

Canadian Journal of Plant Pathology

ISSN: (Print) (Online) Journal homepage: <https://www.tandfonline.com/loi/tcjp20>

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To cite this article: ARIA Dolatabadian, Justine Cornelsen, SHUANGLONG Huang, Zhongwei Zou & W.G. DILANTHA Fernando (2022) Sustainability on the farm: breeding for resistance and management of major canola diseases in Canada contributing towards an IPM approach, Canadian Journal of Plant Pathology, 44:2, 157-190, DOI: [10.1080/07060661.2021.1991480](https://doi.org/10.1080/07060661.2021.1991480)

To link to this article: <https://doi.org/10.1080/07060661.2021.1991480>



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Published online: 16 Nov 2021.



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Sustainability on the farm: breeding for resistance and management of major canola diseases in Canada contributing towards an IPM approach

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Abstract: Genetic diversity is vital for the survival of any population. If humans were all the same, a single strain of a nasty flu virus, like COVID-19, could wipe us all out! In plants, genetic diversity plays a similar role. The variation in the type and number of resistance gene(s) between individuals can cause the difference between surviving a disease or not. Studies on genetic diversity will lead to the identification of novel disease-resistance genes. Canola (*Brassica napus* L.) is an economically and nutritionally important oilseed worldwide. Several serious diseases, including blackleg, clubroot, sclerotinia stem rot, and verticillium stripe, threaten canola production in Canada and worldwide. Traditional methods are not enough for effective control of these diseases. Therefore, the ideal approach is to optimize and utilize the resistance genes found in different *B. napus* cultivars. With the advent of next-generation sequencing and the development of genomics and molecular genetics techniques, it is now possible to rapidly identify and apply resistance genes. This paper reviews current information about disease-resistance genes identified in *B. napus* cultivars, mapping and cloning, their importance, role and function, and their association with plant disease resistance and application in resistance breeding. The feasibility of using current resistance sources in Canadian cultivars for developing new disease-resistant cultivars is also discussed. Sustainability of a farm and an agricultural system could be maintained by breeding for disease resistance, including the resistant varieties and incorporating other integrated pest management strategies along with it.

Keywords: *Brassica napus*, breeding for resistance, blackleg, sclerotinia stem rot, clubroot, verticillium stripe

Résumé: La diversité génétique est essentielle à la survie de toute population. Si tous les humains étaient identiques, une seule souche d'un redoutable virus de la grippe, comme celui de la COVID-19, pourrait nous anéantir! Chez les plantes, la diversité génétique joue un rôle similaire. La variation d'un individu à l'autre quant au type et au nombre de gènes de résistance peut faire la différence entre survivre à une maladie ou en mourir. Des études sur la diversité génétique permettront d'identifier de nouveaux gènes de résistance aux maladies. Le canola (*Brassica napus* L.) est une plante oléagineuse importante sur les plans économiques et nutritionnels, et ce, mondialement. Plusieurs maladies graves, y compris la nécrose du collet, la hernie, la pourriture à scléroties et la rayure verticillienne, menacent la production de canola au Canada et partout dans le monde. Les méthodes traditionnelles ne suffisent pas pour lutter efficacement contre ces maladies. En conséquence, l'approche idéale est d'optimiser et d'utiliser les gènes de résistance trouvés dans différents cultivars de *B. napus*. Grâce à l'arrivée du séquençage de nouvelle génération et au développement des techniques de génétique moléculaire et génomique, il est maintenant possible d'identifier et d'utiliser rapidement les gènes de résistance. Cet article résume l'information la plus récente sur les gènes de résistance identifiés chez les cultivars de *B. napus*, sur la cartographie et le clonage, sur leur importance, leur rôle et leur fonction ainsi que sur leur lien avec la résistance des plantes à la maladie et leur application dans la sélection axée sur la résistance. Nous traitons également de la faisabilité d'utiliser des sources courantes de résistance issues de cultivars canadiens pour développer de nouveaux cultivars résistants à la

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The first four authors contributed equally to this work. This is an invited review in recognition of the Outstanding Research Award granted to Professor Dilantha Fernando by the Canadian Phytopathological Society

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maladie. La viabilité d'une ferme et d'un système de production agricole pourrait être maintenue par la sélection de cultivars axée sur la résistance aux maladies, y compris de cultivars résistants soutenus par l'application d'autres stratégies de lutte intégrée.

Mots clés: *Brassica napus*, sélection axée la résistance, nécrose du collet, pourriture à sclérotines, hernie, rayure verticillienne

Introduction

The Brassicaceae is one of the most widespread and economically significant families in the plant kingdom. The family includes 372 genera and 4060 accepted species (The Plant List 2013) distributed throughout the world. The *Brassica* genus, the most important agricultural genus of the family, includes oil and condiment seeds such as rapeseed and mustard and vegetable crops such as cabbage, broccoli and cauliflower. *Brassica napus*, one of the most valuable species of the genus *Brassica*, was formed about 7500 years ago through interspecific hybridization between *Brassica rapa* and *Brassica oleracea*, followed by chromosome doubling. *Brassica napus* was cultivated by ancient Asian, Mediterranean, and Indian civilizations as early as 2000 B.C. Although *B. napus* has been grown in Europe since the thirteenth century (Colton and Sykes 1992), in 1972 Canada was the first country to grow *B. napus* commercially as an oilseed crop. The term ‘canola’ is a registered trademark of the Canola Council of Canada and comes from the term ‘Canadian oil low acid’ and refers to the edible oil crop characterized by low erucic acid and low glucosinolate content (Raymer 2002).

Globally, 27.9 M tonnes of canola/rapeseed are produced annually. The vegetable oil market generated 203.5 M tonnes in 2018–2019, with palm oil, the largest component at 73.9 M tonnes, followed by 55.7 M tonnes of soy (USDA 2021). Over a 5-year period, canola/rapeseed production has been consistent, whereas palm and soy production increased. The top five rapeseed producing regions based on area sown are Canada, China, India, the European Union, and Australia.

Canola is the number one cash crop (high commercial value) and the most economically important oilseed crop in Canada. In 2020, Canada produced 18.7 million tonnes of canola seed from 8.41 million hectares, mainly located in the Western provinces, also known as the Prairie Provinces, of Alberta, Saskatchewan, and Manitoba. Canada exported 20.2 million tonnes of canola seed, oil, and meal, contributing CAD \$11.19 billion to the Canadian economy (Canola Council of Canada 2021). The amount of canola produced in Canada has doubled over the last 10 years, with

the most recent 5-year average yield being 40 bushels per acre (2.7 tonnes per ha). This is on track to reach the Canadian canola industry’s target of 52 bushels per acre (3.5 tonnes per ha) by 2025 to help meet the global demand for vegetable oil (Canola Council of Canada 2014). Canada is the largest canola exporter by far, producing 20% of the world’s canola and accounting for 74% of export trade (AgMRC 2018). Ninety percent of canola production is exported, with the top four importers being the United States, China, Japan, and Mexico. Hence, Canada is uniquely positioned to provide for the world’s edible oil needs.

Canola can be infected by several fungal, protist, bacterial, and viral pathogens that can cause diseases on roots, crowns, stems, leaves, pods, and seeds. Diseases such as blackleg, clubroot, sclerotinia stem rot, and verticillium stripe (caused by *Leptosphaeria maculans/biglobosa*, *Plasmodiophora brassicae*, *Sclerotinia sclerotiorum* and *Verticillium longisporum*, respectively) are among the major canola diseases in Canada. They cause substantial damage to canola crops, reducing quality and yield or are a serious threat to sustainable canola production. For example, for every unit of increase in blackleg disease severity, a 17.2% loss in seed yield can be expected (Hwang et al. 2016). It has also been reported that canola seed yield dramatically decreases when blackleg severity is equal to or greater than a rating of two (Wang et al. 2020).

Clubroot was first identified on canola in the Prairie Provinces in 2003 (Tewari et al. 2005) and then spread throughout more than 3000 fields in Alberta, Saskatchewan, and Manitoba by 2018 (Strelkov et al. 2019). Clubroot can cause significant yield losses ranging from 30% to 100% in susceptible canola (Tewari et al. 2005; Pageau et al. 2006; Howard et al. 2010; Strelkov and Hwang 2014) and poses a major threat to the Canadian canola industry (Rempel et al. 2014).

In 2016, the average sclerotinia stem rot incidence in the Prairie region was 14–30% (Harding et al. 2017), responsible for 7–15% yield loss. However, in 2019, the sclerotinia stem rot incidence was lower, ranging from 5% to 12%.

Canola verticillium stripe disease was first discovered in Manitoba in 2014. A survey in 2015 demonstrated that

this disease is present in all provinces, but is found most frequently in Manitoba. Since verticillium stripe is a relatively new disease in Canada, there is not sufficient information about the disease prevalence, incidence and severity, or yield loss and management strategies. Therefore, understanding the pathogen's biology and how it interacts with the host will provide valuable information for researchers and farmers to help reduce potential disease impacts.

Given the economic importance of canola in Canada and the economic losses due to diseases, the development of breeding programs and management strategies that integrate alternative measures are crucial to successful canola production. In canola, integrated pest management (IPM) includes the use of the clean, healthy and vigorous seed, crop rotations, pest monitoring and resistant cultivars. It also includes using crop protection chemicals judiciously (Canola Council of Canada 2021). In addition to agronomic practices and biosecurity actions to prevent damage from these diseases, major efforts are required to understand the genetic mechanisms underlying the pathogenicity of the host's pathogens and genetic resistance determinants. Breeding for resistance via classical breeding or genetic engineering are ideal measures that mine and utilize resistance genes (*R* genes) to minimize the harmful effects of pathogens. The development of genomics, molecular genetics, and biological techniques such as next-generation sequencing (Bayer et al. 2015) and genotyping methods such as array or bead-based genotyping (Mason et al. 2015) enables researchers to uncover more genetic associations than ever before (Golicz et al. 2015). They have increased our understanding of the molecular mechanisms underpinning pathogenesis and resistance. These genomics technologies generate a large amount of data and assist in discovering and applying *R* genes rapidly. These data might be efficiently employed in canola breeding towards developing cultivars with elevated disease resistance.

This review focuses on the progress in the fight against blackleg, clubroot, sclerotinia stem rot, and verticillium stripe in Canada. It discusses and summarizes some of the most significant recent advances through genetic and genomics-assisted breeding towards improving resistance to these diseases in canola.

***Leptosphaeria* species**

Leptosphaeria is a teleomorphic genus of fungi belonging to the Ascomycota phylum, Dothideomycetes class and Leptosphaeriaceae family. A species complex,

comprising *Leptosphaeria maculans* and *L. biglobosa*, causes blackleg or phoma stem canker disease on *Brassica* crops. *Leptosphaeria maculans* and *L. biglobosa* are closely related and morphologically similar fungi. Although both cause blackleg disease, *L. biglobosa* is less aggressive and is regarded as a weakly virulent pathotype (West et al. 2001) and causes less damage to oilseed rape than that caused by *L. maculans* (Shoemaker and Brun 2001). The damage is limited to producing surface lesions on the upper stem rather than phoma stem base cankers (Williams and Fitt 1999). *Leptosphaeria maculans* has been recorded on *Brassica* species since 1791, but the severe global damage to *Brassica* crops was only recorded in the last four decades (Rouxel and Balesdent 2005). *Leptosphaeria maculans* has a broad host range within the Brassicaceae family, infecting numerous *Brassica* species, including *Raphanus raphanistrum* (wild radish), *R. sativus* (radish), *Sinapis alba* (white mustard), and cultivated *Brassica* crops, such as *B. rapa*, *B. juncea*, *B. oleracea* and *B. napus* (Alamery 2015). In Canada, *L. biglobosa* was first reported before the 1970s. Later in 1975, *L. maculans* was identified in Saskatchewan (McGee 1978) and then in Manitoba, Alberta, and British Columbia (Gugel and Petrie 1992). There was no widespread blackleg disease in Canada until 1982, when blackleg caused 6–56% yield losses in Saskatchewan (Juska et al. 1997). Since the 1990s, blackleg has been recognized as a major disease in Canadian canola and has resulted in significant yield losses (Gugel and Petrie 1992).

***Leptosphaeria maculans* life cycle**

The *L. maculans* life cycle is complicated and includes phases of biotrophy, necrotrophy, and saprotrophy (Fig. 1). The pathogen survives as a saprophyte on the stubble of infected plants in the fall and winter, during which time it uses sexual reproduction to produce primary inoculum from pseudothecia. In the spring, ascospores are released from the pseudothecia and spread by wind, rain splash, or infected plants. Ascospores enter through the stomata and the pathogen enters its biotrophic phase. Soon after the infection, tan-coloured lesions and black pycnidia form on the leaves, representing a necrotrophic phase of the pathogen. Following the colonization of the intercellular spaces, the pathogen reaches a vascular strand and spreads down the stalk between the leaf and the stem. This colonization leads to the invasion and destruction of the stem cortex, leading to stem canker formation (Hammond et al. 1985), destroying plant tissue. After plant death or harvesting, the

