Current understanding of phoma stem canker and light leaf spot on oilseed rape in the UK

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Summary

Oilseed rape is the third most important arable crop in the UK. Phoma stem canker and light leaf spot are two economically important diseases of this crop. These two diseases cause annual yield losses of winter oilseed rape worth > £100M, despite the use of fungicides. Phoma stem canker is caused by two closely related fungal pathogens *Leptosphaeria maculans* and *L. biglobosa*, whereas light leaf spot is caused by the fungal pathogen *Pyrenopeziza brassicae*. Epidemics of both diseases are initiated in autumn by ascospores released from crop debris from the previous cropping season. However, phoma stem canker is a monocyclic disease, while light leaf spot is a polycyclic disease. Understanding the pathogen biology, disease epidemiology and host resistance are essential for effective control of these two diseases. This mini review summarises current understanding of these two diseases in relation to pathogen biology, disease epidemiology and host resistance.

Key words: blackleg, *Brassica napus*, disease control, host resistance, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, *Pyrenopeziza brassicae*

Introduction

Oilseed rape is the third most important arable crop in the UK. Diseases of arable crops are major threats to food production in the agricultural industry**.** Phoma stem canker and light leaf spot are two economically important diseases of oilseed rape. These two diseases cause annual yield losses of UK winter oilseed rape >£100M despite the use of fungicides (www.cropmonitor.co.uk). Understanding of the pathogen biology, disease epidemiology and host resistance is essential for effective control of these two diseases.

Phoma stem canker

Pathogen biology

In the UK, phoma stem canker is caused by two closely related fungal pathogens *Leptosphaeria maculans* (Lm) and *L. biglobosa* (Lb) that cause different symptoms on leaves and stems of oilseed rape (West *et al*., 2002; Huang *et al*., 2005). Germinated ascospores of Lm and Lb penetrate leaves of oilseed rape through stomata (Huang *et al*., 2003a). On leaves, Lm causes large phoma leaf spot lesions with many pycnidia while Lb causes small dark lesions with no or few pycnidia; on stems, Lm is often associated with damaging stem base cankers, whereas Lb is generally associated with superficial upper stem lesions with dark margins (Williams & Fitt, 1999; Toscano-Underwood *et al*., 2001) (Fig. 1). Therefore, Lm is considered more damaging than Lb and current control of phoma stem canker by variety resistance or

fungicides mainly targets Lm. No current cultivars have been bred for resistance against Lb. Recent work suggested some cultivars that are resistant to Lm are often more susceptible to Lb and Lb can cause severe yield losses (Huang *et al*., 2014; Cai *et al*., 2018). Furthermore, Lb is less sensitive to some triazole fungicides than Lm (Eckert *et al*., 2010; Huang *et al*., 2011). Therefore, effective control of phoma stem canker needs to target both Lm and Lb.

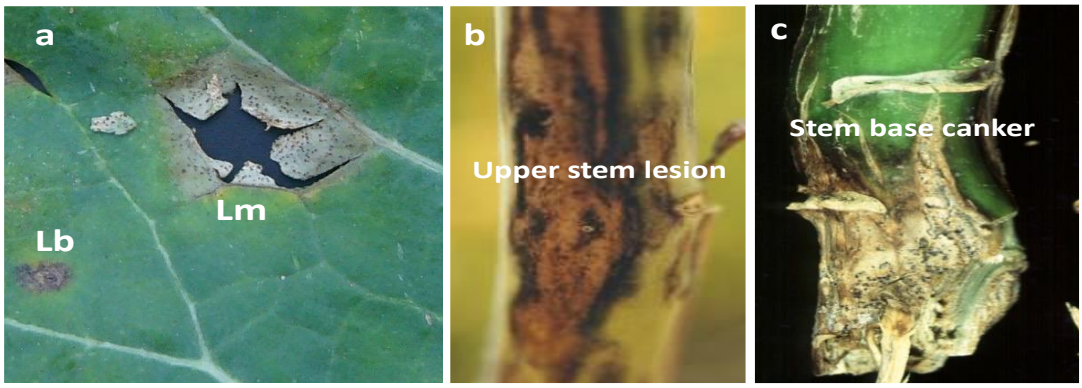

Fig. 1. Symptoms of phoma leaf spot caused by *L. maculans* (Lm) or *L. biglobosa* (Lb) (a) and symptoms of phoma stem canker on upper stem (b) and stem base (c).

