L. (radish). Cruciferous weeds, such as shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.] and stinkweed (*Thlaspi*

arvense L.), are also susceptible to infection by *P. brassicae* (Buczacki and Ockendon, 1979).

PHYSIOLOGICAL SPECIALIZATION

Physiological specialization has long been known to occur in *P. brassicae* (Honig, 1931), with strains of the pathogen differing in their ability to infect specific host genotypes. Traditionally, strains of the pathogen were referred to as 'races' but, as the differential hosts and the pathogen (in cases in which populations rather than single-spore isolates of *P. brassicae* are examined) lack the genetic uniformity necessary to apply the concept of races (Parlevliet, 1985) to the clubroot pathosystem, the term 'pathotype' has been suggested instead (Crute *et al.*, 1980; Voorrips, 1996). Accordingly, in this review, strains of *P. brassicae* differing in virulence will be referred to as 'pathotypes' regardless of the authors' original terminology.

There has been a strong effort to characterize the virulence of *P. brassicae* from canola in western Canada, and studies have examined populations (Cao *et al.*, 2009; Strelkov *et al.*, 2006, 2007) as well as single-spore isolates (Xue *et al.*, 2008) of the pathogen. This work has revealed that pathotype 3, P₂ or ECD 16/15/12, as classified on the differentials of Williams (1966), Somé *et al.* (1996) and the European Clubroot Differential (ECD) set (Buczacki *et al.*, 1975), respectively, is predominant in Alberta (Cao *et al.*, 2009; Strelkov *et al.*, 2006, 2007; Xue *et al.*, 2008). Other pathotypes of *P. brassicae* have also been detected, particularly when single-spore isolates rather than field populations of the pathogen were examined (Strelkov *et al.*, 2006; Xue *et al.*, 2008). Therefore, although pathotype 3 represents 87% of the populations and 72% of the single-spore isolates characterized from Alberta thus far, pathotypes 2, 5, 6 and 8 have also been identified (Howard *et al.*, 2010). This indicates that clubroot-resistant canola germplasm will need to be well managed, through appropriate crop rotation and deployment strategies, as the pathotype composition of *P. brassicae* populations can shift rapidly in response to the selection pressure imposed by resistant host cultivars (Diederichsen *et al.*, 2009).

The fairly large amount of variability observed in the virulence of *P. brassicae* on all three differential sets (Somé *et al.*, 1996; Williams, 1966; and the ECD set) suggests that these differentials do not reflect the full range of pathogenic diversity in pathogen populations from Canadian canola (Howard *et al.*, 2010). This is not surprising as these differentials were originally developed to study pathogen populations from Europe or vegetable brassicas. Therefore, the development of a differential system for the Canadian context may be of great value in resistance breeding and disease management efforts. A coordinated effort has been initiated by several Canadian research groups to assess a large number of host genotypes for their suitability for inclusion in a new differential set for *P. brassicae* strains from canola.

MOLECULAR BASIS OF PATHOGENICITY

An improved understanding of the mechanisms of *P. brassicae* pathogenesis could contribute to the development of novel sources of resistance and other control measures. However, the nature of *P. brassicae* as an obligate parasite hampers the application of the majority of techniques for the study of molecular mechanisms of pathogenesis. To date, there have been only a few reports on the molecular characterization of *P. brassicae* genes expressed during growth of the pathogen in host tissues (Bulman *et al.*, 2006, 2007; Feng *et al.*, 2010; Siemens *et al.*, 2009).

Although the plasmodiophorids are an important pathogen group, information on their genomic makeup is almost completely lacking. To date, approximately 100 partial cDNA fragments or full-length clones of *P. brassicae* have been isolated. Graf *et al.* (2004) isolated a few partial cDNAs that had no significant homology to GenBank sequences. Suppression subtractive hybridization (SSH) has been conducted between RNA from *P. brassicae*-infected and uninfected *Arabidopsis* [*Arabidopsis thaliana* (L.) Heynh] tissue and, from the resulting library, 232 clones were screened. In addition, an oligo-capping procedure was used to screen 305 full-length cDNA clones from the infected tissue (Bulman *et al.*, 2006). A total of 76 new *P. brassicae* gene sequences were identified, the majority of which were extended to full length by rapid amplification of cDNA ends (RACE). Many of the unisquences were predicted to contain signal peptides for translocation of the encoded proteins. Again using SSH, but starting with *B. rapa* rather than *Arabidopsis* clubroot galls, Sundelin (2008) isolated about 140 genes. Half of these gene sequences originated from the pathogen. Of these, 10 clones were newly characterized *P. brassicae* genes. The remainder of the *P. brassicae* genes had been identified previously, particularly from the work of Bulman *et al.* (2006), revealing the consistency of results between these two SSH studies.