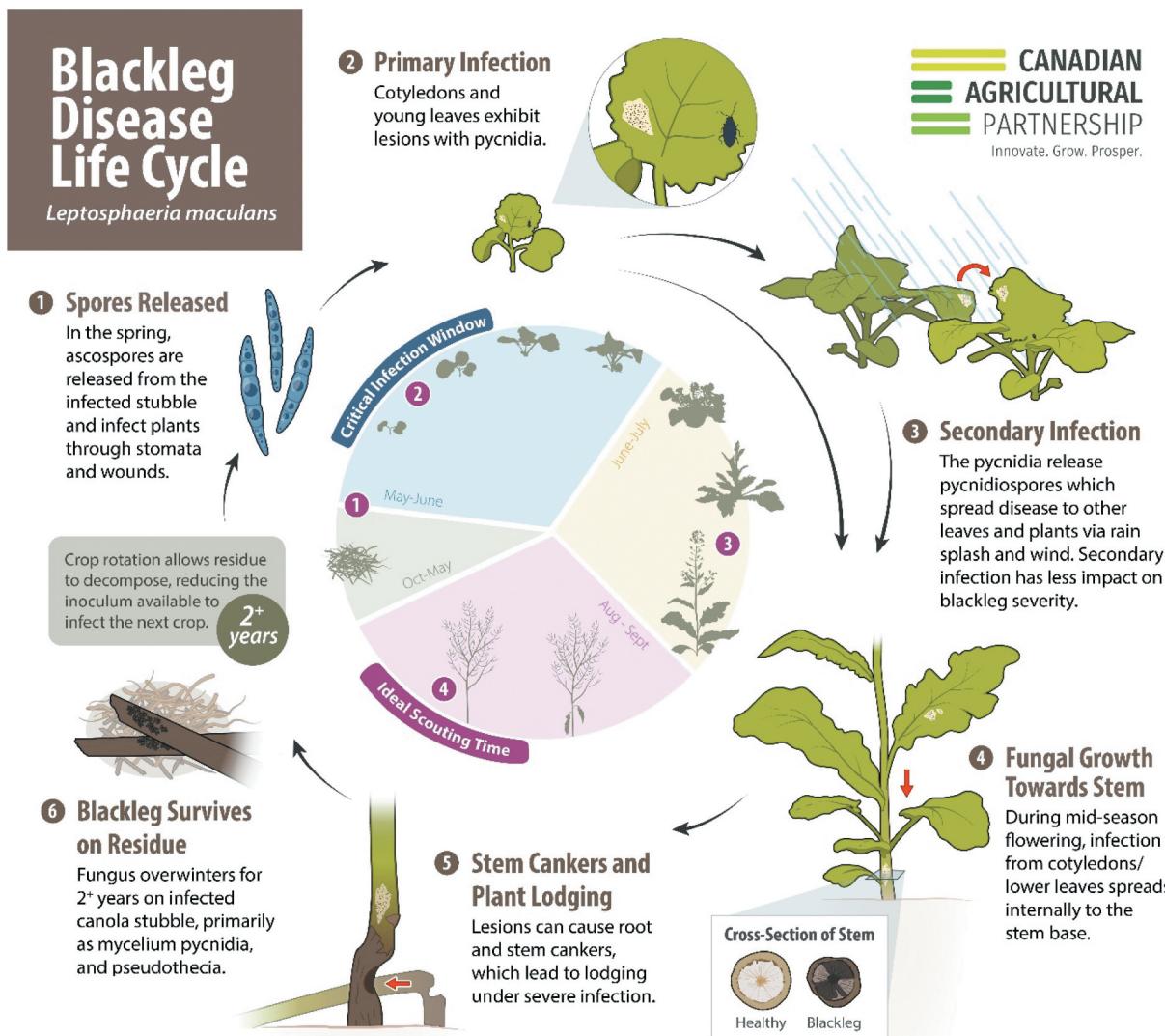


Fig. 1 Blackleg [*Leptosphaeria maculans*] disease cycle in Canadian canola (Canola Council of Canada 2021).

pathogen overwinters as pseudothecia and mycelium on the plant residue and continues producing pseudothecia in its saprotrophic phase.

***Leptosphaeria maculans* spread and disease symptoms**

Leptosphaeria maculans can be either monocyclic or polycyclic. If the primary source of inoculum is ascospores, the pathogen can be monocyclic, but if the primary or secondary source of inoculum is pycnidiospores, the disease may be considered polycyclic (Li et al. 2007). The pathogen can spread by wind-borne dispersal of ascospores and rain splash of conidia, but the major source of the disease contributing to widespread field infection and yield reduction is spores over-wintering on infected canola residue.

Leptosphaeria maculans can attack at various stages of crop development and infect nearly all parts of the plant, including cotyledons, leaves, stems, roots, and pods; nonetheless, the occurrence of seed infection is relatively low; hence seed-borne inoculum is only a major concern in spreading *L. maculans* into places where the pathogen has not been found yet (Van de Wouw et al. 2016a). Symptoms and signs include tan-coloured, round to irregularly shaped leaf lesions usually dotted with numerous small, black pycnidia. On stems, lesions with a dark border can be found at the base of the stem.

***Leptosphaeria maculans* damage**

Stem cankering restricts moisture and nutrient movement so that plants may lodge as the fungus girdles stem

bases, often completely severing or cutting off the plant (West et al. 2001). The first blackleg outbreak was observed in *B. oleracea* (Henderson 1918). Significant yield losses in *B. napus* were later reported in 1961 and 1972 in Canada and Australia (Vanterpool 1961; Gugel and Petrie 1992). In Canada, Australia, and the UK, annual yield losses associated with blackleg disease are 10–20% (West et al. 2001; Fitt et al. 2006; Van De Wouw et al. 2016b; Zhang and Fernando 2018) but can be much higher depending on regions and years. Recent work with a blackleg susceptible cultivar, 'Westar', has shown a decrease in yield per plant by 17.2% for every unit of increase in blackleg disease severity, using the 0–5 stem rating system (Hwang et al. 2016). Hwang et al. (2016) found that for each increase in disease severity index, seed yield declined by 13%. The yield models were updated using moderately resistant cultivars to represent the blackleg losses in producer's fields. Relationships between disease severity, pod number, and seed yield reported in field trials were best described by second-degree quadratic equations (Wang et al. 2020). Yield losses in moderately resistant cultivars ranged from 18% to 99% when disease severity was between a rating of 2–5, compared with ratings between 0 and 1. Blackleg disease yield loss on resistant cultivars is challenging to compare because cultivars react differently to the *L. maculans* races used to inoculate plants. Measuring yield loss allows for a better understanding of economic implications caused by the disease and shows the benefit of disease mitigation and agronomic practices for managing the disease.

Blackleg disease management

The most successful disease management strategies consist of three major components: avoidance techniques, application of approved fungicidal products, and genetic resistance obtained through breeding. The avoidance techniques can be divided into three main categories, rotation with other crops, crop isolation, and avoiding exposure to stubble. Farmers have used crop rotation to prevent or reduce crop losses attributed to diseases as standard practices. With non-Brassica host crops, extending crop frequency to at least 2 years between canola crops is one of the most effective approaches to decrease inoculum levels (Harker et al. 2015a). To further limit the risk of inoculum build-up, resistance breakdown, and yield loss, growing canola less frequently in rotations on the same field is recommended. Kutcher et al. (2013) emphasized that rotations needed to be greater than one canola crop in 4 years. Intensive

canola-growing regions are susceptible to blackleg epidemics (Hegewald et al. 2018). Canola frequency in rotation becomes essential to manage the disease spread, and spatial cropping must be considered.

Crop isolation can be used to minimize the inoculum pressure in new canola crops from known blackleg infected canola residue (Marcroft et al. 2004; Guo et al. 2005). Stubble or crop residue management is another alternative that aims to destroy the source of inoculum. Guo et al. (2008) reported that the number of ascospores and pycnidiospores dispersed was less from tilled plots than no-till plots, indicating that tillage may reduce inoculum levels. Spore dispersal is associated with infected canola residue on the soil surface and conclusive environmental conditions (Guo et al. 2008). Burning residue or tillage and burial of infected canola residue do not always decrease the risk of blackleg. The rotation interval, cultivar grown, and seasonal weather conditions have a much more significant impact on managing blackleg inoculum than tillage (Kutcher and Malhi 2010). Due to the negative impacts on soil health, frequent tillage (Blackshaw et al. 2005) is not commonly practiced in western Canada to manage blackleg-infested residue.

Canadian canola farmers have the option to use chemical formulations as crop protection products to manage blackleg. To date, several fungicides have been introduced to deliver protection to young plants. These fungicides move through the plant's water-conducting system and are taken up into the cotyledons or leaves where they are present and active when needed most. For example, foliar application of azoxystrobin, fluxapyroxad, propiconazole, and pyraclostrobin during the 2–6 leaf stage is recommended to protect young canola plants from blackleg infection (Manitoba Agriculture 2018). Fungicide resistance has already been identified in Australia, where *L. maculans* populations show sensitivity to fluquinconazole (Van De Wouw et al. 2017). Fluopyram seed treatment tested in Canada inhibited cotyledon infection and substantially reduced the disease severity on a susceptible cultivar (Peng et al. 2020). The same research tested fluopyram on a resistant cultivar, but disease severity remained low with or without fluopyram. Seed treatments are effective when cultivar resistance is no longer effective or when growing a susceptible cultivar (Fraser et al. 2020). If cultivar resistance is effective, the benefit of fungicide application is negligible.

Although these strategies are adopted to control the disease, the most promising disease control strategy uses resistant canola cultivars. Cultivars with resistance to

blackleg were first deployed in the 1990s, which helped minimize the disease's impact. In Canada, four classes of blackleg resistance, i.e. resistant (R; 0–29.9% of susceptible check cultivar), moderately resistant (MR; 30.0–49.9% of check), moderately susceptible (MS; 50.0–69.9% of check), and susceptible (S; 70.0–100% of check cultivar) have been established by the Western Canada Canola and Rapeseed Recommending Committee (Canola Council of Canada 2020). Once a cultivar is commercialized, assessments for blackleg are no longer continued. Resistance in R-rated cultivars can be overcome by shifts in pathogen populations, as they are not immune to all pathogen races. After several cropping cycles of growing a particular cultivar, the cultivar may start behaving like a susceptible cultivar, depending on the predominant blackleg races in the field (Liban et al. 2016; Zhang et al. 2016b). Strategic deployment of resistant cultivars to maintain the effectiveness and longevity of resistance sources has become a research priority.

Resistance in *Brassica napus*

Generally, there are two types of resistance in *B. napus* to blackleg: qualitative resistance (also known as major gene resistance) and quantitative resistance (minor gene resistance). Qualitative resistance is race-specific and stops the pathogen from spreading at the initial infection site (Delourme et al. 2004). Quantitative resistance is non-race specific and has an additive effect that partially impedes the pathogen (Hayward et al. 2012). When a cultivar only has quantitative resistance, it will be susceptible to infection by *L. maculans* early in plant development, but disease symptoms and yield impact will be reduced significantly at crop maturity. Quantitative resistance remains a crucial tool in minimizing blackleg disease pressure, but due to the complexity in measuring the effect it plays at reducing disease severity, it remains difficult to properly quantify the role it plays (Van de Wouw et al. 2016a). The optimal cultivar contains both major resistance genes and quantitative resistance genes to defend against a diversity of *L. maculans* races (Brun et al. 2010; Delourme et al. 2014).

***Leptosphaeria maculans-Brassica napus* interaction**

When pathogens invade plants, they can trigger a two-phase immunity response. The first phase involves the detection of pathogen-associated molecular patterns (PAMP) by surface-localized receptors; this phase is

termed PAMP-triggered immunity or PTI. The second phase is called effector-triggered immunity (ETI) (Jones and Dangl 2006). This response relies on the interaction of a plant resistance gene, encoding recognition receptors, with race-specific pathogen effectors encoded by avirulence genes. This interaction usually leads to hypersensitive defense response, usually manifested in rapid cell death, limiting further pathogen growth, and is phenotypically observed as complete resistance (Yu et al. 2017).

The *L. maculans-B. napus* pathosystem follows the gene-for-gene model but with some exceptions (Flor 1971). In the *L. maculans-B. napus* coevolution, avirulence proteins known as effectors, have been recognized by the *B. napus* resistance (*R*) proteins (receptor) encoded by major resistance genes (Hayward et al. 2012). Effector-triggered immunity (ETI) occurs with the gene-for-gene interaction between an avirulence gene and its matched major resistance gene, resulting in disease defense. In other words, major gene resistance is only effective against *L. maculans* races that contain the corresponding avirulence (*Avr*) genes (Flor 1971). Therefore, if the major resistance gene matches the avirulence allele prevalent within the *L. maculans* population, the plant will initiate a defense response or incompatible interaction, killing the cells around the infected cell and stopping the pathogen from spreading further (Rimmer 2006). Coevolution between the host and pathogen have allowed for diversifying selection to promote protein diversity in both partners (Rouxel and Balesdent 2010).

Major resistance genes

The introgression of *R* genes has been employed as one of the key strategies to manage blackleg disease. To date, at least 23 *R* genes against blackleg have been identified (Table 1). Of these, three (*Rlm2*, *LepR3* and *Rlm9*) have been cloned, and some genes are suspected to be identical or allelic forms due to the different populations and markers used in their mapping. *Rlm1* has been mapped to *B. napus* A07 chromosome and induces a resistance response against an *L. maculans* isolate harbouring the *AvrLm1* avirulence gene (Fu et al. 2019). *Rlm2*, which is allelic to *LepR3*, is located on chromosome A10 and interacts with *AvrLm2* (Larkan et al. 2013; Chalhoub et al. 2014). *Rlm3* has been mapped to *B. napus* chromosome A07 and responds to *L. maculans* gene *AvrLm3* (Balesdent et al. 2013). In another interaction, both *Rlm4* and *Rlm7* from chromosome A07 in *B. napus* recognize the *L. maculans* *AvrLm4-7* gene (Parlange et al. 2009).

Table 1. Identified *Brassica* resistance genes (*R*) and *Leptosphaeria maculans* avirulence genes (*Avr*).