Ascospores of Lm and Lb are similar in size and shape, so they cannot be distinguished visually but can be distinguished by ascospore germination patterns. Germ tubes of Lm ascospores often emerge from the interstitial cells of the ascospores and the hyphae grow tortuously with extensive branching, while germ tubes of Lb ascospores often emerge from the two ends of the ascospores and the hyphae grow predominantly straight with little branching (Huang *et al*., 2001 & 2003a) (Fig. 2). Lm and Lb can be also distinguished by colony morphology and production of pigments on PDA; Lb produces fluffy colonies with yellow pigment while Lm produces flat colonies without yellow pigment (Williams & Fitt, 1999). However, checking ascospore germination patterns is time-consuming and technically demanding, and colony morphology and production of pigment on PDA are not always reliable identities. Therefore, confirmation of Lm and Lb isolates needs to use species-specific PCR (Liu *et al*., 2006). After release from pseudothecia, ascospores of Lm and Lb can survive more than 35 days at 20°C in darkness; however, Lm ascospores survived longer than Lb ascospores at 5-20°C in darkness (Huang *et al*., 2003b).

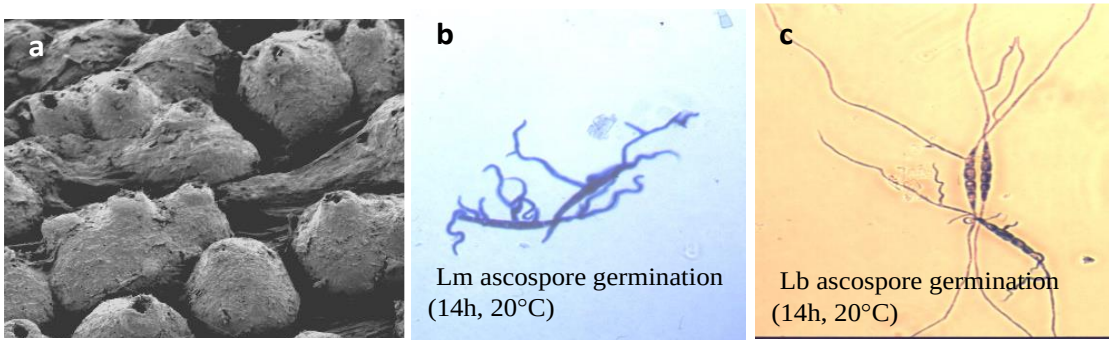

Fig. 2. Mature pseudothecia on stem debris (the ostioles are open after release of ascospores) (a), germinated *L. maculans* (Lm) (b) or *L. biglobosa* (Lb) (c) ascospores (photos are adapted from Huang *et al*., 2001 and Toscano-Underwood *et al*., 2003).

Phoma stem canker epidemiology

Epidemics of phoma stem canker are started by ascospores released from pseudothecia that developed on previous crop debris (Huang *et al*., 2005). Ascospores that land on leaf surfaces germinate and germ tubes penetrate through stomata, causing phoma leaf spots, then the hyphae grow from leaf spot lesions along the leaf petiole to the stems, causing phoma stem canker. (Toscano-Underwood *et al*., 2001; Huang *et al*., 2003a; Huang *et al*., 2006). Although pycnidia (asexual fruiting bodies containing conidia) are produced on leaf lesions, conidia are not important in epidemics in UK field conditions since ascospores are continuously released during the autumn and winter (Huang *et al*., 2005). Therefore, phoma stem canker is considered as a monocyclic disease. Effective control of phoma stem canker needs to reduce the ascospore production on the crop debris and prevent the spread of the pathogen from the leaf to the stem (e.g. by fungicide sprays).

