

Host–pathogen interactions in relation to management of light leaf spot disease (caused by *Pyrenopeziza brassicae*) on *Brassica* species

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Abstract. Light leaf spot, caused by *Pyrenopeziza brassicae*, is the most damaging disease problem in oilseed rape (*Brassica napus*) in the United Kingdom. According to recent survey data, the severity of epidemics has increased progressively across the UK, with yield losses of up to £160M per annum in England and more severe epidemics in Scotland. Light leaf spot is a polycyclic disease, with primary inoculum consisting of airborne ascospores produced on diseased debris from the previous cropping season. Splash-dispersed conidia produced on diseased leaves are the main component of the secondary inoculum. *Pyrenopeziza brassicae* is also able to infect and cause considerable yield losses on vegetable brassicas, especially Brussels sprouts. There may be spread of light leaf spot among different *Brassica* species. Since they have a wide host range and frequent occurrence of sexual reproduction, *P. brassicae* populations are likely to have considerable genetic diversity, and evidence suggests population variations between different geographic regions, which need further study. Available disease-management tools are not sufficient to provide adequate control of the disease. There is a need to identify new sources of resistance, which can be integrated with fungicide applications to achieve sustainable management of light leaf spot. Several major resistance genes and quantitative trait loci have been identified in previous studies, but rapid improvements in the understanding of molecular mechanisms underpinning *B. napus*–*P. brassicae* interactions can be expected through exploitation of novel genetic and genomic information for brassicas and extracellular fungal pathogens.

Additional keywords: crop losses, extracellular pathogens, pathogen population variation, QTL mapping, *R*-gene-mediated resistance.

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Introduction

Plant pathogens account for substantial yield losses worldwide (Savary *et al.* 2012). It has been estimated that pre-harvest pathogens cause yield losses of 9–15% in crop production each year. The losses can be a much greater percentage of yield for certain crops (Teng *et al.* 1984; Oerke 2006). Therefore, crop protection plays a key role in maintaining agricultural production with the increasing demand for food due to population growth (Savary *et al.* 2012). Successful management of plant diseases depends on reliable identification of the pathogens and their dispersal mechanisms, correct evaluation of the disease severity and yield loss, and knowledge about pathogenicity determinants (Strange and Scott 2005). Knowledge about host–pathogen interactions can be exploited to decrease yield losses by minimising pathogen inoculum (cultural practices), targeted inhibition of pathogen growth (chemical control, fungicide applications) and utilising the genetic composition of the host (breeding for cultivar resistance).

Light leaf spot disease, caused by *Pyrenopeziza brassicae* Sutton and Rawlinson (anamorph *Cylindrosporium concentricum*

Grev.), is an economically damaging disease of *Brassica* species and the major fungal disease threat to oilseed rape (*B. napus* L.) in the United Kingdom. Several severe epidemics have been reported in winter oilseed rape in the UK since the first major epidemic recorded in 1974 (Simons and Skidmore 1988). Severe epidemics have affected oilseed rape production in northern continental Europe. In France, the disease was first reported in 1978 and there were severe epidemics in the 1980s and 2000s (Pilet *et al.* 1998; Karolewski *et al.* 2006). In Germany, occurrence of light leaf spot was widespread in the late 1980s (Pilet *et al.* 1998) and incidence on oilseed rape has increased recently (C. A. Klöppel, unpubl. data). Light leaf spot also occurs in Poland, with severe damage during mild winters (Karolewski 1999; Koike *et al.* 2007). *Pyrenopeziza brassicae* is also prevalent on brassicas in the wet, cool climate of New Zealand, with severe outbreaks of light leaf spot reported on vegetable brassicas (Cheah *et al.* 1980; Vegetables New Zealand 2016).

In the UK, light leaf spot was considered the predominant disease of brassicas in Scotland and northern England, where the

disease is favoured by the wet, cool climate (Figueroa *et al.* 1995). Disease severity has varied greatly between cropping seasons and different geographic regions (Fitt *et al.* 1998b; Karolewski *et al.* 2006). According to recent data from winter oilseed rape pest and disease surveys partly funded by the Department for Environment, Food and Rural Affairs (Defra) (CropMonitor 2016), the severity of epidemics has increased progressively across the UK, accompanied by increased yield losses. Light leaf spot has now replaced phoma stem canker (caused by two closely related pathogen species, *Leptosphaeria maculans* (Desm.) Ces. & de Not. and *L. biglobosa* Shoemaker & Brun) as the main disease on winter oilseed rape in the UK. In England, annual yield losses were estimated to range from ~£18M to 160M between 2005 and 2014. This frequent, widespread occurrence has made light leaf spot a high priority for oilseed rape cropping areas in the UK. Light leaf spot is also one of the major diseases affecting vegetable brassicas, including Brussels sprouts (*B. oleracea* var. *gemmifera*) and cabbages (*B. oleracea* var. *capitata*). Losses of Brussels sprouts due to light leaf spot are estimated at ~10% (~£2.8M, with the value of the crop estimated at £28.1M in 2015) per annum in the UK (Defra 2016).