No.	R genes	Chromosome	Species	References	Avr genes	References
1	<i>Rlm1</i>	A7	<i>B. napus</i>	Fu et al. (2019)	<i>AvrLm1-L3</i>	Gout et al. (2006)
2	<i>Rlm2</i>	A10	<i>B. napus</i>	Larkan et al. (2013)	<i>AvrLm2</i>	Ghanbarnia et al. (2015)
3	<i>Rlm3</i>	A7	<i>B. napus</i>	Balesdent et al. (2013)	<i>AvrLm3</i>	Plissonneau et al. (2016)
4	<i>Rlm4</i>	A7	<i>B. napus</i>	Parlange et al. (2009)	<i>AvrLm4-7</i>	Parlange et al. (2009)
5	<i>Rlm5</i>	A10-B?	Re-synthesized, <i>B. juncea</i>	Ghanbarnia et al. (2018)	<i>AvrLm5-9</i>	Ghanbarnia et al. (2018)
6	<i>Rlm6</i>	A7-B4	<i>B. juncea</i>	Yang (2018)	<i>AvrLm6</i>	Fudal et al. (2007)
7	<i>Rlm7</i>	A7	<i>B. napus</i>	Parlange et al. (2009)	<i>AvrLm8</i>	Balesdent et al. (2002)
8	<i>Rlm8</i>	A	<i>B. rapa</i>	Larkan et al. (2016)	<i>AvrLm10_{a, b}</i>	Petit-Houdenet et al. (2019)
9	<i>Rlm9</i>	A7	<i>B. napus</i>	Rimmer (2006)	<i>AvrLm11</i>	Balesdent et al. (2013)
10	<i>Rlm10</i>	B4	<i>B. nigra</i>	Petit-Houdenet et al. (2019)	<i>AvrLm14</i>	Degrave et al. (2021)
11	<i>Rlm11</i>	A	<i>B. rapa</i>	Balesdent et al. (2013)	<i>AvrLmS</i>	Van de Wouw et al. (2009)
12	<i>Rlm12</i>	A1	<i>B. napus</i>	Raman et al. (2020)	<i>AvrLepR1</i>	Ghanbarnia et al. (2012)
13	<i>Rlm13</i>	C3	<i>B. napus</i>	Raman et al. (2021)	<i>AvrLepR2</i>	Raman et al. (2020)
14	<i>LepR1</i>	A2	Re-synthesized <i>B. rapa</i> subsp. <i>sylvestris</i>	Cantila et al. (2021)	<i>AvrLepR4</i>	
15	<i>LepR2</i>	A10-C9	Re-synthesized <i>B. oleracea</i> , <i>B. rapa</i> subsp. <i>sylvestris</i>	Cantila et al. (2021)		
16	<i>LepR3</i>	A10	Re-synthesized <i>B. rapa</i> subsp. <i>sylvestris</i>	Larkan et al. (2013)		
17	<i>LepR4_{a, b}</i>	C3-C8-A6	Re-synthesized, <i>B. oleracea</i> , <i>B. rapa</i> subsp. <i>sylvestris</i>	Yu et al. (2012)		
18	<i>RlmS</i>	A10	Re-synthesized <i>B. rapa</i> subsp. <i>sylvestris</i>	Cantila et al., (2021)		
19	<i>BLMR1</i>	A10	<i>B. napus</i> Surpass 400	Long et al. (2011)		
20	<i>BLMR2</i>	A10	<i>B. napus</i> Surpass 400	Long et al. (2011)		
21	<i>LMJR1</i>	B3	<i>B. juncea</i>	Cantila et al. (2021)		
22	<i>LMJR2</i>	B8	<i>B. juncea</i>	Cantila et al. (2021)		
23	<i>rJlm2</i>	B1	<i>B. juncea</i>	Cantila et al. (2021)		

Out of 23 resistance genes and 14 avirulence genes identified, three resistance genes and eight avirulence genes have been cloned (**bold**).

The *AvrLm4-7* gene is epistatic over *AvrLm3* and *AvrLm9*, which means that *AvrLm4-7* can mask the function of *AvrLm3* and *AvrLm9* (Plissonneau et al. 2016; Van de Wouw and Howlett 2020). Similarly, *Rlm5* and *Rlm9* recognize the same *Avr* gene, i.e. *AvrLm5-9*. The recognition of *AvrLm5-9* by *Rlm9* is masked in the presence of *AvrLm4-7*. The host recognition for *AvrLm5-9* is also masked by *AvrLm4-7* (Ghanbarnia et al. 2018). *Rlm5* is a *B. juncea* *R* gene and resides in a region homologous to chromosome A10 of *B. napus*, while *Rlm9* is on chromosome A07 (Rimmer 2006). Plissonneau et al. (2018) showed that the *AvrLmJ1* gene maps to the same position as the *AvrLm5* locus, so *AvrLmJ1* is *AvrLm5*. *Rlm5* and *RlmJ1* have been found in *B. juncea*, but it is still uncertain whether they reside on the A or B genome (Larkan et al. 2016). In addition, as with the case of *Rlm1* and *LepR3*, it is unclear if *Rlm5* and *Rlm9* are allelic variants or independent genes (Ghanbarnia et al. 2018). *Rlm6*, which has been genetically mapped onto A07 and B04 chromosomes of *B. juncea* (Yang 2018) and interacts with *AvrLm6*, is now utilized in canola cultivars. Due to overlap in the resistance genes mapped by different groups and falling into the same linkage group, *Rlm6* is likely to be the same gene as *Jlm1* (Brun et al. 2010). *Rlm8*, which interacts with *AvrLm8*, resides on the A genome

in *B. rapa* and *B. napus*, but it has not yet been mapped further (Larkan et al. 2016). *Rlm10* interacts with *AvrLm10_a* and *AvrLm10_b* and is present in the B genome of *B. juncea* or *B. nigra* (mapped on chromosome B04) but has not yet been introgressed into *B. napus* (Petit-Houdenet et al. 2019). *Rlm11* resides on the A genome in *B. rapa* and *B. napus* and matches the *L. maculans* *AvrLm11* gene (Balesdent et al. 2013). *Rlm12* (*QRLm.wwai-A01*), a novel locus, conveys adult plant resistance in canola (Raman et al. 2020). There are no *Avr* genes discovered for the *Rlm12* gene so far. In addition, Raman et al. (2021) identified a major locus, designated *Rlm13*, on chromosome C03 that accounts for genetic variation in resistance to *L. maculans* in an Australian canola population derived from the CB-Telfer/ATR-Cobbler. *LepR1* and *LepR2* interact with *AvrLepR1* and *AvrLepR2*, respectively.

Although *LepR2* and *RlmS* have been reported as independent genes, they interact with the same *Avr* gene, *AvrLmS-Lep2*; hence, *LepR2* and *RlmS* could be the same gene or allelic variants (Cantila et al., 2021). Like *Rlm1*, *LepR3* detects the *L. maculans* *AvrLm1* gene, so the hypothetical *AvrLepR3* gene does not exist. In addition, *Rlm1* is a natural allele, while *LepR3* is an introgressed gene from *B. rapa* subsp. *Sylvestris*. *LepR3* is located at the same locus as *Rlm2* (seems allelic

gene for *Rlm2*), but *LepR3* interacts with *AvrLm1* while *Rlm2* interacts with *AvrLm2* (Larkan et al. 2013). *LepR3* and *Rlm2* in ‘Surpass 400’ and ‘Glacier’ cultivars are homologs of the *blmr1* protein in *B. napus* ‘Westar’ (Larkan et al. 2015). *LepR4* interacting with *AvrLepR4* was reported to have two alleles, *LepR4_a* and *LepR4_b*, each having different resistance levels (Yu et al. 2013). It is suggested that *RlmS* (detects *AvrLmS*) and *BLMR1.2* are likely to be the same gene; however, neither gene has been identified to date (Van de Wouw and Howlett 2020). Some *R* genes are thought to be redundant with other *R* genes. For example, *BLMR1* was supposed to be redundant to *LepR3*. *BLMR2* is another example but needs confirmatory analysis (Larkan et al. 2013). In addition to *Rlm6* and *Rlm10* genes, *LMJR1*, *LMJR2*, and *rjlm2* have been identified in the B genome. No *Avr* gene has been isolated/discovered for these *R* genes yet (Cantila et al. 2021).

According to the gene for gene model, each *R* gene in the plant responds to an *Avr* gene in the pathogen. However, in some cases, two *R* genes can detect a single *Avr* gene. For example, *Rlm4* and *Rlm7* detect the *AvrLm4-7* gene, or *Rlm1* and *LepR3* detect the *AvrLm1* gene. In contrast, cases where a single *R* gene detects multiple *Avr* genes have not yet been reported with the exception of *B. nigra*, where *Rlm10* interacts with two *Avr* genes, *AvrLm10_a* and *AvrLm10_b*, resulting in a deviation from the classical gene for gene hypothesis and suggesting a gene-for-two-gene interaction (Petit-Houdenot et al. 2019).

Leptosphaeria maculans avirulence genes

Until now, 14 *Avr* genes have been identified in *L. maculans*. Seven have been mapped to two gene clusters; *AvrLm1-2-6* cluster and the *AvrLm3-4-7-9-AvrLepR1* cluster (Balesdent et al. 2005; Yu et al. 2005; Van de Wouw et al. 2009; Ghanbarnia et al. 2012) and eight *Avr* genes (*AvrLm1-L3*, *AvrLm2*, *AvrLm3*, *AvrLm4-7*, *AvrLm5-9*, *AvrLm6*, *AvrLm10_{a/b}* and *AvrLm11*) have been cloned (Gout et al. 2006; Fudal et al. 2007; Parlange et al. 2009). In brief, *AvrLm1-L3* recognizes both *Rlm1* and *LepR3*. *AvrLm2* and *AvrLm3* match with *Rlm2* and *Rlm3*, respectively. *AvrLm4-7* interacts with *Rlm4* and *Rlm7*, *AvrLm5-9* recognizes both *Rlm5* and *Rlm9* (*AvrLm9* co-segregating with *AvrLm5*). *AvrLm6* corresponds to *Rlm6*. Two avirulence genes, *AvrLm10_a* and *AvrLm10_b*, are required for *AvrLm10-Rlm10*-mediated resistance. *AvrLm11* recognizes *Rlm11*. A recent study identified a new *Avr* gene, *AvrLm14*, which confers a new resistance source from

the American broccoli (*B. oleracea*) genotype ‘Monaco’ (Degrave et al. 2021) (Table 1, Fig. 2). This finding suggests a typical gene-for-gene interaction with the postulated *Rlm14* gene as well. Like other avirulence genes, *AvrLm14* encodes a small, secreted protein in a genomic region harbouring abundant transposable elements and heterochromatin marks H3K9me3. The inferred corresponding major resistance gene (*Rlm14*) in broccoli indicates that *B. oleracea* can also be

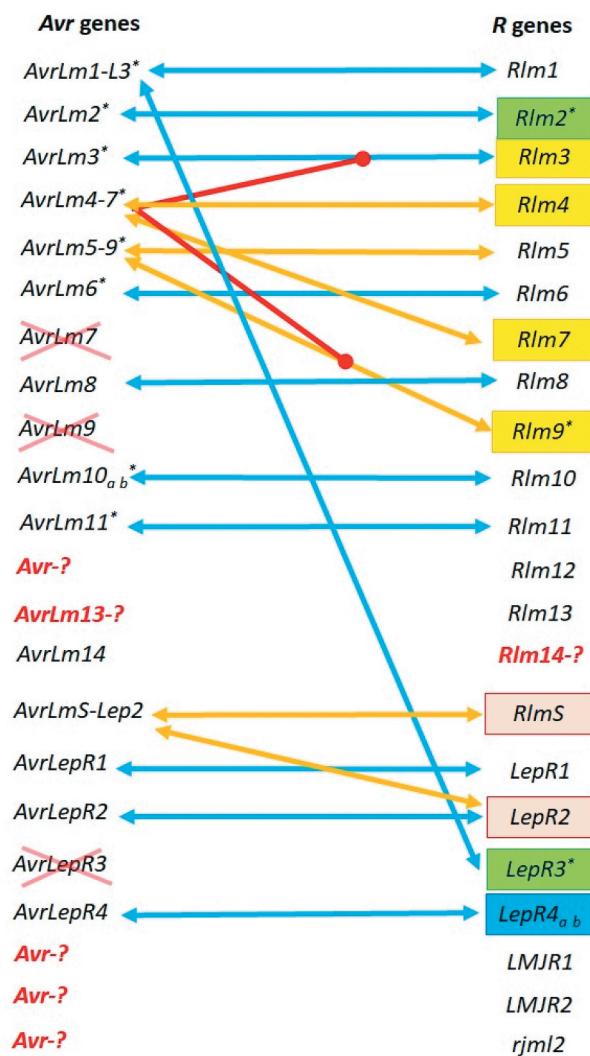


Fig. 2 The interaction between resistance (R) genes and avirulence (Avr) genes mediating blackleg resistance in canola. R genes in the same color boxes are or suspected to be allelic forms. The red oval end arrow indicates Avr genes that mask other interactions. Genes (R and Avr) with an asterisk represent cloned genes. Avr genes with red cross do not exist, and genes with a question mark (?) are hypothetical genes that have not been isolated/discovered.

considered and introduced to diversify the resistance genes in *B. napus* breeding.

Marker development for the detection of avirulence and virulence alleles allows for rapid testing to occur. One example is developing a marker to discriminate *AvrLm9* from *avrLm9* in *L. maculans* isolates (Liu et al. 2020). This allows for rapid identification of avirulence alleles within the *L. maculans* population. By cloning avirulence alleles and developing specific detection markers, testing infected stubble can determine *L. maculans*' avirulence profile. Testing is available to farmers for predominant *L. maculans* races in their field through commercial labs using Kompetitive allele-specific PCR (KASP) markers (Cornelsen et al. 2019). This provides field-level information to facilitate better decision-making regarding effective resistant cultivar use.

Understanding *L. maculans* races and avirulence profiles has become an industry focus in trying to manage the disease. Across western Canada, two dominant *L. maculans* races, *AvrLm2-4-6-7* and *AvrLm2-4-6-7-S* and 55 less common races were detected, indicating high diversity (Liban et al. 2016). Soomro et al. (2020) also found the same top two races in commercial and 'Westar' trap crops in western Canada, confirming that the predominant isolated races have not changed. Monitoring blackleg races spatially and temporally creates the opportunity to study the impact of major resistance genes on the changes of avirulence genes within the population (Kutcher et al. 2007). Changes in the virulence profile of *L. maculans* can indicate the success of major resistance genes deployed, help to estimate the durability of resistance genes deployed, and predict predominant *L. maculans* races that will occur in the future (Van de Wouw et al. 2014). Liban et al. (2016) found that over 85% of isolates contained *AvrLm6* and *AvrLm7*, whereas under 10% of isolates contained *AvrLm3*, *AvrLm9* *AvrLepR2*. This work, compared with previous studies, identified the changes in virulence over time. Knowing the *L. maculans* races at the field level allows for the opportunity for a strategic cultivar rotation system to be applied, where cultivars can be deployed to target specific *L. maculans* races.