Studies showed that severity of phoma stem canker at harvest affects the number of pseudothecia produced on the stem debris after harvest (Lô-Pelzer *et al*., 2009; Bousset *et al*., 2021). Use of resistant cultivars and fungicide sprays to reduce the stem canker severity will help to reduce the number of pseudothecia (i.e. reduce the initial ascospore inoculum) for infecting the next crop. However, the production of pseudothecia on crop debris after harvest is affected by environmental factors such as temperature and rainfall. Weather based models were developed to forecast the timing of ascospore release to guide the timing of fungicide sprays (Huang *et al*., 2007; Salam *et al*., 2007). However, these models do not distinguish the timings of Lm or Lb ascospore release. Previous studies showed that ascospores of Lm matured faster than those of Lb at temperatures 5-10°C while there were no differences between them in maturation rate at 15-20°C (Toscano-Underwood *et al*., 2003). The differences between Lm and Lb in ascospore maturation may lead to differences in timing of ascospore release. There is a need to develop separate models for forecasting Lm and Lb ascospore release to guide targeted fungicide sprays.

Host resistance

Use of either host qualitative or quantitative resistance is probably the most economically and environmentally friendly way to control crop diseases. Qualitative resistance is usually controlled by a single dominant resistance (*R*) gene, whereas quantitative resistance is usually controlled by several minor genes (quantitative trait loci; QTL) (Delourme *et al.,* 2006). Currently used *R* gene-mediated resistance in oilseed rape is race-specific, complete resistance; it is effective only when the avirulent allele of the corresponding effector gene is predominant in the pathogen population (Rouxel *et al*., 2003). Therefore, for effective use of *R* gene-mediated resistance there is a need to monitor the pathogen population. The resistance gene *Rlm7* has been widely used in UK oilseed rape cultivars for control of phoma stem canker; however, Lm isolates virulent against *Rlm7* have been detected; there is a need to continue to monitor Lm populations to avoid 'breakdown' of this novel resistance (Mitrousia *et al*., 2018; Huang *et al*., 2018). There are at least 17 *R* genes in *B. napus* conferring resistance against Lm (e.g. *Rlm1* - *Rlm11*, *LepR1- LepR6*) that have been identified (Yu *et al*., 2008; Larkan *et al*., 2020) and two of them (*Rlm2/LepR3* and *Rlm9*) have been cloned (Larkan *et al*., 2013 & 2020). There are 15 corresponding *Avr* genes in Lm that have been identified and seven of these (*AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4-7*, *AvrLm5/9*, *AvrLm6* and *AvrLm11*) have been cloned (Balesdent *et al*., 2013; Plissonneau *et al*., 2016; Ghanbarnia *et al*., 2018). The effector gene *AvrLm4-7*, a single locus gene in Lm, triggers resistance mediated by two resistance genes *Rlm4* and *Rlm7*. Similarly, the effector gene *AvrLm5-9*, triggers resistance mediated by resistance genes *Rlm5* and *Rlm9*. *R* gene-mediated resistance against Lm confirms complete resistance to Lm isolates carrying an avirulent allele of the corresponding effector gene, preventing such isolates from colonising the leaves and subsequently preventing the growth of

Lm from the leaf to the stem (Huang *et al*., 2006) (Fig. 3). Using a differential set of cultivars/lines with known *R* genes (e.g. *Rlm* or *LepR* genes), avirulent alleles of the corresponding effector genes in each Lm isolate can be determined by cotyledon inoculation (Balesdent *et al*., 2006; Huang *et al*., 2018). On the other hand, using a differential set of Lm isolates with known avirulent alleles of *AvrLm* genes (e.g. *AvrLm1, AvrLm6* genes), the corresponding resistance genes in *B. napus* cultivars/lines can be determined by cotyledon inoculation in controlled conditions (Rouxel *et al*., 2003; Rashid *et al*., 2018). The cotyledon inoculation assay is a reliable method for high-throughput screening of large collections of *B. napus* lines/cultivars or Lm isolates.

