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Insights into fighting against blackleg disease of *Brassica napus* in Canada

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Abstract. Blackleg disease, caused by the ascomycete fungal pathogen *Leptosphaeria maculans*, is a devastating disease of canola (*Brassica napus*) in Australia, Canada and Europe. Although cultural strategies such as crop rotation, fungicide application, and tillage are adopted to control the disease, the most promising disease control strategy is the utilisation of resistant canola varieties. However, field populations of *L. maculans* display a high evolutionary potential and are able to overcome major resistance genes within a few years, making disease control relying on resistant varieties challenging. In the early 1990s, blackleg resistance gene *Rlm3* was introduced into Canadian canola varieties and provided good resistance against the fungal populations until the early 2000s, when moderate to severe blackleg outbreaks were observed in some areas across western Canada. However, the breakdown of *Rlm3* resistance was not reported until recently, based on studies on *R* genes present in Canadian canola varieties and the avirulence allele frequency in *L. maculans* populations in western Canada. The fact that *Rlm3* was overcome by the evolution of fungal populations demands canola breeding programs in Canada to be prepared to develop canola varieties with diversified and efficient *R* genes. In addition, frequent monitoring of fungal populations can provide up-to-date guidance for proper resistance genes deployment. This literature review provides insights into the outbreaks and management of blackleg disease in Canada.

Additional keywords: avirulence, *Leptosphaeria maculans*, resistance.

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Introduction

During the past three decades, the cultivation and production of canola (*Brassica napus*, oilseed rape) have grown rapidly and canola has become the second most important oilseed crop, after soybean, with an estimated production of 67.91 million tonnes globally in 2016 (USDA 2017). In Canada, canola is the number one cash crop, with a production of 18.4 million tonnes in 2016 (Statistics Canada 2016). Canola is mainly cultivated in the western provinces of Saskatchewan, Alberta and Manitoba, with a low to substantial amount of the crop grown in other provinces. Canola contributed $26.7 billion to the Canadian economy in 2016. Annually, Canada exports 90% of canola seeds, oil and meals to ~55 foreign markets worldwide (Canola Council of Canada 2017). In 2015, Canada exported 3.97 million tonnes of canola seeds to China, one of the most important export markets. To ensure continuous growth of the Canadian canola industry, the Canola Council of Canada established a new strategic plan, 'Keep it Coming 2025', to encourage an annual production of 26 million tonnes of canola by 2025.

Blackleg caused by the fungal pathogen *Leptosphaeria maculans* is the most severe disease of canola, causing more than $900 million economic losses per growing season worldwide (Fitt *et al*. 2008). In western Canada, blackleg caused up to 50% yield losses in individual fields during the 1980s, when blackleg susceptible variety, Westar was widely cultivated (Juska *et al*. 1997). Following the first wave of major blackleg outbreaks in the Canadian prairies, blackleg-resistant varieties were released in the early 1990s. Until the early 2000s, blackleg disease was well controlled by using resistant varieties and 4-year crop rotations (Kutcher *et al*. 2013). However, due to the favourable economic returns through canola, many growers adopted 2-year rotations or even grew canola in successive years across the prairies. This led to the erosion of blackleg resistance in some fields since 2002 and became widespread by 2012 (Hwang *et al*. 2016). This literature review summarises the outbreaks and management of blackleg disease in Canada.

Blackleg disease caused by *Leptosphaeria maculans*

Until 2001, strains of *L. maculans* were classified into two pathotypes: the highly virulent, aggressive 'A' group strains that cause stem cankers on canola, and the nonaggressive, weakly virulent, 'B' group strains that do not cause stem cankers on canola (Williams and Fitt 1999). Later, 'A' pathotype isolates were divided into different pathogenicity groups (PG) according to the differential *B. napus* varieties test, whereas 'B' pathotype isolates (PG1 group) were classified as another species, termed *L. biglobosa* (Shoemaker and Brun 2001; Kuusk *et al*. 2002; Chen and Fernando 2006). *Leptosphaeria biglobosa* species were divided into six subclades

and a few of them including *L. biglobosa* 'canadensis', *L. biglobosa* 'brassicae' and *L. biglobosa* 'thlaspii' were present in Canada (Mendes-Pereira *et al*. 2003). To date, these two species have been found to coexist in North America, Australia and Europe, whereas only *L. biglobosa* has been identified in China (West and Fitt 2005; Fitt *et al*. 2006; Magyar *et al*. 2006; Karolewski *et al*. 2007; Brazauskiene *et al*. 2011; Zhang *et al*. 2014).