Novel sources of candidate *R* genes and *R* genes pyramiding

The relationships between *Brassica* species offer a unique opportunity to deploy introgression candidate *R* genes from A, C (Chen et al. 2010) or B (Christianson et al. 2006) genomes. Wild Brassicaceae species, such as

Sinapis arvensis, have been reported as a good source of *R* genes for blackleg (Snowdon et al. 2000). However, identifying novel *R* genes in wild relatives is problematic since most wild species lack reference genomes (Zhang et al. 2020). Since the 2000s, molecular markers, such as SSRs, microsatellites, and InDels, have been applied to discover novel *R* genes and loci for *Brassica* diseases (Honghao et al. 2020). For example, the discovery of novel disease resistance QTL/*R* genes in relation to blackleg (Raman et al. 2016; Fu et al. 2020), sclerotinia (Wu et al. 2016; Atri et al. 2019; Rana et al. 2019) and clubroot (Li et al. 2016; Peng et al. 2018) diseases. Now, many qualitative and quantitative resistance sources have been identified in *Brassica* species (Mithen et al. 1987; Chevre et al. 1996; Chèvre et al. 1997; Christianson et al. 2006; Rimmer 2006; Leflon et al. 2007; Raman et al. 2013; Fredua-Agyeman et al. 2014; Gaebelein et al. 2019). Many more novel *R* genes in the *Brassica* could be discovered by applying genomics approaches and technologies. For example, the *Brassica* pangenome's construction could help identify many more novel candidate *R* genes and develop molecular markers to screen for resistant cultivars in the field (Neik et al. 2020). The identification of novel *R* genes provides a valuable resource in future breeding programs for *Brassica* crops.

R gene's longevity diminishes rapidly when a virulent population overcomes the field's avirulent population (Lof and van der Werf 2017). For example, some *R* genes (*Rlm1*, *Rlm3*, *Rlm6*, *Rlm7*, *LepR1*, and *LepR3*) have been reported to break down and lose effectiveness in the field due to sexual recombination of *L. maculans* and population changes (Van de Wouw et al. 2010; Winter and Koopmann 2016; Van De Wouw et al. 2017). This means there is a high risk of breakdown of a particular source of resistance, so canola farmers should be advised to utilize different *R* genes by crop rotation in canola cultivars to sustainably manage the blackleg infection and resistance breakdown (Zhang and Fernando 2018; Van de Wouw and Howlett 2020; Cantila et al. 2021). Another strategy is gene stacking or pyramiding, in which a host contains several major resistance genes (Mundt 2018). Combining a major resistance gene with quantitative resistance is also considered a gene stack; this has proven vital for extending major resistance genes' effectiveness (Pilet-Nayel et al. 2017). Pyramiding major resistance genes is durable when the virulent population is low and works best when new genes that have not been previously deployed are incorporated (Fuchs 2017). One concern of pyramiding major resistance genes is that the virulent races will develop to defeat all the major genes deployed in the

host (De Vallavieille-pope et al. 2012). This is particularly true when moderate to high gene flow occurs. Gene stacks provide broad-spectrum and durable resistance, but there is not enough information about stacked gene cultivars' longevity.

Leptosphaeria maculans is considered a recombining pathogen that would easily break the effectiveness of stacked major resistance gene cultivars (McDonald and Linde 2002; Marcroft et al. 2012). In Canada, lower rates of pathogen sexual recombination occur, minimizing the impact on avirulence frequency (Ghanbarnia et al. 2011). Based on the *L. maculans* race profile in Canada, Liban et al. (2016) suggested an *Rlm6* and *Rlm7* stacked cultivar would be effective against most *L. maculans* races. Screening of Canadian *B. napus* germplasm found a combination of major resistance genes with quantitative resistance, with only a few gene stacks identified (Zhang et al. 2016b). The ideal resistance gene deployment approach depends on the virulence profile of the *L. maculans* population and race dynamics.

Gene rotation

Rotating cultivar genetics in a field has been one management practice deployed in more significant canola production regions. It is important to use major resistance genes that target the predominant *L. maculans* population and rotate between resistance genes virulent alleles. In Australia, the Grains Research and Development Corporation (GRDC) adopted a major resistance gene labeling system that places cultivars with the same gene into groups to help farmers manage blackleg by cultivar rotation (GRDC 2018). This approach has worked as a predictor system to indicate which major genes are no longer successful in specific cropping regions. An intensive cultivar monitoring trial network is used to help predict which major resistance genes will remain successful and which genes have been overcome by virulent populations (Marcroft et al. 2012).

In Australia, the environmental conditions are ideal for increasing the incidence and development of *L. maculans*, resulting in very severe disease symptoms. Cultivars have short lifespans within specific regions of Australia but can be re-introduced to an area after switching to a different major gene grouping. This monitoring approach in Australia has predicted resistance gene failure and avoids disasters from blackleg disease for farmers (Sprague et al. 2006a). Reusing blackleg resistance genes in areas previously had been overcome has been considerable success in Australia's cultivar rotation system. It also alleviates pressure on canola

breeders to develop blackleg resistance cultivars with new novel traits.

In Canada, when canola farmers found increased levels of blackleg within their R-rated cultivar, the recommendation was to rotate to a different cultivar (Kutcher et al. 2011). Unknowingly, they could have selected a cultivar with the same major resistance genes that would not match the field's predominant blackleg races. Zhang et al. (2016b) identified the major resistance genes deployed in Canadian cultivars/germplasm. In 2017, the Canadian canola industry had adopted a new resistance labeling scheme to identify the major resistance genes deployed within a cultivar, allowing farmers to rotate cultivars based on major resistance gene groups (Table 2). Cultivars continue to be labeled with the R/MR resistance rating, but plant breeders and the seed industry now have the option to include a major resistance gene label: A, B, C, D, E1, E2, F, G, J, K, L, N, P, or X. Each group represents blackleg major resistance gene(s), while 'X' represents a labeled cultivar with an unknown resistance gene (Zhang and Fernando 2018; Canola Council of Canada 2020). Groups have been determined based on resistance gene interactions and similar responses to *L. maculans* avirulence genes. The Canadian system has been validated at the farmer field level in Western Canada (Cornelsen et al. 2021). Van de Wouw and Howlett (2020) outline the differences in resistance gene groups between the Canadian and Australian systems. One main difference is that farmers in Canada know the exact gene used within their cultivar, whereas Australian farmers only know what major resistance gene group the cultivar contains. Marcroft et al.'s (2012) cultivar rotation work indicate that

Table 2. The Canadian Blackleg Major Resistance Gene labelling system classifies major resistance genes by Resistance Gene Groups (RG).

Resistance Gene Group (RG)	Major Resistance Genes
A	<i>Rlm1</i> or <i>LepR3</i>
B	<i>Rlm2</i>
C	<i>Rlm3</i>
D	<i>LepR1</i>
E ₁	<i>Rlm4</i>
E ₂	<i>Rlm7</i>
F	<i>Rlm9</i>
G	<i>RlmS</i> or <i>LepR2</i>
J	<i>Rlm5</i>
K	<i>Rlm6</i>
L	<i>Rlm8</i>
N	<i>Rlm11</i>
P	<i>LepR4</i>
X	Unknown

Australian farmers have at least nine resistance genes available in commercial cultivars. This approach may mitigate the risk of rapid resistance erosion and extend cultivars' life in Canada (Harker et al. 2015b; Fernando et al. 2016; Liban et al. 2016). Similar strategies have been deployed in Australia and France with great success (Ansani-Melayah et al. 1998; Marcroft et al. 2012). A major resistance gene rotation system helps preserve advanced genetics and takes the pressure off developing new cultivars with novel sources of resistance (Van de Wouw et al. 2014).

Challenges in breeding for blackleg resistance

The major challenge in breeding for blackleg resistance is genetic variation within the host and pathogen (Neik et al. 2017); therefore, resistance levels vary with different host species or pathogens isolate. Although *Brassica* species have a complex genome (Liu et al. 2014), *B. napus* has a comparatively lower genetic diversity compared to its progenitors (*B. oleracea* and *B. rapa*) (Wu et al. 2014) due to strong genetic bottlenecks (Mason et al. 2016). However, homoeologous rearrangements/recombination contribute significantly toward genetic diversity in re-synthesized *B. napus* (Gaeta and Pires 2010); hence identifying *R* genes in *B. napus* remains challenging. Also, cloning of *LepR3/Rlm2* revealed that the *R* genes could be arranged as members of allelic variants or tandem repeats (Larkan et al. 2013). This clustering of *R* genes can also make *R* gene identification difficult as these *R* genes tend to collapse in genome sequence assemblies (Neik et al. 2017).

Moreover, it has been reported that *R* genes diversity can be affected by the genetic background of *B. napus*. For example, winter and spring-type cultivars or re-synthesized lines have higher and lower *R* genes diversity (Rouxel et al. 2003). Among them, re-synthesized lines provide a good source of *R* genes diversity than cultivars (Becker et al. 1995).

From the pathogen side, *L. maculans* revealed a high genetic diversity within populations. For instance, a high gene flow and high dispersal of spores among populations across large geographical distances were found in France (Travadon et al. 2011). The genetic diversity of *L. maculans* depends on the specific host morphotype from which the pathogen is adapted. For example, *L. maculans* 'brassicae' and *L. maculans* 'lepidii' isolated from *Brassica* and *Lepidium* sp. respectively were identified as one of the seven subgroups of the species complex based on ITS-RFLPs (Mendes-Pereira et al. 2003). Thus, understanding the *L. maculans* avirulence

profile within a field becomes a key component of effectively learning how to deploy resistance genes. Deploying the same major *R* gene repeatedly creates a virulent allele within the pathogen population (Kutcher et al. 2011). When major *R* genes are deployed broadly on many hectares, intense selection pressure is put on the pathogen population, increasing virulent individuals' frequency (Van de Wouw and Howlett 2020). The virulent allele bypasses the plant's major resistance gene mechanisms, and the pathogen can cause infection. A well-documented case of resistance breakdown was in Australia's Eyre Peninsula, where cultivars with resistance gene *RlmS*, from *B. rapa* subsp. *Sylvestris*, were released commercially in 2000. The cultivars had a high level of blackleg resistance and were grown widely until their breakdown in 2003, only 3 years after commercialization (Sprague et al. 2006b). Since then, the avirulence frequency surveillance of *L. maculans* isolates has occurred to help predict future cultivar breakdowns (Van de Wouw et al. 2016b). The shifts in virulence documented within Australia's surveillance program have provided valuable information on cultivar selection to avoid resistance breakdown (Van de Wouw et al. 2014). Within Canadian canola growing regions, the *AvrLm3* avirulence allele has become scarce in the *L. maculans* population due to overuse of the *Rlm3* resistance gene in Canadian *B. napus* germplasm (Zhang et al. 2016b). Rashid et al. (2021) found rapid loss of avirulence and shifts to virulence by *L. maculans* isolates in Canada in as little as one year. Collection of isolates from commercial fields across the Canadian prairies found *AvrLm2*, *AvrLm4*, *AvrLm6*, and *AvrLm7* in most isolates but the frequencies for detecting *AvrLm1*, *AvrLm3*, *AvrLm9*, and *AvrLep2* appeared low (Soomro et al. 2020).

Another challenge is uncertainty about some *R* genes if they are the same gene with different nomenclature or allelic variants of the same gene. For instance, although *LmR1*, *cRLMm*, *Rlm4*, *cRLMRb*, and *LEMI* genes have been mapped on chromosome A7 in *B. napus* (Ferreira et al. 1995; Mayerhofer et al. 1997; Rimmer et al. 1999; Balesdent et al. 2001; Rimmer 2006), it remains unknown if they are the same gene until one is cloned. Similarly, it has been well documented that *LepR3* interacts with *AvrLm1* (Larkan et al. 2013); however, phenotypic studies indicated that *AvrLm1* might also be recognized by *Rlm1* (Rouxel and Balesdent 2013). Also, the relationship between *Rlm4* (Raman et al. 2012) and *Rlm7* (Balesdent et al. 2002) is still uncertain. Cloning studies uncovered that *AvrLm4* and *AvrLm7* are two distinct alleles of the same gene, named *AvrLm4-7*,

and can induce resistance in *B. napus* that carry this allele either *Rlm4* or *Rlm7*. It is also possible that *Rlm3*, *Rlm4* and *Rlm7* may all be allelic variations of the same gene. Furthermore, the interaction between *Rlm3* and *AvrLm3* or interaction between *Rlm9* and *AvrLm5-9* is suppressed by *AvrLm4-7* when attacked by the *L. maculans* isolate harbouring this allele (Plissonneau et al. 2016). Therefore, knowing what major resistance genes are in *B. napus* germplasm and the avirulence alleles within the *L. maculans* population creates the opportunity to strategically deploy resistance genes to successfully match the predominant *L. maculans* races.

Sclerotinia sclerotiorum

Sclerotinia sclerotiorum is a devastating necrotrophic fungal plant pathogen that infects a broad range of hosts, including several valuable oilseed crops, such as soybean, sunflower, and canola (Boland and Hall 1994). *Sclerotinia sclerotiorum* is one of the members of the Sclerotiniaceae family and is thought to reproduce predominantly through self-fertilization. Several names, including cottony rot, watery soft rot, stem rot, drop, crown rot, blossom blight, and white mold, have been used to refer to diseases caused by this pathogen (Bolton et al. 2006).

Sclerotinia sclerotiorum life cycle

The resting structures of the pathogen are termed sclerotia. Sclerotia may survive in the soil for several years (Khangura and Beard 2015). Sclerotia produce either mycelium or ascospores to infect canola plants under wet and humid conditions (Bardin and Huang 2001) (Fig. 3). Sclerotia developed mycelia infect underground tissues, whereas ascospores infect aboveground tissues (Bardin and Huang 2001; Lane et al. 2019). In western Canada, the favourable conditions for *Sclerotinia* germination generally occur in June when canola plants are at the bolting stage. Canola petals that have fallen onto leaves or into leaf axils are required to initiate the ascospore infection process (Turkington and Morrall 1995). Following petal colonization, the fungus produces aggregates of appressoria used as infection cushions to penetrate live plant tissues (Jamaux et al. 1995). The subsequent infection process coincides with the production of the major fungal virulence factors. As the infection progresses, brown or bleached necrotic lesions appear around penetration sites and spread to other parts of the plant. Later in the growing season,

necrotic lesions girdle the stem and cause lodging or collapse of the whole plant (Khangura and Beard 2015; Wang et al. 2015). At the end of the growing season, new sclerotia formed inside the infected stem will be served as an inoculum source to infect the subsequent host crops.

Sclerotinia sclerotiorum damage

The soil-borne fungal pathogen *S. sclerotiorum* is the causal agent of sclerotinia stem rot (SSR). Sclerotinia stem rot is one of the major diseases distributed across all major *B. napus* growing regions, including Australia, Canada, China, France, Germany, and India. It is generally considered one of the most damaging canola diseases (Kharbanda and Tewari 1996). When environmental conditions are favorable for the disease development, canola yield losses caused by SSR can exceed 50% in severely infected fields and 10–15% across large areas. The average disease incidence of canola fields surveyed in western Canada was 14–30% (Canola Council of Canada). Over the past five years, the highest average SSR disease prevalence and incidence in western Canada was observed in 2016 due to high precipitation during the flowering stage, which resulted in a 7–15% yield loss (Canola Council of Canada). For every unit percentage of SSR incidence, the potential yield loss was 0.5% (del Río et al. 2007).