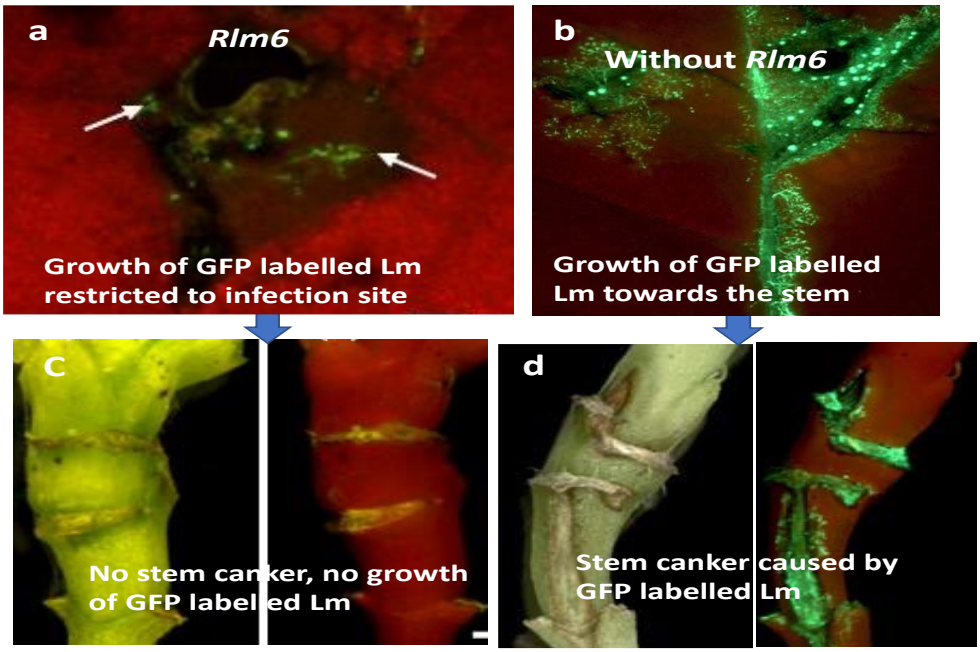

Fig. 3. Cultivar DarmorMX carrying the resistance gene *Rlm6* preventing the growth of GFP labelled *L. maculans* (Lm) carrying the corresponding effector gene *AvrLm6* from leaf lesion (a) along the leaf petiole to the stem, so no stem canker developed (c); GFP labelled Lm growing from leaf lesion along the leaf petiole (b) towards the stem of cultivar Eurol without *Rlm6*, so stem canker developed (d). (photos are adapted from Huang *et al*., 2006 and 2009)

Quantitative resistance (QR) against Lm is race non-specific and considered more durable than *R* gene-mediated resistance (Delourme *et al*., 2006; Huang *et al*., 2016). Therefore, identification of QTL for quantitative resistance against Lm is desirable for resistance breeding. Sixteen QTL related to quantitative resistance against Lm in different environments have been identified (Kumar *et al*., 2018). One QTL for resistance against Lm growth along the leaf petiole towards the stem of young plants in controlled environments was also detected in adult plants in field experiments (Huang *et al*., 2019), suggesting that resistance to the growth of Lm in leaves of young plants contributes to the quantitative resistance in stems of adult plants. Recent work suggests that quantitative resistance against Lm can be race-specific during the late stages of stem colonisation (Jiquel *et al*., 2021). QR does not prevent the infection and colonisation of leaves by Lm; however, it can reduce the growth of Lm from the leaf to the stem and within the stem and prevent Lm from spreading into the stem pith, subsequently reducing the stem canker severity and thereby reducing its impact on yield (Huang *et al*., 2009; Brun *et al*., 2010; Huang *et al*., 2014). As QR is a partial resistance, it cannot provide effective protection in the presence of large amounts of inoculum of different pathogen races in an

environment favourable for disease development. There is a need to combine *R* genes with QR to provide effective cultivar resistance (Brun *et al*., 2010; Huang *et al*., 2018).