Leptosphaeria maculans has been recorded on crucifers since 1791, but the severe damage to *Brassica* species was only recorded in the last four decades (Rouxel and Balesdent 2005). The fungus can survive on infected stems or other parts of crop residues for several years in the form of mycelia, pycnidia and pseudothecia (West *et al*. 2001; Li *et al*. 2007*b*). *Leptosphaeria maculans* is able to attack nearly all parts of the plant, including cotyledons, leaves, stems, roots and pods, and cause leaf lesions and stem cankers (Fig. 1). *Leptosphaeria maculans* has both sexual and asexual stages on host plants and can either be monocyclic or polycyclic depending on the source of inoculum (Li *et al*. 2007*a*). In the case of ascospores as the primary inoculum, the disease is considered as monocyclic. However, the disease may be considered as polycyclic when pycnidiospores are the primary inoculum or as secondary inoculum (Li *et al*. 2007*a*). The period of ascospore release varies from region to region and generally coincides with the emergence of young plants (Savage *et al*. 2013). Ascospores are released in June in western Canada (Guo and Fernando 2005), May in Australia (Khangura *et al*. 2001) and late September–early October in western and central Europe (Huang *et al*. 2005). The epidemiology of blackleg differs between continents and regions because of differences in climate, growing season, cultivars and especially fungal populations (West *et al*. 2001; Fitt *et al*. 2006). Although the incidence of seed infection by *L. maculans* and *L. biglobosa* is relatively low, seed-borne inoculum is a major concern in transporting *L. maculans* into countries where *L. maculans* has not been identified, such as China (Fitt *et al*. 2006; Zhang *et al*. 2014; Fernando *et al*. 2016; Van de Wouw *et al*. 2016*a*, 2016*b*).

In Europe, ascospore showers are believed to be the major inoculum (Fitt *et al*. 2006). In Australia, the major inoculum of blackleg is ascospores, in combination with pycnidiospores (Barbetti 1976; Marcroft *et al*. 2004). In western Canada, pycnidiospores are the most important sources of inoculum in infection and disease development (Petrie 1995; Guo and