In addition to yield loss, *S. sclerotiorum* infections may also result in the downgrading of canola seeds due to the presence of sclerotia (Canola Council of Canada). SSR can progress rapidly in the swath in wet years, and the sclerotia may also rot the swath. Moreover, after harvest, the sclerotia bodies left in the field pose a high risk on western Canada's soybean/canola rotation.

Sclerotinia stem rot management practices

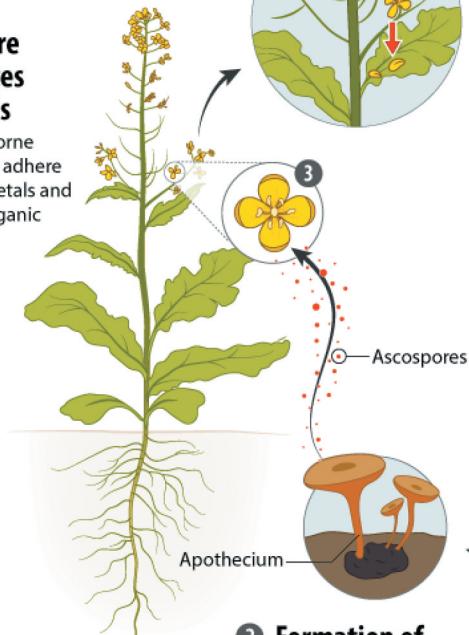
Management of SSR disease in canola relies heavily on cultural practices (Kharbanda and Tewari 1996; Murray and Brennan 2012). Common practices include reducing sclerotia in the soil by lengthening rotation with non-host crops or creating an in-field environment unfavourable to SSR development. Due to the complexity of environmental influences, crop rotation is a cultural practice that needs to be carefully evaluated to limit SSR incidence. However, crop rotation must be coupled with efficient weed management practices to minimize the chances of establishing *S. sclerotiorum* in the absence of a host crop. Tilling practices that bury sclerotia deep in the soil are another way to reduce the ability of

Sclerotinia Stem Rot Disease Cycle

(Caused by the fungus
Sclerotinia sclerotiorum)

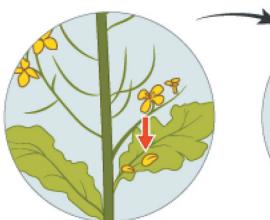
③ Ascospore Distributes on Petals

The windborne ascospores adhere to flower petals and/or other organic material.



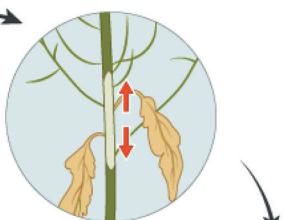
④ Germination and Distribution of Infection

Ascospores germinate, infect the petal, and spread to adjacent tissues of healthy leaves and stems by direct contact.



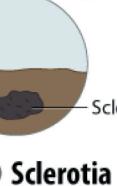
⑤ Distribution of Fungal Lesion

The lesions progress up and down the stem. At this stage, wilted leaves can be visible.



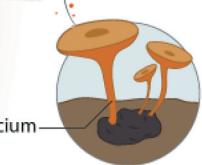
⑥ Formation of New Sclerotia

The infected stem becomes bleached and brittle and forms new sclerotia. The sclerotia return to the soil at harvest and the cycle repeats.



② Formation of Apothecia

Spore-producing apothecia germinate from sclerotia under moist plant canopy and release ascospores.



① Sclerotia Overwinter in Soil

The stem rot fungus (*Sclerotinia sclerotiorum*) overwinters as sclerotia in the soil or in stubble at the soil surface.

Fig. 3 Sclerotinia stem rot [*Sclerotinia sclerotiorum*] disease cycle in Canadian canola (Canola Council of Canada 2021).

sclerotia to produce apothecia in the next year, but the buried sclerotia's survival rate remains high (Kharbanda and Tewari 1996). Manipulating soil-water content is another technique for SSR control, but it is not feasible in western Canada as most canola fields are non-irrigated.

Additionally, chemical amendment of soil is another option to reduce carpogenic germination of sclerotia. For example, Perlka (calcium cyanamide) significantly reduced apothecia by 87% in the field experiments (Huang et al. 2006). Other SSR control practices focus on manipulating the local field environment to create a nonconductive condition for SSR development. The relative humidity is a key factor affecting the infection of *S. sclerotiorum* in canola. When proximal relative humidity was greater than 80%, SSR development in

the crop was very aggressive in field experiments (Koch et al. 2007). The high relative humidity is essential during the flowering stage, as senescing flower petals are the primary target of *S. sclerotiorum* ascospores. Relative humidity within the crop can be reduced using suitable crop density, allowing airflow between individual plants.

Among all available SSR management tools, fungicide application is the most effective tool widely used (Canola Council of Canada). Fungicide application can significantly reduce SSR incidence compared with the untreated control. There is a broad range of fungicides that are available for SSR control in canola. Globally, several fungicide classes have been registered to control SSR on canola, including quinone outside inhibitors (QoIs), demethylation inhibitors (DMIs), and succinate

dehydrogenase inhibitors (SDHIs) (Derbyshire and Denton-Giles 2016). These fungicide classes exhibit diverse modes of action, and they can inhibit critical pathways to the pathogen infection cycle. Most fungicides exhibit single-site activity, and the risk of developing fungicide-resistant isolates is relatively high. The first report on *S. sclerotiorum* isolates resistant to fungicides was published in 2001 (Gossen et al. 2001). Canola farmers should rotate among the registered active ingredients to slow down the selection for fungicide-resistant isolates and maintain fungicide efficacy. Some fungicides such as Proline GOLD bring together two active ingredients to protect the crop under severe SSR pressure when applied at label rates (Bayer Crop Science). It is believed that fungicide resistance development is much slower if fungicides with varying modes of action are used.

Due to the interactions of numerous variables affecting SSR epidemiology, it is challenging for farmers to decide the necessity and timing of fungicide application (Bradley et al. 2006). There has been lots of research focused on developing risk assessment tools that can be used to predict SSR outbreaks. Variables such as crop density, cropping history, amount of airborne inoculum, frequency of petal infestation, soil moisture content and relative humidity should be considered when developing such tools (Boland and Hall 1988; McCartney et al. 1999; Canola Council of Canada). Petal testing is helpful for SSR forecasting. Different petal testing kits have been commercially available to Canadian canola farmers since the early 1990s to determine the presence/absence of *S. sclerotiorum* in the canola crop. However, the prediction power of petal testing is very low because the petal testing method itself is affected by environmental variables. A PCR-based detection method is also available to determine the presence/absence of *S. sclerotiorum* DNA. In addition to chemical-based fungicides, RNAi-based fungicides via transcripts knockdown offer a potential new generation of environmentally safe fungicides to manage SSR, but more studies are required to understand the effectiveness of RNAi-based fungicides in the field environment and their modes of action (McLoughlin et al. 2018; Wytinck et al. 2020).

Although a few canola cultivars with enhanced resistance to *S. sclerotiorum* are available in Canada (Falak et al. 2011), the resistance level in these cultivars is not enough to fight against severe SSR infections and additional disease management tools are needed to reduce yield loss caused by SSR infections. Fungicide application is still recommended if a crop with partial SSR

resistance is at high risk of SSR development. Microorganisms to suppress sclerotinia stem rot provide a biological control tool to control the disease. In western Canada, two biological products with different modes of action are available: Serenade OPTI and Contans (Guide to Field Crop Protection 2021). Serenade OPTI is a bacterial formulation that disrupts the cell membrane growth of ascospores and newly developing hyphae. The active ingredient of Contans is a fungus, and it is applied pre-emergence to reduce the viability of sclerotinia in an infested field.

Genetic resistance to sclerotinia stem rot

Genetic resistance through breeding is the most environmentally friendly method for controlling crop diseases (Moose and Mumm 2008). Complete or strong genetic resistance has not been identified in *B. napus* (Ding et al. 2021). A high level of genetic resistance has been discovered in other *Brassica* species, including *B. oleracea*, *B. juncea*, *B. rapa* var. *chinensis*, *B. incana*, *B. rupestris*, *B. insularis* and *B. villosa* (Li et al. 2009; Mei et al. 2011, 2013; Uloth et al. 2013, 2014; Taylor et al. 2018). Among the *Brassica* species, *B. oleracea* has the greatest resistance, and *B. rapa* has the lowest, while *B. juncea*, *B. napus*, and *B. nigra* are intermediate (Mei et al. 2011). Research suggested that winter oil seed rape (OSR) cultivars are more resistant than canola because of higher glucosinolates (Zhao et al. 2004). Various research groups have identified quantitative trait loci (QTLs) associated with SSR resistance in *Brassica* species. However, none of these QTLs confer complete resistance to SSR and the resistance level varies under different environments (Derbyshire and Denton-Giles 2016). Studies that assayed different growth stages often found QTLs specific to each life stage (Zhao and Meng 2003; Wu et al. 2013). The most useful QTLs for SSR resistance breeding are probably effective in mature flowering plants and common QTLs identified in several independent SSR mapping studies (Li et al. 2015).

Bi-parental genetic mapping has been widely used to identify QTLs for SSR resistance. However, this method is mainly dependent on the genetic diversity of the parental population and phenotyping methods. There are many SSR screening methods available to evaluate genetic resistance, but these methods are rarely repeatable. To overcome the limitations of bi-parental mapping, a genome-wide association study (GWAS) has been used to identify QTLs in a panel of germplasm collections. With the significant progress on *B. napus* genome sequencing and the development of *Brassica* single

nucleotide polymorphism (SNP) array technology, QTLs associated with SSR resistance or susceptibility have been identified by GWAS (GyawaGyawali et al. 2016; Wei et al. 2016; Wu et al. 2016). By combing through eight leaf resistance QTLs and 27 stem resistance SSR QTLs, Li et al. (2015) identified two common stem resistance QTLs on chromosomes A9 and C6. However, GWAS has a much higher false-positive rate than bi-parental QTL analysis. Therefore, many research teams have used the combination of GWAS and QTL analysis to achieve more powerful QTL discovery. Also, transcriptome analysis is another addition to bi-parental mapping and GWAS to identify candidate genes.

In addition to genetic resistance QTLs, genetic factors associated with petals and flowering time also demonstrated the potential to reduce SSR susceptibility. Due to the critical role of petals in initiating ascospore infections, apetalous *B. napus* plants, as the result of genetic mutation, can reduce SSR development under a controlled environment (Zhao and Wang 2004; Young and Werner 2012). However, both apetalous and fully petalled cultivars are equally infected by *S. sclerotiorum* in field conditions (Young and Werner 2012). Wu et al. (2016) reported that SSR resistance was significantly negatively correlated with flowering time and identified four common QTL regions on chromosomes A2, A6, C2 and C8 that affect SSR resistance and flowering time. Using a recombinant inbred line population and high density SNP arrays, Zhang et al. (2019) further identified two syntenic QTL blocks on A2 and C2 chromosomes for SSR resistance and flowering time in *Brassica napus* and developed a syntenic method to finalize a list of 33 candidate genes for future investigations and potential incorporation into breeding programs.

These common QTLs provided clues to dissect the genetic linkage mechanisms between these two traits. Lodging is another factor that is associated with SSR infections. Cultivars with good lodging resistance have the potential to reduce *Sclerotinia* severity (Canola Council of Canada).

Breeding for sclerotinia stem rot resistance

Most commercial canola cultivars in Canada are susceptible to SSR. Although variations in resistance or susceptibility are present among canola cultivars, developing SSR-resistant canola lines is challenging due to the lack of highly resistant canola germplasm. Another limitation of SSR resistance breeding is the challenges to develop reliable phenotyping approaches to screen breeding populations (Taylor et al. 2018).

Moreover, different screening approaches in some cases have produced conflicting results. The main SSR screening methods reported include stem inoculations, petiole inoculations, detached leaf incubations, detached stem inoculations and canopy infections using infested wheat or sorghum grain or ascospores (Taylor et al. 2018). However, none of these methods generates high-quality data that differentiate resistant, susceptible, and partially resistant entries. Therefore, the development of robust screening methods is desirable to assist SSR resistance breeding.

The highest available level of SSR field resistance in *B. napus* was achieved by breeding efforts in China. Chinese *B. napus* cultivars such as Zhongyou 821 and Zhongshuang 9 demonstrated stable partial SSR resistance and have been used as resistance donors by some breeding programs worldwide. In Canada, canola cultivars such as 45S51, 45S52, 45CS40 with enhanced resistance to *S. sclerotiorum* are available (Falak et al. 2011), but resistance levels in these cultivars are not enough to withstand high disease pressures. Pyramiding multiple partial resistance QTLs has the potential to achieve more excellent resistance. SSR resistance identified in *B. juncea* and *B. oleracea* (wild cabbage) germplasm can be introduced into *B. napus* through distant hybridization and molecular marker-assisted selection (Li et al. 2009; Mei et al. 2011, 2013). Major QTLs conferring a high level of resistance to *S. sclerotiorum* from wild cabbage were transferred to *B. napus* (Mei et al. 2015, 2020). By transferring three major resistant QTLs from *B. oleracea* and pyramiding them in Zhongshuang 9, Mei et al. (2020) identified offspring lines that exhibited about 35% higher resistance level than Zhongshuang 9.

Despite the challenges, significant progress has been made to improve SSR resistance in recent years. With the availability of more high-quality *Brassica* reference genomes and bioinformatics tools to assist disease resistance breeding and in-depth understanding of resistance mechanisms to support gene target discovery, both public and private breeding programs in Canada and worldwide continue to bring efforts together to improve genetic resistance to SSR.