Light leaf spot

Pathogen biology

Unlike phoma stem canker caused by two related fungal pathogens, light leaf spot is caused by one fungal pathogen *Pyrenopeziza brassicae* (Fitt *et al.*, 1998; Boys *et al*., 2007; Karandeni Dewage *et al*., 2018). In the autumn, ascospores of *P. brassicae* germinate on leaf surfaces of oilseed rape and germ tubes penetrate directly through the cuticle. After initial infection, the pathogen enters a long period of asymptomatic growth when it grows within the sub-cuticular space between the cuticle and the epidermis of the oilseed rape leaves (Davies *et al*., 2000; Li *et al*., 2003; Boys *et al*., 2007). The first visible symptom of light leaf spot is the development of white acervuli (asexual sporulation) on leaf surfaces (Fig. 4a). The measurement of light leaf spot severity is normally based on the amount of *P. brassicae* sporulation on the plants, as the percentage area covered with sporulation (*Pilet et al*., 1998; Boys *et al*., 2012; Karandeni Dewage *et al*., 2018). In addition to causing light leaf spots on oilseed rape leaves, *P. brassicae* can also infect stems and pods (Fig. 4b,c). Infection of pods causes premature ripening and pod-shatter, leading to substantial yield losses. Furthermore, infection of oilseed rape plants by *P. brassicae* can also result in leaf deformations (leaf curling, leaf distortion, petiole elongation) and stunting of the plants, which can reduce plant vigour, increase susceptibility to frost damage and reduce photosynthetic leaf area resulting in yield loss (Boys *et al*., 2007; Karandeni Dewage, 2018).

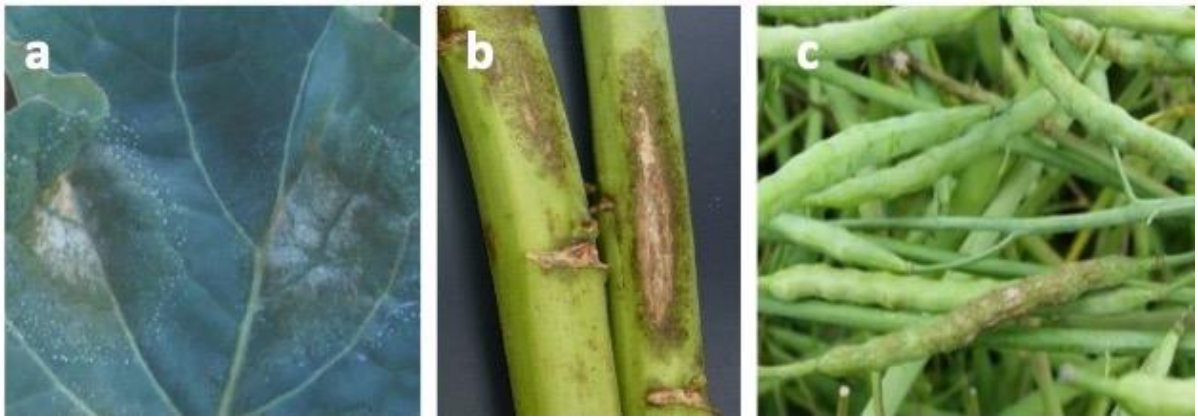

Fig. 4. Symptoms of light leaf spot caused by *P. brassicae* on leaf (a), stems (b) and pods (c) of oilseed rape.