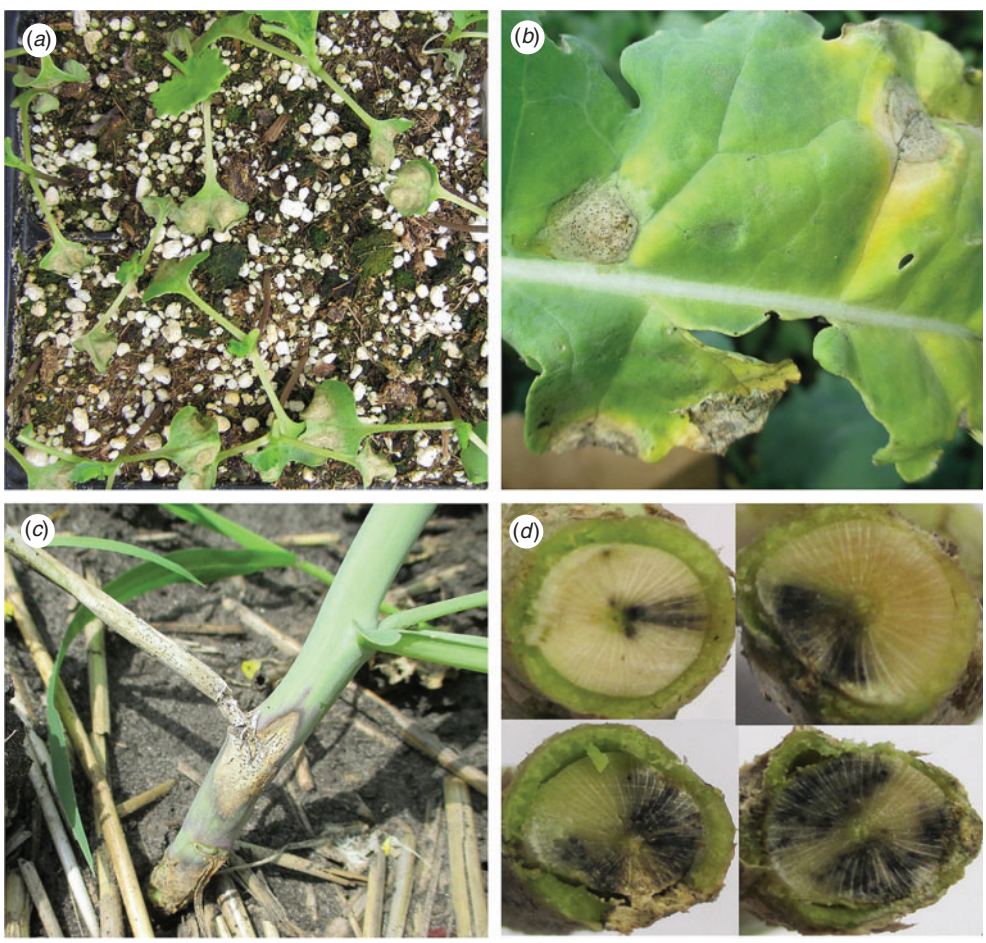


Fig. 1. Disease symptoms caused by blackleg (*Leptosphaeria maculans*) on canola. Disease lesions on (*a*) cotyledons and (*b*) leaves. The pathogen grows from leaves towards (*c*) stems and colonises the (*d*) stem base.

Fernando 2005; Ghanbarnia *et al*. 2011; Dilmaghani *et al*. 2013). Ascospores are mainly dispersed by wind thus can travel long distances, whereas pycnidiospores can only travel short distances by rain-splash. Therefore, in Australia, the recommended distance between canola fields is more than 400 m as canola plants grown within 400 m are in higher risk of infection than that of more than 400 m (Marcroft *et al*. 2004). In western Canada, however, the recommended distance between canola fields is at least 50–100 m to reduce the impact of inoculum movement (Guo and Fernando 2005). After harvest, infected plant residues remain in the fields and supply inoculum for the following season. The life cycle of *L. maculans* in western Canada is illustrated in Fig. 2.

Blackleg disease in Canada

The first wave of severe blackleg outbreaks in the 1980s

In Canada, *L. maculans* was first identified in Saskatchewan in 1975 (McGee and Petrie 1978), and later in Manitoba, Alberta and British Columbia (Gugel and Petrie 1992). Prior to the 1970s, only *L. biglobosa* was identified in Canada and blackleg was not a major concern in rapeseed production. First widespread blackleg disease was observed in 1982, when blackleg caused 6% yield losses in Saskatchewan, with the highest of 56% yield losses in some fields (Juska *et al*. 1997). Westar, a variety that performed much better than all previously registered varieties but very susceptible to blackleg, was widely cultivated in Canada during 1984 and the late 1980s (Juska *et al*. 1997). Lack of blackleg management experience combined with the incentives and pressures to achieve higher canola production, Canadian growers adopted tight rotations to grow blackleg susceptible variety Westar. This led to accumulation of infected stubbles in the field and wide spread of severe blackleg outbreaks in the Canadian prairies. In 1987, yield losses from blackleg reached 10% in Alberta. Similarly, blackleg caused 10% yield losses in Manitoba in 1988 (Juska *et al*. 1997). In 1989, disease incidence of blackleg reached 52% in Saskatchewan (Fig. 3).

Fighting against blackleg disease in the early 1990s

When the entire canola industry was threatened by severe blackleg outbreaks in the 1980s, strategies such as the application of cultural practices, development of disease-resistant varieties, and fungicide applications were adopted by growers. Westar was abandoned in 1991 and new varieties lower in yield but resistant to blackleg were released and cultivated in the early 1990s (Kutcher *et al*. 2010*a*). Since 1995, many blackleg resistant varieties such as Quantum, Q2, Hi-Q, and Conquest were released. It is now known that most of these varieties carried the same single resistance gene, *Rlm3*. In 1994, fungicides became available for blackleg control in Canada (Juska *et al*. 1997). At the same time, cultural practices such as crop rotation, deep tillage, delayed seeding, and seed testing for blackleg infestation were applied in disease control. These strategies largely contributed to the reduced disease incidence and disease severity. For example, in the early 1990s, provincial canola yield losses from blackleg declined to 1% in Alberta (Gugel and Petrie 1992).