Fungicide applications are becoming less effective in controlling light leaf spot in the UK, and reduced sensitivity to azole fungicides has been reported in *P. brassicae* populations (Carter *et al.* 2014). Host resistance can serve as an effective disease management strategy, provided sufficient diversity is present within commercial cultivars (Boys *et al.* 2007, 2012) and variation of *P. brassicae* populations is considered. Study of genes responsible for resistance against *P. brassicae* can greatly improve understanding of this pathosystem and help to identify new sources of resistance. There have been studies on identification and characterisation of resistance genes operating against *P. brassicae* and more progress can be expected since the *Brassica* genome sequences have become available.

In this review, we evaluate current knowledge of light leaf spot disease, identify knowledge gaps and explore future prospects for sustainable management of this disease. We discuss what is known about *B. napus*–*P. brassicae* interactions, the importance of studying the host range and population variation of *P. brassicae*, and the potential for identification and characterisation of genes for resistance against *P. brassicae*, in the light of advances in *Brassica* genomics and bioinformatics.

Light leaf spot epidemiology

Light leaf spot epidemics are usually initiated by airborne ascospores of *P. brassicae*, which are forcibly released from apothecia (cup-shaped fruiting bodies) produced on diseased plant stem, pod or leaf debris (Fig. 1a) (Gilles *et al.* 2001a, 2001c). In Europe, epidemics on winter oilseed rape crops are generally initiated in the autumn by ascospores produced on debris from previous crops. However, ascospores produced on crop debris at other times may be important in initiating epidemics on crops of vegetable brassicas or secondary epidemics on oilseed rape (McCartney and Lacey 1990; Gilles *et al.* 2000, 2001c; Karolewski *et al.* 2012). These airborne ascospores may be sampled by volumetric spore samplers as a means of forecasting risk of severe light leaf spot epidemics.

Since they are difficult to identify microscopically in spore samples, use of species-specific quantitative PCR provides a more reliable method for measuring airborne inoculum concentrations (West *et al.* 2008; Karolewski *et al.* 2012).

When *P. brassicae* ascospores land on leaves of susceptible *Brassica* crops, they germinate and directly penetrate the cuticle, aided by cutinases (Ashby 1997). They then colonise the subcuticular niche, where extensive hyphal networks can be observed microscopically (Fig. 1b), although there are few visual symptoms on leaves of crops during this endophytic, apoplastic phase in the epidemic cycle (Rawlinson *et al.* 1978b; Boys *et al.* 2007, 2012). Early infections during autumn and winter can kill seedlings, decrease plant vigour and increase susceptibility to frost damage (Fitt *et al.* 1998b). When sufficient subcuticular biomass has accumulated, *P. brassicae* produces asexual conidia in acervuli (pustules; Fig. 1c), often arranged in circles, from which the anamorph is named (*Cylindrosporium concentricum*). These asexual conidia are dispersed by water (rain-splash) and they serve as secondary inoculum for spread of this polycyclic disease (Fitt *et al.* 1998b; Gilles *et al.* 2000, 2001c). Initially, patches of light leaf spot may be observed in crops but patches may merge as epidemics increase (Evans *et al.* 2003). Since the pathogen interferes with the plant hormone system, frequent symptoms include leaf distortion (Fig. 1d), stunting, and green island formation (Ashby 1997).

Light leaf spot epidemics are favoured by wet weather, which encourages production and dispersal of conidia; therefore, the disease is particularly severe on oilseed rape crops in Scotland and northern England (Fitt *et al.* 1998b; Gilles *et al.* 2000), where stunting of susceptible cultivars, with considerable yield losses, may be observed (Fig. 1e). The pathogen may be spread up crop canopies of oilseed rape not only by splash-dispersed conidia but also by new generations of ascospores produced on affected crop debris and through infection of meristematic tissues that are then carried upwards as crop stems extend (McCartney and Lacey 1990; Gilles *et al.* 2001c). Lesions develop on oilseed rape stems (Fig. 1f, g); although they are generally superficial and do not affect yield, affected stems provide an important source of inoculum for initiating epidemics during the following cropping season. When light leaf spot spreads onto pods (Fig. 1h), pods mature and shatter early, leading to yield loss. In vegetable brassicas such as cabbage, broccoli and Brussels sprouts, apart from yield losses caused by infection early in the cropping season, blemishes caused by infection later in the season (Fig. 1i–l) reduce the marketability of the produce.