Plasmodiophora brassicae

Clubroot is one of the most important soilborne diseases caused by the biotrophic protist *Plasmodiophora brassicae* in cruciferous plants, especially in *B. rapa*, *B. oleracea*, and *B. napus*. The pathogen induces

abnormal cell enlargement and division in infected roots, resulting in characteristic deformed galls (clubs). These malformations can interfere with water and nutrient uptake and transportation, resulting in yellowing, wilting, and stunting of the above-ground tissue, and even plant premature death. Clubroot disease can cause significant yield and quality loss, up to 10–15% worldwide (Dixon 2006, 2009). This disease is considered a limiting factor in oilseed production in Australia, Japan, China, and European countries (Voorrips 1995; Wallenhammar et al. 2000). Clubroot disease has been reported on cruciferous vegetables in eastern Canada and British Columbia province for over a century (Howard et al. 2010). The first clubroot disease in a canola field was diagnosed near Edmonton, Alberta, in 2003 (Tewari et al. 2005). Because of the great importance of clubroot disease in canola, we will overview the current clubroot situation in Canada, disease cycle, pathotype, genetic resistance identification, and management strategies.

Clubroot disease in Canada

Historically, clubroot disease has had the most significant impact on cruciferous vegetables in the main vegetable production regions of British Columbia, Ontario, and Quebec (Howard et al. 2010). Clubroot disease was not reported in canola until 2003 when 12 commercial canola fields were infested near Edmonton (central Alberta area) (Tewari et al. 2005). According to a report published in 2020 in the annual *Canadian Plant Disease Survey*, clubroot had spread to a total of 3353 canola fields in Alberta since the first case reported in 2003 (Strelkov et al. 2020). Four soil samples out of 30 commercial canola fields were diagnosed for the presence of the clubroot pathogen in Saskatchewan in 2008 (Dokken et al. 2009). In 2013, two canola crops in Manitoba were first observed with clubroot disease symptoms (Desjardins et al. 2014). In 2017, clubroot symptoms were first observed in canola in one field near Verner, Ontario (Al-Daoud et al. 2018). In Alberta, 268 new confirmed infestations of clubroot disease were recorded in 2019 (Strelkov et al. 2020). In Saskatchewan, the visible symptoms of clubroot disease have been confirmed in 75 commercial canola fields since 2017, including 24 fields in 2020. The 2020 Manitoba canola disease survey reported that positive clubroot symptoms were present in 10 rural municipalities, bringing the total to 44 clubroot-infested canola fields since the first records began in 2013 (<https://www.canolacouncil.org/canola-watch/2021/01/14/updated-clubroot-maps-and-survey-results/>).

From 2017 to date, a continuous increase in cases of clubroot-infected canola fields was reported in both Saskatchewan and Manitoba (Canola Council of Canada 2021). As such, clubroot disease poses an emerging threat to entire 8.0-million-hectare canola industry in the Prairie region.

Thus, clubroot is established on canola and vegetable crops in western Canada. This is of great concern as western Canada is the most important region for canola production. A previous study indicated that clubroot disease could cause 80–91% yield loss in canola plants in Quebec (Pageau et al. 2006). In Alberta, yield loss of the severely clubroot-infected canola crops was estimated to range from 30% to 100%. In addition, clubroot caused significant seed quality declines, reducing oil content by 4.7–6.1% and 1000-seed weight by 13–26% (Strelkov et al. 2007; Hwang et al. 2010). Clubroot can quickly spread by the movement of soil containing soil-borne resting spores. Currently, no economic control measures can remove this pathogen from a field once it has become infested. Therefore, coordinated research activities are required to understand *P. brassicae* biology, dissemination, disease cycle, pathogenic diversity, pathogenic mechanisms, and host resistance to control clubroot effectively.

The clubroot disease cycle

Clubroot galls produce long-lived resting spores, which can survive in soil up to 20 years, with a half-life of around 4 years (Wallenhammar 1996). The *P. brassicae* pathogen consists of three main stages in the life cycle: resting spore survival in soil, primary root hair infection, and secondary cortical infection (Naiki and Dixon 1987). Here, we will outline the life cycle of *P. brassicae* briefly from the detailed review by Kageyama and Asano (2009) (Fig. 4). The resting spores over winter, germinating to release an oval-shaped biflagellate zoospore in the spring, with germination rates increased in response to the presence of host root exudates. The motile zoospore can swim in the film of water in the soil to penetrate the cell wall of root hairs using mechanical force, initiating the primary infection. Usually, primary infection of root hairs does not cause macroscopic symptoms (Howard et al. 2010). The primary infection of the root hairs is followed by development of the primary plasmodia, which will divide to produce zoosporangia, from which 4–16 secondary zoospores are released for later cortical infection. The secondary zoospores penetrate the host plant's root

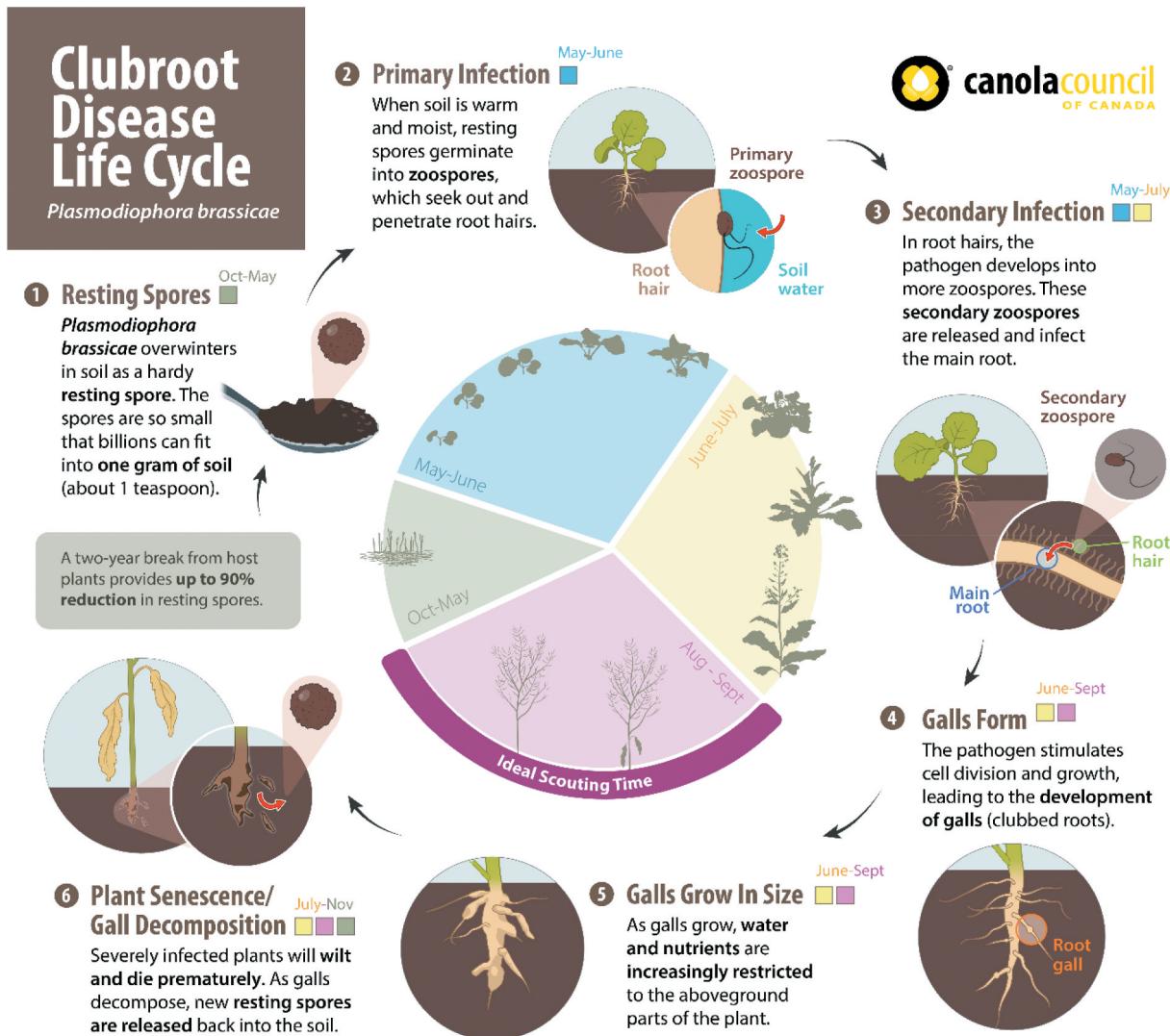


Fig. 4 Clubroot [*Plasmodiophora brassicae*] disease life cycle in Canadian canola (Canola Council of Canada 2021).

epidermis and invade the main roots' cortical cells, representing secondary infection. The establishment of secondary infection will lead to the development of secondary plasmodia within the cell and root cortex, which results in visible symptoms of club-shaped galls in the roots and yellowing, wilting, and stunting of aboveground tissues. The infection of the cortex by *P. brassicae* will affect water and nutrient transportation and the pathogen-induced hormonal disturbances or hypertrophic cells. Within the root galls, the secondary plasmodia cleave into millions of resting spores. The breakdown and disintegration of root tissues will release the resting spores into the soil for the next cycle of infection. Luckily, due to the limitation of zoospore motility in soil, the pathogen

usually completes only one life cycle per canola growing season in western Canada and cannot spread rapidly.

Plasmodiophora brassicae pathotype diversity in Canada

Plasmodiophora brassicae strain virulence and physiological specialization have been studied to differentiate the pathogen in its ability to infect a specific host plant, starting in 1931 by Honig. The term 'pathotype' has been proposed to replace the traditional classification of pathogen strains into 'races', since a lack of genetic uniformity occurred between the different hosts and pathogen isolates with a different interaction. Since

then, several sets of the differential host have been introduced to identify the pathotype of *P. brassicae* strains. Williams (1966) used two *B. oleracea* hosts ('Jersey Queen', 'Badger shipper') and two *B. napus* (Rutabage: 'Wilhelmsburger', and 'Laurentian') to assess the virulence (pathotype) in the pathogen. Later, Buczacki et al. (1975) included five of each *B. rapa*, *B. oleracea*, *B. napus* hosts to classify the *P. brassicae* population as a European clubroot differential set (ECD). Three *B. napus* cultivars ('Nevin', 'Wilhelmsburger', 'Brutor') were used to identify pathogen strains in France (Somé et al. 1996). These three systems were widely used to compare the *P. brassicae* pathogens identified from canola; however, some pathotype distinctions may be relevant for canola while others may not effectively identify all the relevant strains. A new Canadian differential set (CCD) has been developed, consisting of 13 *Brassica* hosts, including eight from the ECD, 'Brutor', 'Laurentian', 'Mendel', 'Westar' (susceptible cultivar) and '45H29' (the first clubroot-resistant canola cultivar released in the Canadian market in 2009) (Strelkov et al. 2018). Seventeen pathotypes were detected with the CCD set compared to five and two pathotypes, respectively, on the differentials of Williams and Some et al.; this indicates that the CCD set can differentiate those pathotypes highly relevant to canola while producing reliable and consistent results.

Based on the Williams (1966) differential set, pathotype 3 was predominant on canola in western Canada (Alberta), while pathotypes 2 and 6 were commonly identified in eastern Canada on cruciferous vegetables and canola (Strelkov et al. 2006, 2007; Strelkov and Hwang 2014). Specifically, pathotypes 2, 3 and 5 were found in the *P. brassicae* population in Alberta, while pathotype six was identified in British Columbia. Pathotypes 3 and 5 have also been from canola crops in Manitoba and Saskatchewan, respectively (Cao et al. 2009; Strelkov and Hwang 2014). Previous studies indicated that pathotype 6 was common in Ontario, and pathotypes 2 and 5 were found in Quebec (Strelkov et al. 2006; Xue et al. 2008; Cao et al. 2009). In 2013, a new pathotype 5X characterized from two fields in central Alberta (North of Edmonton) could overcome the resistance of commercial canola cultivars used in the Canadian market (Strelkov et al. 2016). This pathotype was designated as pathotype 5 based on the Williams differential set. However, as it showed increased virulence on clubroot-resistant cultivars, it is referred to as pathotype 5X. Pathotype analysis of *P. brassicae* isolates from the resistant canola cultivars in the Prairies indicated five pathotypes identified

according to Somé et al. (1996) in 2017–2018, including P1, P2, P3, P4, and P5. Pathotypes 4 and 5 *sensu* Williams were also reported in western Canada for the first time (Hollman et al. 2021). Manitoba Agriculture reported *P. brassicae* 3A, as defined on the CCD set, in the Rural Municipality of Pembina in 2019 (<https://www.gov.mb.ca/agriculture/crops/seasonal-reports/pubs/club-root-pathotype-3a.pdf>), the first resistance-breaking pathotype found on the Prairies outside of Alberta. The traditionally labeled 'CR' canola cultivars cannot prevent clubroot infection against the pathotype 3A strain. The R-rated canola cultivars showed good resistance to pathotypes 2, 3, 5, 6 and 8 as designated on the Williams set. However, they will not effectively defend against the newly identified *P. brassicae* strains of pathotype 3A and 5X. A recent survey (2017–2019 clubroot samples from Ontario and six Canadian provinces) reported that pathotypes '2X' and '3X' were found in canola. More isolates of pathotypes '2X', '3X', and '5X' were found in eastern Canada and showed virulence on the first generation of CR canola cultivars in Canada. The investigation also suggested that pathotype 9 *P. brassicae* isolates were identified in Manitoba. Rapid shifts in pathotype were confirmed from pathotype 6 to 2 in Ontario and from pathotype 2 and 5 to '2X' and '5X' in Quebec were also suggested (McDonald et al. 2020). Understanding the pathotypes of *P. brassicae* in the Canadian canola fields will help to guide the selection and assessment of recently released resistant canola cultivars. In addition, it is important to monitor changes in the predominant pathotypes in the fields over time, which can result in new pathotypes and host resistance breakdown. Corresponding canola cultivars will need to be recommended against the predominant *P. brassicae* pathotypes, since recently labeled 'CR' cultivars cannot protect against all the pathotypes present in the field. The screened novel resistance genes or sources could be incorporated into breeding programs, producing new canola cultivars/lines equipped against the new or more virulent pathotypes of *P. brassicae* strains.