Blackleg resistance erosion in Canada

Breeding for blackleg resistance is fundamental to successful disease management (Li and McVetty 2013). In *B. napus*, there

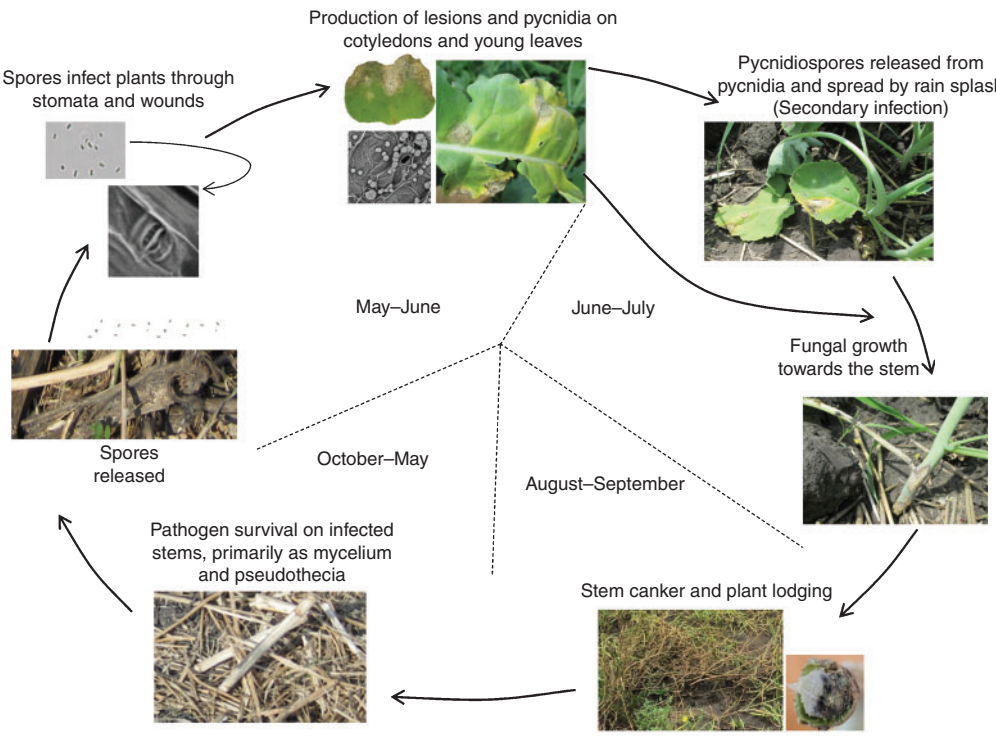


Fig. 2. Life cycle of *Leptosphaeria* maculans in western Canada. Ascospores are mainly released in June.