Only a few genes have been postulated or demonstrated to be related to the pathogenicity of *P. brassicae* (Table 1). Among others, a gene encoding a serine protease (*PRO1*) has been proven experimentally to be important for resting spore germination (Feng *et al.*, 2010). *PRO1* is a single-copy gene present in a broad

range of *P. brassicae* pathotypes. *PRO1* transcript was detected during plant infection, and the quantity of transcript fluctuates according to the stage of pathogenesis. *PRO1* exhibited high activity at a temperature of 25 °C and a pH of 6.0–6.4, whereas Dixon (2009) indicated that the optimal temperature for germination of resting spores was 24 °C and the optimal pH was 6.0–6.7. *PRO1* protein enhanced the stimulating effect of the root exudates on resting spore germination. This indicates that *PRO1* may play a role in clubroot pathogenesis by stimulating resting spore germination through its proteolytic activity. Because of this apparent role in pathogenesis, *PRO1* is a potential target for clubroot management, as inhibition of the expression of this single-copy gene could eliminate the corresponding function without interference from other gene copies (Feng *et al.*, 2010).

HOST METABOLISM AND SYMPTOM DEVELOPMENT

One of the most significant symptoms of *P. brassicae* infection is the formation of large galls or swellings on the roots of susceptible hosts. Studies of pathogen-induced changes in host metabolism and symptom development have been hampered, however, by the difficulties associated with working with this obligate parasite. Early work on the metabolic changes resulting from infection by *P. brassicae* revealed that sugars, such as glucose, fructose, mannose and trehalose, are found at much higher levels in infected hypocotyls than in healthy plants, whereas the concentration of galactose is lower (Keen and Williams, 1969). More recently, transcriptomic and proteomic approaches have been employed to elucidate the mechanisms of *P. brassicae* infection and gall formation. Two of these studies (Devos *et al.*, 2006; Siemens *et al.*, 2006) analysed changes in gene expression and protein content, respectively, in *Arabidopsis*, whereas the third (Cao *et al.*, 2008) examined changes in protein composition in *B. napus*.

Siemens *et al.* (2006) chose two time points to analyse *P. brassicae*-induced changes in *Arabidopsis* gene expression: 10 days after inoculation (dai), when colonization by the pathogen had occurred but symptoms had not developed, and 23 dai, when root morphology had been extensively altered and galls had formed. Using a full-genome Affymetrix chip, more than 1000

Table 1 Identified genes with a potential function in the pathogenicity of *Plasmodiophora brassicae*.

Gene name	Genbank accession number	Description	Reference
<i>Y10</i>	AB009880	Expression exclusively correlated with the vegetative plasmodial stage	Ito <i>et al.</i> (1999)
<i>PbTPS</i>	Unknown	A trehalose-6-phosphate synthase gene with expression correlated with an accumulation of trehalose in resting spores	Brodmann <i>et al.</i> (2002)
<i>PbSTKL1</i>	AB231687	Expression increased strongly beginning 30 days after inoculation and coincident with resting spore formation	Ando <i>et al.</i> (2006)
<i>PbBrip9</i>	EU345432	Strongly expressed at disease stages corresponding to the occurrence of sporulating plasmodia	Siemens <i>et al.</i> (2009)
<i>PbCC249</i>	AF539801		
<i>PRO1</i>	GU082362	A serine protease that stimulates resting spore germination	Feng <i>et al.</i> (2010)

Arabidopsis genes were found to be differentially expressed at each time point in infected vs. noninfected roots. Differentially expressed genes included those associated with defence, sugar phosphate metabolism, plant growth and the cell cycle. Genes involved in auxin homeostasis were upregulated in response to infection, whereas those involved in cytokinin homeostasis were downregulated, providing support for the involvement of plant growth regulators in clubroot development (Siemens *et al.*, 2006). A key role for cytokinins was further suggested by the observation that cytokinin oxidase/dehydrogenase-overexpressing lines of *Arabidopsis* were resistant to *P. brassicae*.

The proteomic studies employed two-dimensional gel electrophoresis and quantitative image analysis in conjunction with mass spectrometry-based protein identification to analyse changes in protein abundance at 4 dai in *Arabidopsis* (Devos *et al.*, 2006), and at 0.5, 1, 2 and 3 dai in *B. napus* (Cao *et al.*, 2008). In *Arabidopsis*, 12% of the visualized proteins exhibited an altered abundance in infected vs. noninfected roots, including proteins involved in cell defence, cell differentiation and detoxification of reactive oxygen species (ROS) (Devos *et al.*, 2006). In *B. napus*, changes in the levels of 20 proteins could be detected after infection by *P. brassicae*. These included proteins with roles in lignin biosynthesis, cytokinin metabolism, glycolysis, intracellular calcium homeostasis, as well as the detoxification of ROS (Cao *et al.*, 2008). Interestingly, the ROS detoxification system appeared to be overwhelmed in the first hours (0.5 dai) after infection, but recovered by 1–2 dai.