Light leaf spot epidemiology

In the UK, epidemics of light leaf spot are initiated in autumn by wind-dispersed ascospores released from apothecia that developed on crop debris (Fitt *et al.*, 1998; Gilles *et al*., 2001a; Boys *et al.*, 2007). After initial infection, the light leaf spot pathogen *P. brassicae* produces acervuli (asexual fruiting bodies containing conidia) on leaf lesions, resulting in secondary infections on other leaves, stems and pods through rain-splashing of conidia. Furthermore, within the cropping season, apothecia (sexual fruiting bodies containing ascospores) develop on senescent *P. brassicae*-infected leaves and release ascospores, also contributing to secondary disease spread (Gilles *et al*., 2001b; Karolewski *et al*., 2004). Therefore, light leaf

spot is a polycyclic disease, which has several infection cycles within one cropping season. Effective control of light leaf spot requires both the ascospore production on the crop debris and the production of conidia (acervuli) on the crops to be controlled. This makes it is more difficult to control light leaf spot than to control phoma stem canker.

In addition, after initial infection, *P. brassicae* has a long period of asymptomatic growth (Boys *et al.*, 2007; Karandeni Dewage *et al.*, 2018). Although the infection of oilseed rape leaves by *P. brassicae* occurs in autumn (Sept/Oct), the symptoms are often not visible in crops until late winter (Jan/Feb) or early spring (March/April). Furthermore, severe symptoms are often not visible until after incubation of leaves sampled from crops. Current control of light leaf spot often relies on fungicides. However, this long period of asymptomatic growth of *P. brassicae* makes it is difficult to time the fungicide application. When the light leaf spot symptoms are visible, it is often difficult to achieve effective control by fungicides, either because the disease is then too severe or the weather conditions are not favourable at optimal spray times. Furthermore, development of fungicide insensitivity has been observed in *P. brassicae* populations (Carter *et al.*, 2014). With limited available fungicides and environment protection issues, the demand for effective host resistance to control this disease is increasing.

Host resistance

Compared with phoma stem canker, host resistance against the light leaf spot pathogen *P. brassicae* is less well understood. Studies showed that both major gene-mediated qualitative resistance and minor gene-mediated quantitative resistance operate against *P. brassicae* (Bradburne *et al*., 1999; Pilet *et al.*, 1998; Boys *et al*., 2012). Bradburne *et al*., (1999) reported two major genes for resistance against *P. brassicae* with two different resistance phenotypes; one gene corresponding to no asexual sporulation (*PBR1*) mapped on linkage group A1, and the other gene corresponding to black necrotic flecking (*PBR2*) mapped on linkage group C6. Using a DH mapping population 'N26' developed by crossing cultivar Imola (derived from resistant lines studied by Bradbourne *et al*.,1999) and line 218-11, a major gene locus for resistance against *P. brassicae* has been characterised and mapped to the bottom end of the *B. napus* chromosome A1 (Boys *et al*., 2012). This resistance is characterised by the presence of black necrotic flecking along the leaf vein/petiole or leaf lamina with no asexual sporulation of *P. brassicae* (Fig. 5). Recently, the genomic region related to this major gene-mediated resistance has been narrowed down from >1.2Mbp to c. 42Kbp using new KASP (Kompetitive Allele Specific PCR) markers (Karandeni Dewage, 2018). There is a need to identify and clone this major resistance gene, not only for improving our understanding of host resistance against *P. brassicae* but also for providing molecular markers for resistance breeding.