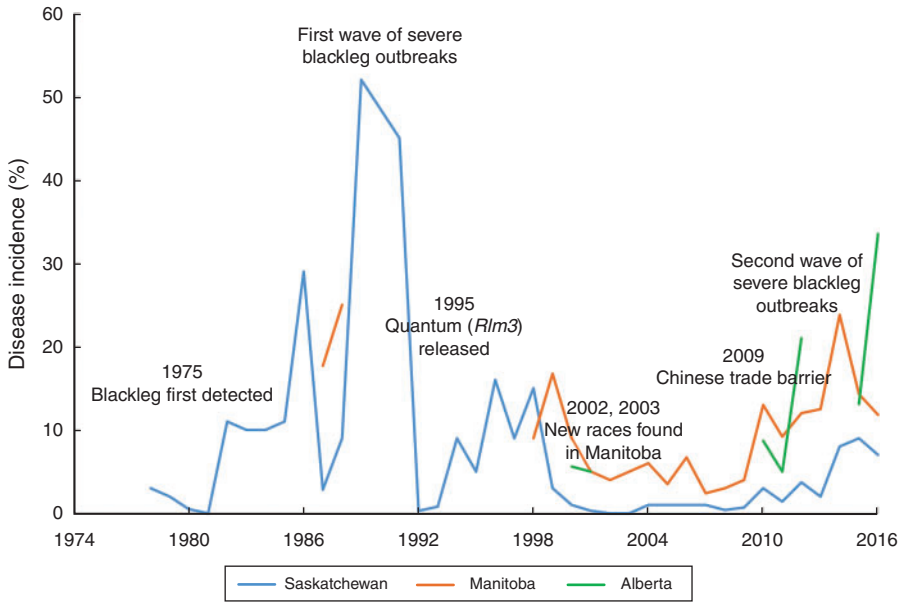


Fig. 3. Incidence of blackleg disease in western Canada (Saskatchewan, Manitoba, and Alberta), 1975–2016. Year 1990 was removed from horizontal axis as data is not available. Data was obtained from Canadian Plant Disease survey (http://phytopath.ca/publication/cpds/, accessed 27 June 2017) and Juska *et al*. (1997).

are two types of resistance against blackleg, qualitative resistance (*R* gene, major gene) mediated by single major genes and quantitative resistance (adult plant resistance) controlled by multiple genetic factors (quantitative trait loci - QTL) (Rimmer 2006; Raman *et al*. 2013). Although blackleg-resistant varieties have been released since the early 1990s and all commonly grown varieties and high erucic acid rapeseed have moderate to high level of blackleg resistance, the erosion of resistance in some fields was identified in 2002 and 2003, when severe infection was observed in blackleg-resistant varieties. In recent years, Canadian plant disease survey results suggested blackleg incidence is on the rise from 2010 to 2016 (Fig. 3). However, it is difficult to determine which genes have been broken down due to resistance genes and resistance types in these varieties were generally unknown until recently. A study conducted in 2012 revealed the presence of *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm9*, *RlmS*, *LepR1 and LepR2* in Canadian canola varieties, with *Rlm3* gene being predominant (Zhang *et al*. 2016). This study further identified *Rlm1*, *Rlm2* and *Rlm3* were the top three most frequently used *R* genes in Canadian *B. napus* varieties. In addition, the study also revealed the presence of moderate to high level adult plant resistance in more than 50% of Canadian canola varieties (Zhang *et al.* 2016). In the fungal populations, however, the frequency of the corresponding avirulence gene of *Rlm3*, *AvrLm3* was very low (Liban *et al*. 2016; Zhang *et al*. 2016; W. G. Dilantha Fernando, unpubl. data). More specifically, low frequency of the *AvrLm3* allele was observed in 2010 and 2011, followed by a very low frequency or lack of *AvrLm3* allele in fungal populations in 2012, 2013, 2014 and 2015 (Liban *et al*. 2016; Zhang *et al*. 2016; W. G. Dilantha Fernando, unpubl. data). These findings strongly suggested the erosion of *Rlm3* in western Canada.

The race shift in L. maculans *populations*

Prior to 2005, *L. maculans* isolates were classified into PG2, PG3, PG4, and PGT based on their interaction phenotypes on a few differential *B. napus* varieties, including Glacier (*Rlm2* and *Rlm3*), Quinta (*Rlm1* and *Rlm3*), and Westar (no resistance). However, a few limited PG cannot fully illustrate population variations of the pathogen. To better address population variation of *L. maculans*, a new term, race structure was introduced by Balesdent *et al*. (2005) to describe population structures of *L. maculans* populations. To date, at least 14 avirulence (*Avr*) genes have been identified in *L. maculans* and a few of them have been cloned (Liban *et al*. 2016). Knowledge on these *Avr* genes has largely enabled molecular and phenotypic methods for the analysis of race structures in field *L. maculans* populations.