Collectively, these transcriptomic and proteomic studies, together with other research, revealed that clubroot infection is associated with the initial promotion of root growth and auxin production, before the growth of secondary plasmodia (Devos *et al.*, 2005). As galls begin to form, they become a major metabolic sink for auxins and cytokinins. In addition, the expression of auxins is reduced and that of auxin hydrolases is increased during gall development (Schuller and Ludwig-Müller, 2006). It is these disturbances in the levels of plant growth regulators that are largely responsible for the hypertrophy and hyperplasia observed in infected root tissues. Ultimately, the root galls function as strong metabolic sinks in infected plants, drawing nutrients and other resources away from the shoots and leaves (Ludwig-Müller *et al.*, 2009), and contributing to above-ground symptom development. Additional research, however, will be required to dissect the exact impact and significance of many of these metabolic changes, particularly with respect to the eventual outcome of the interaction between host and pathogen.

PATHOGEN DIAGNOSIS AND QUANTIFICATION

One of the key challenges in *P. brassicae* research and clubroot disease management has been the absence of rapid and reliable methods for the detection and quantification of the pathogen in

soil and biological material. Clubroot infestation has historically been detected by means of plant bioassays, in which bait plants are grown in the suspect soil for a 5–6-week period and then examined for the presence of root galls (Faggian and Strelkov, 2009). Although this approach represents a reliable method to detect moderate to high levels of viable *P. brassicae* inoculum, it is time consuming, labour intensive, prone to the effects of environmental conditions and requires fairly large amounts of glasshouse space. Moreover, although bioassays may provide an indication of the relative amount of pathogen in soil samples, they cannot provide a quantitative measure of infestation, nor can they be applied to host tissue or in circumstances in which soil material is limited. In recent years, therefore, numerous other approaches have been developed to detect clubroot in plant and soil samples.

Alternative diagnostic approaches have included microscopic examination of the root hairs to assess the incidence of root hair infection (Hwang *et al.*, 2011; MacFarlane, 1952), as well as fluorochrome staining of the *P. brassicae* resting spores to distinguish them from soil particles (Takahashi and Yamaguchi, 1988, 1989). These techniques, however, can also be time consuming and must be conducted by trained research personnel. Serological methods to detect *P. brassicae* have also been developed (Lange *et al.*, 1989; Wakeham and White, 1996), but, if based on polyclonal antiserum, the results obtained could be influenced by variation in the specificity and sensitivity of the antiserum used. The development of serological assays based on monoclonal antibodies may represent a desirable alternative to polyclonal-based assays, as monoclonal antibodies show improved specificity and are potentially available in almost infinite quantities (Faggian and Strelkov, 2009).

More recently, various diagnostic tests based on the polymerase chain reaction (PCR) have been developed for the detection of *P. brassicae*. Most of these protocols have targeted the ribosomal genes (rDNA) and internal transcribed spacer (ITS) regions of the pathogen genome (Cao *et al.*, 2007; Chee *et al.*, 1998; Faggian *et al.*, 1999; Wallenhammar and Arwidsson, 2001), although one test was based on the amplification of an isopentenyltransferase-like gene (Ito *et al.*, 1999). In PCR-based detection protocols, *P. brassicae*-specific primers and reaction conditions are developed to ensure that they amplify DNA only from the pathogen. If appropriately validated, these tests can provide a rapid and reliable method to determine whether or not *P. brassicae* DNA is present in a variety of samples. In western Canada, one such PCR-based protocol developed by Cao *et al.* (2007) has been commercialized by a diagnostic laboratory and is used for the routine detection of *P. brassicae* in plant and soil materials submitted by farmers, municipalities and industry personnel. This test, which represents the first one-step PCR-based protocol for the detection of the pathogen, can consistently detect 100 fg or less of *P. brassicae* DNA, which corresponds to 1×10^3 resting spores per

gram of soil, or an index of disease of 11% or lower, when the soil is bioassayed (Cao *et al.*, 2007). Although providing consistent results, this protocol, like all other earlier PCR-based tests, provides an indication only of the presence or absence of the pathogen, rather than the amount of inoculum present in a particular sample.