Using a *B. napus* DH mapping population derived from a cross between cultivars Darmor-bzh and Yudal (DY population), 10 QTL related to resistance against *P. brassicae* have been identified (Pilet *et al.*, 1998). Recently, using a DH population, the Q population (a synthetic *B. napus* line × *B. napus* cultivar Tapidor, developed at the John Innes Centre), several QTL related to resistance to *P. brassicae* sporulation have been identified in glasshouse and field experiments (Karandeni Dewage *et al*., 2018). Identification of common QTL detected in the DY population and the Q population will be valuable for breeding cultivars with environmentally stable resistance. Sources identified for resistance against *P. brassicae* are limited; studies on major gene-mediated resistance mainly used cultivar Imola and those on quantitative resistance mainly used two mapping populations (DY population and Q population). There is a need to identify new sources of resistance against *P. brassicae* for both improving breeding and improving understanding of mechanisms of host resistance.

The mechanisms of major gene resistance or quantitative resistance against *P. brassicae* remain largely unknown. Major gene-mediated resistance may operate against *P. brassicae* through membrane-located receptors. These initiate programmed cell death (e.g. black necrotic

flecking) at the time when *P. brassicae* initiates production of asexual spores, preventing secondary infection. The phenotype of resistance in Imola (which has a major resistance gene) is black necrotic flecking with no sporulation (Boys *et al*., 2012). For polycyclic diseases like light leaf spot, reducing secondary infection is important for effective disease control. Quantitative resistance against *P. brassicae* may operate by reducing asexual sporulation, because light leaf spot severity data used for detection of resistance QTL are based on assessment of the % area of leaves covered with sporulation (Pilet *et al*., 1998; Boys *et al.*, 2012; Karandeni Dewage *et al*., 2018). Ascospores released from apothecia that developed on senescent *P. brassicae* infected leaves contribute to secondary disease spread; thus delayed leaf senescence may provide quantitative resistance against *P. brassicae* by reducing the sexual sporulation of the pathogen, resulting in reduced levels of secondary inoculum. Since *P. brassicae* enters the host directly through the cuticle and grows in the sub-cuticular space between the cuticle and the epidermis of the oilseed rape leaves, studies showed that extracellular cutinases (Pbc1), extracellular proteases (Psp1) and cytokinins can be considered as pathogenicity factors of *P. brassicae* during penetration and sub-cuticular growth (Davies *et al*., 2000; Li *et al*., 2003; Batish *et al*., 2003).

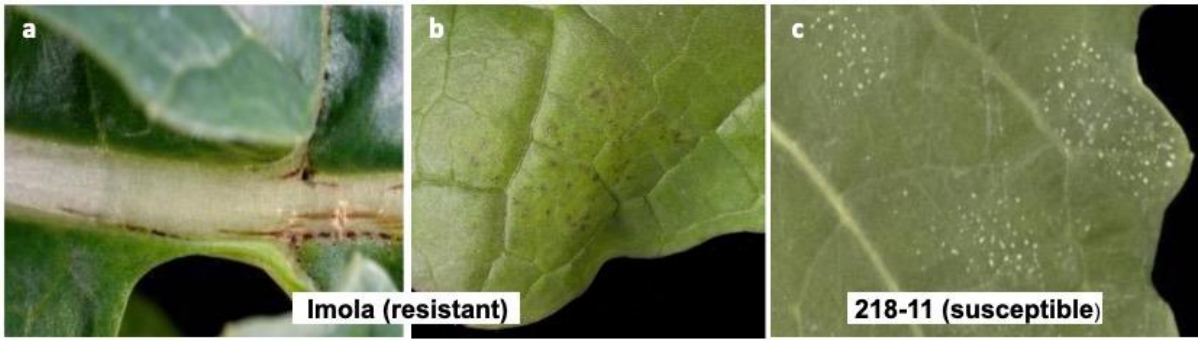

Fig. 5. Black necrotic flecking symptoms along the leaf vein (a) or on leaf lamina (b) of cultivar Imola carrying a major resistance gene against *P. brassicae* (a,b) and sporulation without black flecking on leaf lamina of susceptible line 218-1 (c) (photos are adapted from Boys *et al.*, 2007; Karandeni Dewage *et al*., 2018).