The genetic diversity and complexity of the *L. maculans* population in western Canada has changed over time. Although varieties with moderate to high levels of blackleg resistance were cultivated in the Canadian prairie, a shift in the *L. maculans* populations became evident in the early 2000s. All isolates collected from Manitoba and Saskatchewan in 1991 belonged to the PG2 group (Kutcher *et al*. 1993). PG2 isolates remained the most common isolates found in western Canada until the year 2000, but new PGT isolates were identified in isolates collected between 1998 and 2000, and a new PG3 isolate was detected in Manitoba in 1999 (Fernando and Chen 2003; Chen and Fernando 2006; Rimmer 2006). Population structure analysis of *L. maculans* isolates collected between 1997 and 2005 in western Canada demonstrated high frequency of a few avirulence genes such as *AvrLm4*, *AvrLm6* and *AvrLm7* (Kutcher *et al*. 2010*b*). Dilmaghani *et al*. (2009) reported a high frequency of *AvrLm3* and *AvrLm9* in 2005 and 2006 fungal populations in western Canada. However, the frequency of *AvrLm3* and *AvrLm9*

decreased to a very low level in 2010 and 2011 as reported by Liban *et al*. (2016).

Since 2012, blackleg incidence in Manitoba and Alberta were more than 10%. In Manitoba, the highest disease incidence was observed in 2014, 23.8% of plants surveyed showed stem canker. Lower level (less than 5%) of blackleg incidence was observed in Saskatchewan until 2013, but it increased since 2014. To better understand the second severe outbreak of blackleg disease, W. G. Dilantha Fernando (unpubl.) conducted a study to assess disease incidence and avirulence allele distribution of *L. maculans* populations in Manitoba, Canada from 2010 to 2015. Among fungal populations, high frequencies of *AvrLm2*, *AvrLm4*, *AvrLm5*, *AvrLm6*, *AvrLm7*, *AvrLm11*, and *AvrLmS* alleles were detected, whereas low frequencies or lack of *AvrLm1*, *AvrLm3*, *AvrLm9*, *AvrLepR1*, and *AvrLepR2* alleles were observed. From 2010 to 2015, a decrease in the frequency of *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm9*, and *AvrLepR1* alleles was identified, which indicated defeat of the corresponding *R* genes. A total of 180 races were identified in 964 isolates, with three major races, AvrLm-2-4-5-6-7-11, AvrLm-2-4-5-6-7-11-S, and AvrLm-1-4-5–6-7-11-(S), accounting for 24.9% of the isolates. The decrease in the frequency of these avirulence alleles could be explained by the strong selection pressure exerted by these *R* genes in Canadian canola varieties.

Trade barriers due to blackleg disease

Blackleg disease can cause trade barriers on canola seeds exports to major markets. Due to the potential risk of introducing blackleg disease into China, in 2009, restrictions on Canadian canola exports to China was imposed by the Chinese government, resulting in reduced canola exports to China from 3.1 million tonnes in 2009 to 1.5 million tonnes in 2010 (Canola Council of Canada). Following the trade issues triggered by blackleg in 2009, the canola industry and government of Canada have supported many research projects to achieve a science-based solution to mitigate losses and risks from blackleg disease. These research projects covered almost all aspects of the canola supply chain from host resistance (novel resistance genes, defence mechanism), fungal population genetics, and agronomic practices through to seed transportation and processing. In 2016, Canadian and Chinese governments agreed to continue their discussion on a permanent science-based solution for blackleg issues (Canola Council of Canada).