In contrast, the advent of quantitative PCR (qPCR) technologies has enabled the quantification of *P. brassicae* in a manner that was not previously possible. In a recent study, a qPCR assay was used, in conjunction with the analysis of signature fatty acids, for the *in planta* quantification of *P. brassicae* (Sundelin *et al.*, 2010). The amounts of arachidonic acid (20:4) from whole-cell fatty acid analysis and DNA from qPCR were found to be well correlated in infected plants, suggesting that these parameters may represent useful tools for studies requiring the quantification of the pathogen. Similarly, a qPCR-based protocol has been developed for the quantification of *P. brassicae* resting spores that occur as external contaminants of seeds and tubers of various crops (Rennie *et al.*, 2011), in order to assess the risk posed by such inoculum. Assays for the quantification of *P. brassicae* based on qPCR methodologies have the potential to greatly facilitate many aspects of clubroot-related research, as primers and protocols could be modified as necessary to meet the specific requirements of particular studies.

GENETIC RESISTANCE

With the exception of *B. juncea* and *B. carinata*, genotypes with resistance to one or more pathotypes of *P. brassicae* can be found in all of the major brassica crops (Diederichsen *et al.*, 2009). Both qualitative (Crute *et al.*, 1980; Wit and van de Weg, 1964) and quantitative (Chiang and Crête, 1970; Figdore *et al.*, 1993; Grandclément and Thomas, 1996; Voorrips, 1996) types of resistance have been reported. Most of these sources of resistance, however, are pathotype specific.

Early studies indicated that at least three dominant, pathotype-specific resistance genes were located in the brassica A genome. These were termed A, B and C by Wit and van de Weg (1964) and *Pb1*, *Pb2* and *Pb3* by James and Williams (1980); however, the correspondence of their gene positions could not be demonstrated. More recent work on *B. rapa* revealed that between one and three major genes and one quantitative trait locus (QTL) are involved in conferring resistance to *P. brassicae*. The major genes were named *CRa* by Matsumoto *et al.* (1998), *CRb* by Piao *et al.* (2004), *Crr3* by Hirai *et al.* (2004), and *Crr1* and *Crr2* by Suwabe *et al.* (2003, 2006). Furthermore, an additional QTL was identified by Suwabe *et al.* (2006) and termed *Crr4*. Therefore, a total of eight resistance loci were mapped to the A genome. Whether the genes that have been identified to date are homologous, however, is unknown.

Some accessions of *B. oleracea* with pathotype-independent resistance to *P. brassicae* have also been reported (Voorrips,

1996). Indeed, most studies on the C genome have revealed that clubroot resistance in *B. oleracea* is quantitative and under polygenic control by one or two major QTLs and some QTLs with minor effects (Figdore *et al.*, 1993; Grandclément and Thomas, 1996; Landry *et al.*, 1992; Moriguchi *et al.*, 1999; Nomura *et al.*, 2005; Rocherieu *et al.*, 2004; Voorrips *et al.*, 1997). A few studies, however, have indicated that clubroot resistance in *B. oleracea* is qualitative and controlled by either dominant (Chiang and Crête, 1983) or recessive (Yoshikawa, 1993) genes. It is possible that both quantitative and qualitative resistance mechanisms may be at play in this species.

In *B. napus*, most studies have found oligogenic control of resistance to *P. brassicae* (Crute *et al.*, 1980). If this is true, the pyramiding of resistance genes in *B. napus* genotypes will be more practical than in other species. Gustafsson and Fält (1986) proposed models based on three, four and five resistance genes, where the most favoured model was based on four genes. A complex type of inheritance, with dominant genes from *B. rapa* and recessive genes from *B. oleracea*, can be expected in a resynthesized *B. napus*, with resistance from both ancestral species (Diederichsen *et al.*, 1996). Segregation analysis has suggested that resistance in resynthesized *B. napus* is controlled by at least two dominant and unlinked genes (Diederichsen and Sacristán, 1996). Manzanares-Dauleux *et al.* (2000) located one major gene (*Pb-Bn1*) for resistance against two *P. brassicae* isolates on chromosome N03, as well as one additional minor QTL for each isolate on chromosomes N12 and N19.