Clubroot disease management

A diverse crop rotation, with a minimum two-year break between canola crops, allows for inoculum levels to decrease. Since clubroot is a soil-borne disease, sanitation is an important biosafety aspect to prevent clubroot disease from spreading. Anything that can bring soil with spores into pathogen-free soil, such as equipment, tractor tires, boots, and tools, should be sanitized before entering fields. Water is another medium that can spread

the disease by transferring the infested soil to other clean fields. Therefore, over-irrigation and waterlogging should be restricted in fields. High soil moisture will enhance infection of plants by the biflagellated zoospores, and thus, over-irrigation and water logging should be avoided. Increasing the soil pH level by raising the alkalinity in the soil can reduce the spore density to prevent clubroot disease development (Donald and Porter 2009). Soil liming elevating the soil pH value (>7) under lower infestation or when discovered early will reduce the impact and severity of clubroot disease in the canola field. Crop residues that preserve the pathogen spores as inoculum sources should be removed from the field or destroyed by burning after harvest. Controlling host weeds is another important practice to manage clubroot disease. Common weeds such as stinkweed, shepherd's purse, flixweed, mustards, or volunteer canola, which can host the clubroot disease, should be removed within three weeks of emergence to prevent spore production and dissemination (Canola Council of Canada 2021).

Genetic resistance and scouting should as priorities to protect the canola plants at the beginning of each season. In some canola cultivars, labels are included to indicate to which of Canada's predominant *P. brassicae* strains or pathotypes a particular host is resistant. Since the first clubroot canola hybrid '45H29' was released by Pioneer, over 50 canola clubroot-resistant cultivars have been registered in Canada (Canola Council of Canada 2021). As the virulence of *P. brassicae* populations can shift rapidly in response to the selection pressure from canola cultivars, the development or breeding for new clubroot-resistant canola cultivars is required. By monitoring the pathotypes and diversity of the *P. brassicae* populations in fields, rotation with different cultivars may reduce clubroot incidence and severity. Carefully scouting to detect early infestations can alert canola farmers to choose the appropriate resistant cultivars, which is important to protect the plant from pathogen infection.

Protozoicide was proposed to be a more appropriate term than fungicide when using chemicals to manage the *P. brassicae*, as this pathogen is not a true fungus (Donald and Porter 2019). Chemicals, such as fluazinam and cyazofamid, can effectively control clubroot disease under higher disease severity (Donald and Porter 2009; Peng et al. 2011). Some commonly used chemicals against *P. brassicae* have been registered, such as Nebijin, pentachloronitrobenzene, Nano Silver Hydrogen peroxide, etc. (Gahatraj et al. 2019). However, the relatively high cost, application rate, and limited availability make chemical control a poor option

for managing clubroot disease in canola. Biological control, such as *Bacillus subtilis* and *Gliocladium catenulatum* applied in soil-drench, can reduce clubroot severity by more than 80% (Peng et al. 2011). Several *Trichoderma* species have also been reported as biocontrol agents that can protect crops from the damage by soilborne diseases (Elad et al. 1987). In conclusion, it is vital to forestall the pathogen's impact and spread by using sanitation, liming of the soil, regular scouting, and incorporating resistant cultivars and a lengthened crop rotation to manage clubroot disease in canola.

Genetic resistance to clubroot in *Brassica* species

The development of genetically resistant cultivars to defend against biotic and abiotic stresses is highly desired to minimize yield losses and manage clubroot effectively in different crops. Thus, identifying genetic resistance could be an effective and environment-friendly strategy to manage clubroot disease in *Brassica* crops. Extensive studies have been done to identify both qualitative and quantitative clubroot resistance across *Brassica* spp. To date, at least 15 CR loci have been identified from *B. rapa*, including *CRa* (Matsumoto et al. 1998), *CRb* (Piao et al. 2004), *Crr3* (Hirai et al. 2004), *CRk* (Matsumoto et al. 2012), *PbBa3.1* and *PbBa3.3* (Chen et al. 2013), *Rcr1*, *Rcr2*, *Rcr4*, and *Rcr5* (Chu et al. 2014; Huang et al. 2017, 2019; Yu et al. 2017b) on chromosome A03. CR resistance loci *Crr1*, *Rcr9*, *CRs*, *Crr2*, *Crr4*, *CRc*, *CRb^{Kato}* and *Rcr8* (Yu et al. Yu, et al., 2017; Sakamoto et al. 2008; Suwabe et al. 2003; Laila et al. 2019; Kato et al. 2012; Suwabe et al. 2006) have been identified to be located on chromosomes A08, A01, A06 and A02 in *B. rapa*. However, much less dominant CR genes were present in the C genome of *B. oleracea* (Zhang et al. 2016a), where only five loci have been reported, including *CR2a*, *CR2b*, *Pb3*, *Pb4* and *PbBo1* (Landry et al. 1992; Voorrips 1995; Grandclément and Thomas 1996; Rocheirieux et al. 2004). A major gene *Pb-Bn1* was mapped on chromosome A04 of *B. napus*, which conferred resistance to single-spore isolates of *P. brassicae* (Manzanares-Dauleux et al. 2000). Using a double haploid population derived from ECD 04, one major and two recessive genes have been identified in *B. napus* (Diederichsen et al. 2006). A clubroot resistance gene *Rcr6* was identified from *B. nigra* (B genome) using bulk segregant RNA sequencing technology, and which resides in the genomic region homologous to chromosome A08 in *B. rapa* (Chang et al. 2019). Recently, a similar strategy was used to identify two new clubroot

resistance genes, *Rcr3* and *Rcr9^{wa}*, in *B. rapa* (Karim et al. 2020). *CRa* has been cloned and found to encode a TIR-NBS-LRR (toll/interleukin-1 receptor-domain containing nucleotide-binding site leucine-rich repeat) class protein (Ueno et al. 2012; Marone et al. 2013). *Crr1* on chromosome A08 has been cloned and demonstrated to consist of two gene loci as a major locus *Crr1a* and minor locus *Crr1b* (Suwabe et al. 2012). Quantitative resistance has been frequently reported in *Brassica* spp. in response to clubroot disease.

At least 22 quantitative trait loci (QTLs) have been identified in the C genome of *B. oleracea* (Diederichsen et al. 2009; Piao et al. 2009; Peng et al. 2018). So far, at least 22 QTLs involved in clubroot resistance have been reported in *B. napus* (Diederichsen et al. 2009; Piao et al. 2009). To develop and breed clubroot-resistant *B. napus* lines for long-term clubroot management, a pool of CR genes from diverse *Brassica* spp. should be utilized. This also provides the most straightforward and sustainable manner for disease control. The available qualitative major genes or quantitative resistance could be introduced and inherited from the *Brassica* crop-breeding programs. Rotation of different CR genes or pyramiding the major resistance genes could confer excellent resistance to multiple pathotypes of *P. brassicae*.

Moreover, investigating the genetic resistance specifically involved in the primary root hair and secondary cortical infection may be valuable for understanding compatible and incompatible interactions between *B. napus* and *P. brassicae*. In addition to the embryo rescue technique, the amphidiploid species *B. napus* can be re-synthesized through interspecific hybridization between two progenitors, *B. rapa* and *B. oleracea*. Thus, the genetic resources from other *Brassica* spp. could be transferred into new *B. napus* cultivars with improved clubroot resistance.

Molecular interaction between *Brassica* spp. and *P. brassicae*

Plant pathogens, assisted by effector proteins, have been found to evade the sophisticated plant immune system. Characterization of effector proteins from *P. brassicae* with the potential roles in the infection and colonization in host *Brassica* plants is critical to understanding clubroot pathogenesis. Baxter et al. (2010) verified the first effector protein PbBSMT (SABATH-type methyltransferase) in *P. brassicae*, which can methylate salicylic acid (SA) to a volatile methylated form (MeSA). Along with the well-sequenced *P. brassicae* genome, over 500 of the 9730 genes encode secreted proteins, playing

essential roles in infection and disease development (Schwelm et al. 2015). A protein (PbGH3) can affect auxin and jasmonic acid in the infected roots, and chitin-related enzymes having carbohydrate/chitin-binding domain (CBM18 family) in the *P. brassicae* secretome can be resilient to the resting spores (Schwelm et al. 2015). In addition, a recent report indicated that *PbchiB2* and *PhChiB4* derived from the CBM18 family participated in the spore transition to a uninucleate primary plasmodium and spore formation (Muirhead and Pérez-López 2021). Two secreted proteins (PBCN_002550 and PBCN_005499) were found to induce cell death associated with hydrogen peroxide accumulation and electrolyte leakage during the primary infection process (Chen et al. 2019). A total of 32 small, secreted proteins (SSPs) from *P. brassicae* were highly expressed during the secondary infection process in host plants (Pérez-López et al. 2020). Several other motifs/domains associated with proteins, including cysteine-rich, Rxlr motif, protease inhibitors, nuclear localization, and the Pexel motif, are worth exploring in the future (Pérez-López et al. 2018). The potential for future work with pathogen effectors and the plant responses is exciting; however, the subsequent studies, such as intracellular analysis, genetics, and biochemistry, require more innovation and novel approaches. Understanding the virulence of *P. brassicae* and its interaction with host plants may facilitate genetic modification and resistance breeding to improve Brassica crop's performance against clubroot disease.

Challenges in breeding for clubroot disease

The main challenge in clubroot resistance breeding is the genetic variation within the *Brassica* species and within the pathogen. As reviewed above, most of the qualitative resistance genes come from other *Brassica* species, for example, mostly *B. rapa*. This will cause several challenges in a breeding program: (1) fertility problems at crossing due to self-incompatibility is a common phenomenon in the *Brassica* family; (2) requirement of the embryo rescue technique to resynthesize the new *B. napus* line from *B. rapa* and *B. oleracea*; (3) the need for vernalization before crossing in some *Brassica* cultivars; and (4) the length of time it takes to transfer the resistance to *B. napus* from other close relatives. In addition, controlling clubroot by using major gene resistance will impose selection pressure on the pathogen population. Natural selection, DNA mutation, and recombination during sexual reproduction are all forces that can shift/change the pathogen population in the

fields. It will increase some existing pathotypes' frequency, change their virulence, and even produce some new pathotypes. Finally, the currently used resistance genes will be overcome. The shifts in pathogen populations are quicker than anticipated and may result in resistance breaking down quickly. Consequently, a breeder will spend extra time looking for new resistance sources. This requires characterization and mapping of the *R* genes from donor cultivars/lines and finally introgression into elite lines. The breeding process could also potentially introduce undesirable characteristics into elite lines, negatively affecting other core breeding program traits.

Quantitative resistance is polygenic and non-specific and provides more durable disease resistance. Quantitative resistance frequently has only a partial effect and is difficult to select. Along with the exponential development of molecular markers over the last two decades, QTL related to disease resistance have been integrated into breeding strategies for producing resistant cultivars. The identified QTL are often additive; however, epistatic effects of QTL have also been identified. Some resistance QTL can only be detected under certain environmental conditions such as soil, climate, and pathogen populations. The genotype-environment interaction has significantly affected the effectiveness of detected resistance QTL. Particularly, the detected QTL could be isolate specific or effective against a broad spectrum of isolates. The recently reported QTL for clubroot resistance could be species-specific or plant cultivar or population-specific. In addition, sometimes QTL are linked to unexpected phenotypic traits, which increases the difficulty of incorporating the disease resistance of the donor into elite lines of a breeding program. In the canola breeding strategy, we must seriously consider other traits, including seed yield, seed quality and composition, pod shatter resistance, lodging, and maturity. Thus, these multi-functional QTL introduced into breeding germplasm will require a lot of time for evaluation/marker-assisted selection of the other traits in the following steps. It still requires choosing adequate resistance QTL to optimize better combinations and limit the QTL erosion effect. The design of breeding strategies should require a better understanding of the genetic basis, ecological effect, and agronomic determination of clubroot disease adaption. Pyramiding of QTLs is more difficult and complicated than the qualitative resistance (*R*) practically. Finally, the best breeding strategies for clubroot resistance include integrating qualitative resistance (*R* genes) with quantitative resistance and incorporating it with other diverse disease control measures.

Clubroot of canola has become one of the most important diseases in western Canada. Now, this disease is also spreading in eastern Canada and likely will continue to spread into other provinces. An IPM strategy is required to preserve sustainable canola production in prairies provinces. The research efforts on understanding canola-clubroot interaction, *P. brassicae* pathotype diversity, qualitative and quantitative resistance, crop rotation, agricultural practice, biocontrol agents, and soil management have significantly mitigated this disease on canola crops.

***Verticillium* species**

Verticillium is an anamorphic genus of fungi belonging to the Ascomycota phylum, Sordariomycetes class, Hypocreomycetida sub-class (Depotter et al. 2016) in the Plectosphaerellaceae family. The genus is thought to contain 51 species (Kirk et al. 2008), with at least five species known to cause a wilt disease in plants called verticillium wilt: *V. dahliae*, *V. longisporum*, *V. albo-atrum*, *V. nubilum*, and *V. tricorpus*. *Verticillium longisporum* (ex. *V. dahliae* var. *longisporum* Stark; comb. nov. Karapapa) and *V. dahliae* (a close relative of *V. longisporum*) both cause verticillium stripe disease in *Brassica* crops. While *V. dahliae* has an extensive host range and can cause vascular symptoms in more than 200 economically important plant species, *V. longisporum* is mostly restricted to oilseed rape (Zou et al. 2020). *Verticillium longisporum* is an amphidiploid hybrid resulting from hybridization between two haploid ancestors. The species has four parental lines (*V. dahliae* lineage D2, *V. dahliae* lineage D3, unknown species Species A1 and Species D1) belonging to three different *Verticillium* species (A1/D1, A1/D2 and A1/D3) (Depotter et al. 2016). Due to its hybrid ancestry, *V. longisporum* is more virulent on oilseed rape than *V. dahliae* (Eynck et al. 2009).

***Verticillium longisporum* life cycle**

Verticillium longisporum is a soil-borne vascular fungal pathogen. It has a hemibiotrophic life cycle (Depotter et al. 2016), including biotrophic and necrotrophic stages. Its life cycle can be divided into dormant, biotrophic and heterotrophic phases. The fungus life cycle starts with the production of microsclerotia, mycelia, or conidia (Fig. 5). Microsclerotia are clusters of melanized thick-walled fungal cells known as overwintering or dormant structures, which allow the fungus to survive

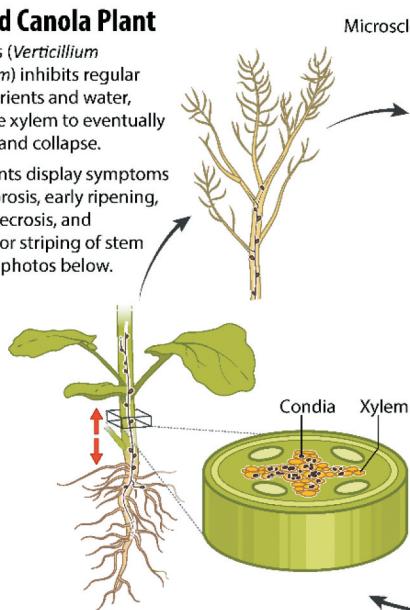
Verticillium Stripe Disease Cycle

(Caused by the fungus *Verticillium longisporum*)

④ Diseased Canola Plant

The fungus (*Verticillium longisporum*) inhibits regular flow of nutrients and water, causing the xylem to eventually turn black and collapse.