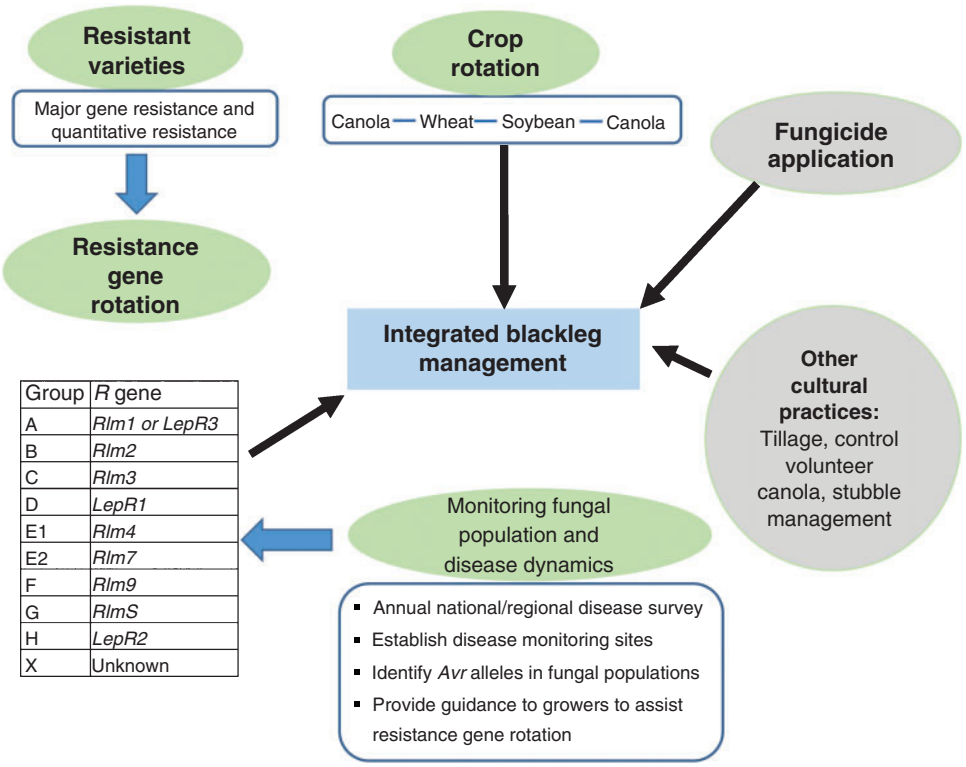
Integrated blackleg management strategies

In spite of the effectiveness of resistance genes in disease control, rapid erosion of blackleg resistance in commercial crops due to the increase in the frequency of the virulent isolates has been reported. In France, *Rlm1* resistance was overcome within 5 years of release of *Rlm1*-carrying varieties (1996–1999) (Rouxel *et al*. 2003). Similarly, in Australia, 'sylvestris' resistance (*Rlm1* and *LepR3*) was lost within 3 years of commercial release in the Eyre Peninsula (Sprague *et al*. 2006). This is not unusual as there is a typical boom and bust cycle in blackleg resistance under field conditions (Rouxel *et al*. 2003; Brun *et al*. 2010; Delourme *et al*. 2014). The phenomenon that a well performing variety with single major resistance gene is grown over a large area is described as the boom phase of the cycle. For example, in western Canada, a typical boom phase is the early 1990s to the 2000s, when *Rlm3*-carrying canola varieties were widely grown and blackleg was well controlled by genetic resistance. Extensive use of *Rlm3* led to changes in the pathogen population, resulting in the increase in disease severity, or breakdown of the resistance. The bust cycle then comes when the variety was not grown in the field, and the frequency of virulent isolates decrease over time (Brun *et al*. 2010; Delourme *et al*. 2014). In western Canada, the finding that *Rlm3* was overcome by the evolution of fungal populations further highlighted the high evolutionary potential of the pathogen (Zhang *et al*. 2016). This is not surprising as *L. maculans* has a mixed reproduction system, and avirulence genes are located in unstable genomic regions (McDonald and Linde 2002; Soyer *et al*. 2014).

Foliar fungicide applications have been proven to be of limited value to maintain canola yield (Huang *et al*. 2011; Liu 2014). A few studies have investigated the effect of fungicide on *L. maculans* and *L. biglobosa*, and most of these studies revealed that *L. maculans* was more sensitive to fungicides than *L. biglobosa* (Griffiths *et al*. 2003; Eckert *et al*. 2010; Huang *et al*. 2011). Among different *L. maculans* isolates, variations in sensitivity to QoI fungicides (fungicides with the action mode of action of Quinone outside inhibitor) were observed in Canada (Liu 2014). The timing of fungicide application is crucial in blackleg control as the fungicides are not able to control the disease once the pathogen has reached the stem (Steed *et al*. 2007; Peng *et al*. 2012; Liu 2014). Although foliar fungicides have been shown to reduce disease severity and increase yield in blackleg susceptible canola varieties, there is no economic benefit of using fungicide in resistant canola varieties (Bailey *et al*. 2000; Liu 2014). Reduction of disease with yield gain on MR or R-rated cultivars can only be achieved when there is severe erosion of resistance in a cultivar due to pathogen shifts (from *Avr* to *avr*) (Liu 2014). Although foliar fungicide products including pyraclostrobin (Headline®, BASF), propiconazole (Tilt®, Syngenta) and azoxystrobin (Quadris®, Syngenta) are available, growers in western Canada only consider in applying fungicide when the pathogen caused significant production issues (Peng *et al*. 2012). In Australia, azole-based fungicides were widely used in seed treatments between 2005 and 2014, foliar fungicide (prothioconazole + tebuconazole) for in-crop *L. maculans* control was not available until 2012. Unlike Canada, the use of a seed-dressing fungicide in Australia has been shown to gain an economic yield benefit (Marcroft and Potter 2008).