It was predicted that, with climate change and increasing temperature, the severity of light leaf spot epidemics on oilseed rape crops may lessen by the 2050s in the UK (Evans *et al.* 2010; Fitt *et al.* 2011) and Germany (Siebold and von Tiedemann 2012). However, during the past decade, there has been a considerable increase in the severity of light leaf spot epidemics in northern Europe, perhaps due to changes in *P. brassicae* populations to render ineffective some sources of *Brassica* resistance (AHDB Cereals and Oilseeds 2016) and some previously effective fungicides (Carter *et al.* 2014).

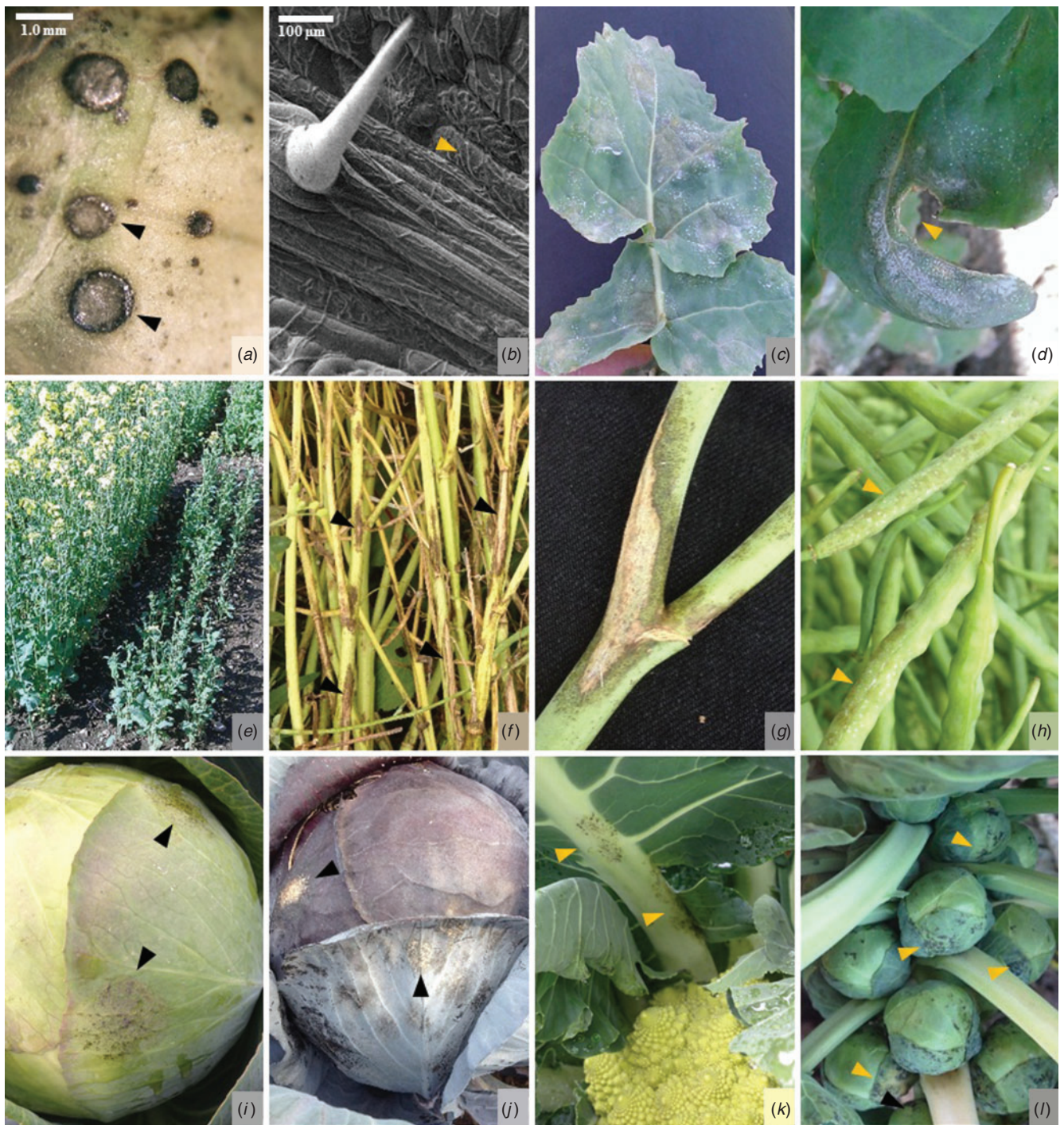


Fig. 1. Symptoms of light leaf spot caused by *Pyrenopeziza brassicae*: (a) apothecia of *P. brassicae* on a Brussels sprout leaf; (b) scanning electron micrograph of leaf section from *Brassica napus* cv. Apex (susceptible), showing abundant *P. brassicae* subcuticular hyphal growth on a leaf vein and surrounding tissue; (c) *B. napus* leaf with light leaf spot lesions showing discoloration of affected leaf areas and formation of acervuli; (d) *B. napus* leaf showing distortion at tip due to infection by *P. brassicae*; (e) susceptible cultivar showing stunting due to light leaf spot (right) next to a less affected cultivar (left) with normal crop height in Scotland; (f) *B. napus* stems with extensive light leaf spot symptoms; (g) light leaf spot on *B. napus* stem; (h) light leaf spot on *B. napus* pods showing formation of acervuli; (i) light leaf spot on white cabbage *B. oleracea* convar. *capitata* var. *alba*; (j) red cabbage *B. oleracea* convar. *capitata* var. *rubra*; (k) Romanesco broccoli *B. oleracea* convar. *botrytis* var. *botrytis*; and (l) Brussels sprouts *B. oleracea* var. *gemmifera* (described symptoms are marked with arrow heads).

Pyrenopeziza brassicae

Taxonomy

Pyrenopeziza brassicae is a haploid, heterothallic (sexual reproduction occurs only between strains of the opposite mating type) fungus classified within the class Leotiomycetes (inoperculate discomycetes) in the phylum Ascomycota. Ascospores of *P. brassicae* were first identified on culture media (Hickman *et al.* 1955) followed by the first report of their occurrence under natural conditions (Staunton and Kavanagh 1966), and later described as the teleomorph of *C. concentricum* (Rawlinson *et al.* 1978b; Mycobank undated). Great variation in morphology is shown between different *P. brassicae* isolates.

Pathogenicity determinants