Populations of *P. brassicae* that can overcome most of the commercially available sources of resistance have been present in Europe for decades. Such pathogen populations appeared in Chinese cabbage crops in Japan only a few years after the introduction of clubroot-resistant cultivars (Kuginuki *et al.*, 1999). European populations of *P. brassicae* display great genetic and pathogenic variation, and therefore can quickly overcome resistance sources from *B. rapa* and *B. oleracea*. Resistance genes from stubble turnips (*B. rapa*) have been used in resistance breeding of various brassica crops, including Chinese cabbage, oilseed rape and *B. oleracea*. Although most turnip lines carry more than one resistance gene, cultivars of the other brassica crops with resistance derived from turnip generally carry a single, dominant resistance gene that is pathotype specific. For example, breeding programmes for clubroot resistance in oilseed rape in Canada in the 1980s utilized nonspecific resistance from *B. rapa* (syn. *B. campestris*), which was backcrossed into cultivars of *B. napus* possessing pathotype-specific resistance genes (Vigier *et al.*, 1989).

The assessment of the impact of genetic resistance on the frequency of primary (root hair infection) and secondary (root) colonization of Chinese cabbage demonstrated that root hair infection occurred more frequently with a compatible isolate (susceptible reaction) compared with an incompatible isolate

(resistant reaction) and, subsequently, fewer cells within the root became infected when the host was resistant (Tanaka *et al.*, 2006). The compatible isolate formed secondary plasmodia with many nuclei and, eventually, resting spores in the host root tissue, whereas plasmodia formed by the incompatible isolate remained immature with only a small number of nuclei and no production of resting spores. A similar response was observed in resistant and susceptible canola lines (Hwang *et al.*, 2011). These results suggest that resistance in these host species is associated with the suppression of infection and subsequent plasmodial development during both primary and secondary colonization.

In contrast, primary and secondary infection and development were observed in both resistant and susceptible lines of *B. oleracea*. Symptoms of cortical invasion by *P. brassicae* in resistant and susceptible hosts included cell wall breaks, the presence of vesicles or inclusion bodies within the cell walls, cell wall thickening in association with plasmodesmata, and enlarged and/or disorganized host nuclei (Donald *et al.*, 2008). The main difference between the resistant and susceptible host reactions was that, in the resistant hosts, the secondary thickening and cell walls of the xylem were not degraded. This study supports previous reports of an amoeboid form of the pathogen, in addition to the recognized two-phase life history of *P. brassicae*. Furthermore, it indicates that resistance in *B. oleracea* does not prevent the development of this amoeboid form. Instead, the reduced number of cell wall breakages in the resistant line suggests that movement of the amoeboid form is restricted, but not prevented, in the resistant host (Donald *et al.*, 2008). Studies to determine whether the differences in the mechanism of resistance observed in *B. napus* and *B. oleracea* are gene specific or host specific are currently underway (B. D. Gossen and M. R. McDonald, unpublished).

The recent introduction of genetically resistant canola hybrids into the Canadian market represents an important clubroot management tool for farmers. As a consequence of proprietary considerations, however, the nature of the resistance in these various cultivars is unknown. This has complicated efforts aimed at resistance stewardship, as it is not possible to develop rational strategies for the rotation of resistance sources in infested fields. Moreover, the number of resistance genes that are available to breeders at the present time is limited (Hirai, 2006), and substantial genetic and pathotype variation is present in the *P. brassicae* populations of western Canada (Cao *et al.*, 2009; Strelkov *et al.*, 2006; Xue *et al.*, 2008). The deployment of a cultivar with single-gene resistance against a genetically diverse pathogen on a large scale imposes a strong selection pressure for pathogen genotypes that are able to overcome this resistance. Resistance to *P. brassicae* in cruciferous crops, including oilseed rape, has broken down in the past (Kuginuki *et al.*, 1999; Seaman *et al.*, 1963); therefore, genetic resistance should be only one component of an integrated clubroot management strategy for canola on the Canadian prairies.

CONCLUSIONS

In less than a decade, clubroot of canola has emerged as one of the most important diseases of this crop in western Canada. From a dozen *P. brassicae*-infested fields, representing less than 800 ha, in 2003, the pathogen has spread to more than 560 fields, representing over 35 000 ha, in central Alberta, and has also been identified in southern regions of this province, as well as in the neighbouring province of Saskatchewan. A concerted research effort in western Canada has built on earlier work conducted in other parts of the world, contributing to an enhanced understanding of clubroot and its causal agent. Although much progress has been made, many questions remain unanswered, and farmers' capacity to manage this disease is still not complete. The advent of novel technologies will undoubtedly facilitate additional research, further accelerating progress in this important area. The sequencing of the *P. brassicae* genome, for instance, will serve to advance our understanding of pathogen biology and host-pathogen interactions by facilitating the identification of *P. brassicae* effectors, the development of molecular markers and the detection of resistance genes in host genotypes. Ultimately, however, successful control of *P. brassicae* will require a multifaceted approach, making use of all available tools and knowledge to mitigate the devastating impact of this pathogen on cruciferous crops such as canola.

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