R-gene rotations and resistance groups

Marcroft *et al*. (2012) demonstrated that rotation of *R* genes can minimise disease pressure by manipulating fungal populations. Since 2012, resistance group(s) based on their *R*-gene complement has been assigned to all commercial canola varieties in Australia. This information is updated and released biannually to growers in the GRDC Blackleg Management Guide (Van de Wouw *et al*. 2016*b*). To understand the performance of each resistance group across canola-growing regions in Australia,



Group	*R* gene
A	*Rlm1 or LepR3*
B	*Rlm2*
C	*Rlm3*
D	*LepR1*
E1	*Rlm4*
E2	*Rlm7*
F	*Rlm9*
G	*RlmS*
H	*LepR2*
X	Unknown

Fig. 4. Integrated blackleg management strategy.

disease monitoring sites have been established and assessed for blackleg disease. This allows GRDC to provide a warning to growers if high level of disease severity is observed in the resistant group. Rotations of cultivars with different components of resistance genes have become evidently effective, but it requires the identification of resistance genes in commercial canola cultivars (Marcroft *et al*. 2012). The combination of major gene resistance and quantitative resistance to *L. maculans* in canola varieties is able to provide improved durability of blackleg resistance (Brun *et al*. 2010; Marcroft *et al*. 2012; Delourme *et al*. 2014). Similarly, it is possible to apply an *R*-gene rotation strategy in the Canadian prairie to control blackleg, given the growing understanding and knowledge of host resistance in canola varieties, pathogen avirulence in *L. maculans* populations, and their interactions. Research scientists and the industry are interested in adopting this strategy to better control the disease, however more efforts are required to develop varieties with diversified *R* genes and understand *Avr* alleles in fungal populations. In February 2017, the Western Canada Canola and Rapeseed Recommending Committee adopted this strategy in principle, so seed companies could use a resistance group on their label. If *R*-gene rotation strategy is available, there is a need to develop an integrated blackleg management strategy to maximise effectiveness of the *R*-gene rotation strategy (Fig. 4). In this integrated strategy, crop rotation is essential to reduce fungal inoculum, whereas fungicide application and tillage could be taken into consideration in some cases, a prudent *R*-gene rotation strategy based on good understanding of resistant varieties and fungal population and disease dynamics is the key to successful blackleg disease management.

Conclusions and future prospects

In Canada, the *Rlm3* gene has successfully protected the canola industry from blackleg disease during the early 1990s to the early 2000s. However, breakdown of *Rlm3* was observed due to the high evolutionary potential and emergence of new races in the blackleg fungal populations. At a recent Western Canada Canola and Rapeseed Recommending Committee meeting in Saskatoon, Canada, a decision was made to introduce new blackleg resistance labels on varieties to introduce an *R*-gene rotational strategy in Canada. Based on known *R* genes, and assigned to groups, these labels will offer more detail on a variety's resistance package. Such labels have successfully been used in other countries helping the growers with less breakdown of resistance in their canola varieties, and allowing the growers and the seed industry to manage the disease through genetics. The authors feel that it is a step forward in the right direction in the reduction of disease caused by *L. maculans* in canola/rapeseed. Deployment of canola varieties with diversified known *R* genes or novel resistance genes, and ideally, with the combination of quantitative resistance is of great significance for public and private breeding programs. To facilitate a proper and effective utilisation of *R* genes in disease control, it is important to monitor *R* genes in canola varieties and *Avr* allele frequency in field fungal populations. For the long-term, integrated disease control strategies with the efficient utilisation of resistance genes, *R*-gene

rotation, crop rotation, and fungicide application need to be deployed.

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