Canola plants display symptoms of leaf chlorosis, early ripening, stunting, necrosis, and shredding or striping of stem tissue. See photos below.



③ Distribution of Disease

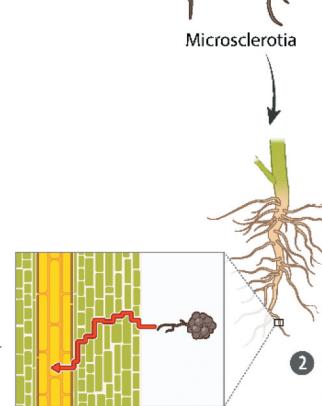
Hyphae and single cell spores called condia are produced locally in the xylem and move up the vascular system.

⑤ Release of Microsclerotia

The pathogen moves into non-vascular tissue where multicellular microsclerotia are formed. The stem tissue is fragile, allowing for it to easily shred. The stem epidermis peels back to expose the microsclerotia. The microsclerotia are released in the soil and the cycle repeats.

① Germination of Fungal Propagules

Fungal propagules called microsclerotia are present in soil or dead plant tissue. Root exudates stimulate microsclerotial development.



② Systemic Invasion and Multiplication

Microsclerotia enter the plant vascular system through fungal hyphae and multiply.

Fig. 5 Verticillium stripe [*Verticillium longisporum*] disease life cycle in Canadian canola (Canola Council of Canada 2021).

for more than 10 years (Depotter et al. 2016). Following the biotrophic phase, microsclerotia germinate and develop fungal hyphae that infect lateral roots and root hairs by penetrating the root cortex and then spread further into the vascular system (Zhou et al. 2006). Single-cell spores called conidia are also produced locally in the xylem and moved through the vascular tissues. The fungus lives in the vascular system most of its lifecycle and exploits the xylem sap for nutrition during the biotrophic phase (Lopez-Millan et al. 2000). During later stages of host colonization, as the plant begins to mature, the necrotrophic phase starts. The fungus moves from the xylem into non-vascular tissues, colonizes the stem parenchyma, and feeds on senescing leaf and stem tissues (Reusche et al. 2012), where it later forms microsclerotia in the stem pith and under the stem epidermis (Knüfer et al. 2017). At harvest, the microsclerotia are released and settle down into the soil together with plant residue. Microsclerotia are the primary dispersal agent of the pathogen and can disperse

between and within fields with soil, water, and crop debris.

Verticillium longisporum damage

Verticillium stripe is a monocyclic disease, meaning that the pathogen produces only one cycle of development per crop cycle or year. Infected plants develop initial symptoms consisting of wilting, stunting and chlorosis on leaves and lateral branches, and discoloration of the stem and early senescence (Fradin and Thomma 2006). Late season senescence, stem discolouration, potential wilting, lodging, and prematurely ripening are the major symptoms in Canada. In *Brassica* species, *V. longisporum* does not develop wilting symptoms; therefore, the common name of verticillium stripe is more appropriate than verticillium wilt. In the final stages of the disease, the pathogen forms dark microsclerotia in the root tissue and stem cortex beneath the epidermis causing the tissues to turn grey and black. The

microsclerotia in the stems cause a peeling back of the stem epidermis so that the stem takes on a shredded appearance (Eastburn and Paul 2007). The interference with water and nutrient uptake can cause the crop to show stunting and premature senescence. Yield losses have been reported to be as high as 80% for single infected field plants, but losses for fields increase where weather conditions promote earlier colonization of plants (Dunker et al. 2008). Field losses of 10% to 50% was also reported by other researchers (Günzelmann and Paul 1990; Paul 2003). So far, the disease has not proven to reduce yields significantly on a regional basis. Yield losses are primarily associated with reduced oil content (Johansson et al. 2006) and oilseed grain size (Zhou et al. 2006).

Breeding for resistance and resistance sources

Since verticillium stripe is a soilborne, root-infecting pathogen, the disease management strategies will resemble those for other soilborne diseases. Since control of verticillium stripe with chemical fungicides is not effective (Klosterman et al. 2009), non-chemical strategies and agronomic practices such as biosecurity measures, soil and equipment sanitation, soil solarization, soil amendments, soil steam sterilization, soil fertility, and cropping systems are alternative effective management options (Panth et al. 2020). In combination with these options, breeding for disease resistance is a promising approach to control diseases of Brassica crops (Bailey-Serres et al. 2019). Pathogens' virulence depends on the extent of genetic variation of the host for resistance. Strategies to reduce disease severity can be divided into two approaches: temporal (application of different resistance sources across time, i.e. crop rotations) and spatial (which can vary from multiple resistance sources occurring in a single cultivar (pyramiding), in different cultivars within the same field (cultivar mixtures) or different fields (mosaics)) (Rimbaud et al. 2018). Since the goal of breeding for resistance is generalized resistance to the pathogen biotypes, these temporal and spatial approaches, which can be so-called 'gene rotation', can constrain the evolution of pathogen populations and thus increase genetic resistance durability.

Today, with the introduction of next-generation sequencing technology, the development of long-read sequencing technologies such as PacBio Single Molecule, Real-Time (PacBio) sequencing (Roberts et al. 2013) and Oxford Nanopore Technologies (Jain et al. 2016), along with high-throughput physical mapping technologies such as BioNano optical mapping

(Howe and Wood 2015) and Chromosome Conformation Capture (Hi-C) (Van Berkum et al. 2010; Li et al. 2017), valuable genomics resources have been generated for a better understanding of the molecular aspects of host-pathogen interactions and for identification of candidate quantitative trait loci (QTL)/candidate *R* genes. These technologies have been applied widely for the QTL mapping of disease resistance traits and identifying candidate genes through genome-wide association studies (GWAS) in *Brassica* crops (Neik et al. 2020). To date, a few verticillium stripe *R* genes have been identified. Although several crops have been reported with polygenic *V. longisporum* resistance (Kemmochi et al. 2000; Fradin and Thomma 2006; Rygulla et al. 2008), no monogenic resistance has been found yet. The *Vel* gene, initially identified in tomato (Kawchuk et al. 2001), is the only *R* gene described against verticillium disease (Fradin et al. 2009, 2011). Verticillium quantitative resistance exists mainly in the *Brassica* C genome of parental *B. oleracea* lines and may be introgressed in canola breeding lines (Depotter et al. 2016). For example, typical verticillium symptoms were not detected on broccoli grown in infested soil. However, resistance was not consistent when plants were inoculated by conidiospore suspension using the root dipping method (Zeise and Tiedemann 2002). Nevertheless, some cultivars from the United States exhibited resistance against 15 *Verticillium* isolates from different hosts using the same root dipping inoculation method (Bhat and Subbarao 2001). For both winter and spring-type canola cultivars, breeding for resistance has been hindered by a lack of sufficient host resistance for *V. longisporum* (Happstadius et al. 2003); nonetheless, breeding for disease resistance is still the most practical and economical means of disease control.

In one study, Happstadius et al. (2003) screened 299 accessions representing 11 cultivar groups of *B. oleracea* and eight additional accessions of the wild species *B. cretica*, *B. incana*, *B. insularis* and *B. villosa* for resistance to verticillium stripe. They found that a total of 235, or 77% of the accessions tested, had a corrected disease index (DI_{corr}) less or equal to the oilseed rape cultivar 'Express' ($DI_{corr} = 2.81$), the reference cultivar. In addition, *B. incana* was the only accession of a wild species with an enhanced resistance level ($DI_{corr} = 2.01$). They also reported that when the progeny of the produced re-synthesized rapeseed lines was investigated for verticillium stripe resistance, three lines showed a significantly lower disease index than the reference cultivar. A promising level of resistance to *V. longisporum* also has been identified in *B. oleracea* (accession Kashirka 202) (Dixelius et al. 2005; Rygulla et al. 2007a) and in re-synthesized *B. napus* produced by

hybridization of resistant *B. oleracea* (C genome donor) and *B. rapa* via the embryo rescue technique (Rygulla et al. 2007a, b). Furthermore, in a comprehensive screening study performed by Eynck et al. (2009), most of the tested *B. napus* accessions showed a susceptible to moderate resistant response, and most of the *B. rapa* genotypes were highly susceptible; however, an elevated level of resistance was found in the *B. oleracea* pool. In addition, re-synthesized *B. napus* lines produced based on these data also exhibited enhanced resistance to *V. longisporum*. Enhancer of vascular Wilt Resistance 1 (EWR1) is a gene that enhances the resistance against verticillium wilt in *Arabidopsis* (Yadeta et al. 2011, 2014). EWR1 may be used to reduce *Brassica* plants' susceptibility to *V. longisporum* as it has homologs that are only found within the Brassicaceae family (Yadeta et al. 2014). The genetic basis of *Verticillium* resistance and disease traits has been elucidated using *Arabidopsis thaliana* (Ellendorff et al. 2009). For example, *Vet1*, a locus on chromosome 4, was shown to confer resistance to chlorosis, and it also delayed flowering after *Verticillium* infection (Veronese et al. 2003). In addition, two QTL were associated with resistance to chlorosis caused by *V. longisporum* (Johansson 2006).

In *B. napus* (re-synthesized from a cross between white cabbage (*B. oleracea* ssp. *oleracea* convar. *capitata*) and a winter turnip rape (*B. rapa* ssp. *oleifera*)), two QTL that significantly correlated with *V. longisporum* resistance were identified on chromosomes C4 and C5 (Rygulla et al. 2008). In another study, three QTL, two on the C5 and one on the C1 chromosome, were identified in the *B. napus* cultivar 'Express 617', indicating that quantitative resistance sources resulted from parental cabbage lines (Obermeier et al. 2013). Recently, Single Nucleotide absence Polymorphism (SNAP) markers were used to trace presence-absence variation (PAV) and to associate PAV with resistance against *V. longisporum* by Gabur et al. (2020), who reported some loci influencing *V. longisporum* resistance in biparental and multi-parental mapping populations. They also stated that PAV was observed in 23–51% of the genes within confidence intervals of QTL for *V. longisporum* resistance. High-priority candidate genes identified within QTL were all affected by PAV. Another QTL region containing seven *R* genes was found to be affected by PAV on chromosome C9 for *V. longisporum* resistance in a *B. napus* nested association mapping population (Gabur et al. 2020).

Since the first report of the presence of *V. longisporum* in Manitoba in 2014 by the Canadian Food Inspection Agency (CFIA), several studies and research projects

have been conducted; however, there is still much that is unknown about this pathogen and disease in Canada, making further research particularly necessary. Among the different research themes, the discovery of genetically diverse resistance sources in different *B. oleracea* and *B. rapa* accessions, and the identification and characterization of *R* genes/QTL are particularly important. The introgression of *R* genes from *V. longisporum*-resistant donors can be expected to result in re-synthesized *B. napus* lines with a quantitative and, therefore, potentially more durable polygenic resistance (Rygulla et al. 2007b).

Challenges in breeding for verticillium stripe resistance

In canola breeding, interspecific crosses between *B. oleracea* and *B. rapa* genotypes are the first step to enlarge the available disease *R* genes pool. Re-synthesizing novel *B. napus* genotypes has been widely used to introduce *R* genes against different pathogens (Rygulla et al. 2007a). For example, in oilseed rape, the introduced *L. maculans* and *V. longisporum* *R* genes were derived from the *B. rapa* and *B. oleracea* genome donors, respectively. A few *B. oleracea* gene-bank accessions were identified with high resistance levels to *V. longisporum* (Happstadius et al. 2003). Considering this strategy, many *R* genes/QTL types have been identified in canola and are being used to improve cultivars' resistance. However, only a few *R* genes and QTL have been identified for verticillium stripe disease, and monogenic resistance has not yet been found. This might be because molecular mechanisms underlying verticillium resistance are still unknown.

Identifying and characterizing a novel *R* gene depends on identifying a phenotype, identifying genetic markers, and understanding how the novel resistance will behave under different genetic backgrounds and pathogenic pressures (Mehraj et al. 2020). Besides, since the re-synthesized lines have lower substandard agronomic performance (Becker et al. 1995) and contain high amounts of undesired seed erucic acid and glucosinolates, they are not broadly used in breeding programs.

Moreover, interspecific crosses could reduce the degree of linkage drag in the primary re-synthesized lines. Introducing germplasm from re-synthesized lines into elite breeding material requires extensive backcrossing (Rygulla et al. 2007b). These studies can open many downstream research opportunities to elucidate the mechanisms and genes involved in resistance. Therefore, understanding the genetic diversity of the pathogen, host

resistance, and plant-pathogen interactions using molecular and sequencing technologies and, more importantly, genomic breeding approaches and genome editing tools, could lead to the better understanding of the mechanisms of resistance to *V. longisporum* and enhance the development of disease-resistant canola cultivars.

Conclusion

Canola is one of Canada's most valuable crops, but its production is threatened by diseases such as blackleg, clubroot, sclerotinia stem rot, and verticillium stripe. These diseases not only affect production in Canadian canola but also are important canola diseases worldwide (Zheng et al. 2020). Advances in understanding and managing these diseases must be a global effort, as collaboration beyond borders will help the industry stay ahead and alleviate the diseases' impact. Joint initiatives that share differential sets, germplasm, and pathogen isolates are in motion to provide research groups the resources they need to advance their work on achieving overall disease management.

Several genes conferring to these diseases have been mapped through collaborations and the use of conventional and molecular techniques that include omics approaches as tools. Some are being transferred into agronomically elite cultivars for farmers to use. Technological advances have provided farmers with the new tools they need to manage these diseases in ever-changing climate and regulatory environments. There is a constant need to develop disease-resistant cultivars using various tools and strategies for integrated pest management and sustainable crop production, making sustainability the priority in canola production.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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