Information on pathogen populations is crucial for effective use of host resistance against *P. brassicae*. However, currently there is no information about virulent races in *P. brassicae* populations in the UK. Observation of cultivar resistance in field experiments in different regions suggests the existence of different *P. brassicae* races in different regions. For example, the resistance in cultivar Cracker 'broke down' in 2014 in Scotland but it was still effective in England in 2016 (Fig. 6). However, there is little information available on specific interactions between *B. napus* and *P. brassicae*. It is not known how *P. brassicae* has overcome host resistance in Cracker. There is an urgent need to investigate host resistance and virulent races in *P. brassicae* populations for effective use of cultivar resistance to avoid breakdown of novel sources of host resistance.

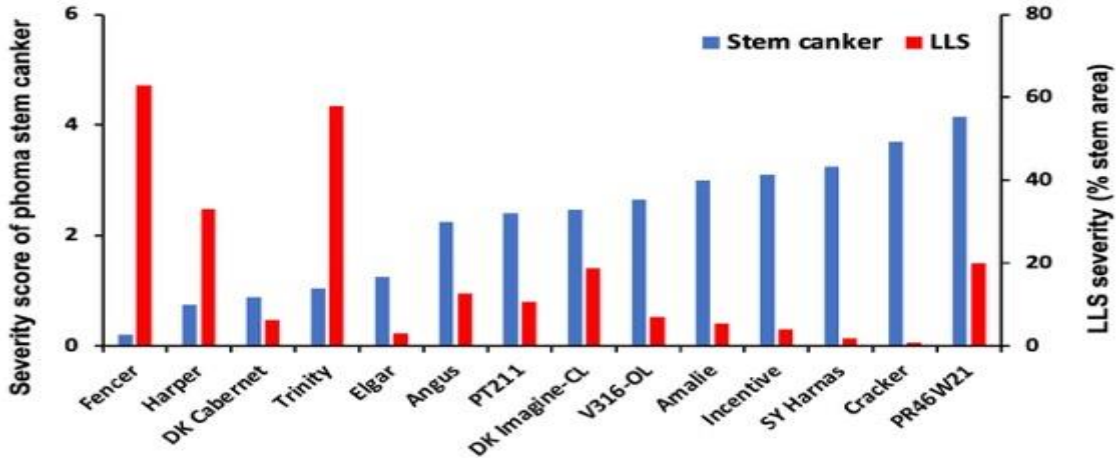

Fig. 6. Comparison of stem canker and light leaf spot severities on different cultivars in a field experiment in 2016 at Morley, Norfolk, UK.

Discussion

Phoma stem canker and light leaf spot are two economically important diseases of oilseed rape in the UK. Over the last 12 years, yield losses in England caused by phoma stem canker are almost stable while yield losses caused by light leaf spot have increased significantly from <£20M in 2005 to > £100M in 2018 (www.cropmonitor.co.uk). Light leaf spot has now become the most damaging disease of oilseed rape in the UK. However, little work has been done on understanding host resistance against *P. brassicae*. By contrast, much work has been done on understanding host resistance against phoma stem canker pathogen Lm. There have been at least 17 *R* genes for resistance against Lm identified and two of them have been cloned (Yu *et. al*., 2008; Larkan *et al*., 2020). There have been 15 corresponding Lm *Avr* effector genes identified and seven of them have been cloned (Balesdent *et al*., 2013; Plissonneau *et al*., 2016; Ghanbarnia *et al*., 2018). However, only two major genes for resistance against the light leaf spot pathogen *P. brassicae* have been identified and neither of them has been cloned (Bradburne *et al*., 1999; Boys *et al.*, 2012). Furthermore, there is no information about *P. brassicae* effector genes. More research is needed to improve understanding of host resistance and of *P. brassicae* virulent races for better control of light leaf spot. Due to the long period of asymptomatic growth and multiple cycles of *P. brassicae* within a cropping season, control of light leaf spot is more challenging than control of phoma stem canker.

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