Phytopathogenic fungi have adopted several mechanisms to invade and utilise the host for their growth and development. These include hydrolytic enzymes and secreted peptides, including effectors and toxins (Rohe *et al.* 1995; Laugé and De Wit 1998; Brunner *et al.* 2013). The involvement of cutinases in *P. brassicae* infection has been studied and cutinolytic activity of the pathogen was suggested to assist penetration (Davies *et al.* 2000). The asymptomatic growth phase of the pathogen starts with the formation of a hypha, followed by proliferation of fungal hyphae to produce mycelial plates within the subcuticular space (Rawlinson *et al.* 1978b). Prior to and during this stage, two-way communication occurs between the pathogen and the host plant in which the pathogen attempts to utilise the host metabolism for its growth and reproduction, whereas the host may defend itself against the pathogen after recognition of pathogen signals (Boys *et al.* 2007). It has been shown that extracellular cutinases (Pbc1) (Li *et al.* 2003), extracellular proteases (Psp1) (Batish *et al.* 2003) and cytokinins (Ashby 1997) are key pathogenicity determinants involved during the penetration and subcuticular growth of the pathogen.

Population variation and host range of *P. brassicae*

Determination of the genetic structure of plant pathogen populations is crucial for the development of strategies to improve disease management and deployment of resistance. The population structure of *P. brassicae* was studied by Majer *et al.* (1998), who found considerable genetic variation by using AFLP markers. The occurrence of genetic variation (subpopulation diversity, H_S) within a geographic region suggests that there is frequent sexual reproduction of the pathogen. Natural formation of *P. brassicae* apothecia is common in oilseed rape crops (Lacey *et al.* 1987; Gilles *et al.* 2001b). Being heterothallic, *P. brassicae* has two mating types originally described by Illott *et al.* (1984) and designated *MAT 1-1* and *MAT 1-2*, according to the nomenclature of Yoder *et al.* (1986) (Courtice and Ingram 1987). *MAT 1-1* and *MAT 1-2* were later referred to as *MAT-2* and *MAT-1*, respectively (Singh and Ashby 1998, 1999). According to reviews of ascomycete mating-type gene nomenclature, the two idiomorphs of the single mating-type locus, *MAT1*, were designated *MAT 1-1*, consisting of an open reading frame (ORF) encoding a protein with an α -box motif, and *MAT 1-2*, consisting of a

single ORF encoding a protein with an high mobility group (HMG) motif (Turgeon and Yoder 2000; Pöggeler 2001). Pathogens such as *P. brassicae* with a mixed reproduction system have a high potential to evolve through recombination of alleles during the sexual stage and to fix newly combined alleles in the population by asexual reproduction (McDonald and Linde 2002). Consequently, the pathogen may rapidly overcome host major resistance (*R*) genes in a few years after resistance is deployed at a large scale, and frequency of virulent alleles may increase with asexual cycles of the pathogen. Therefore, *P. brassicae* poses an increasing risk to oilseed rape and other *Brassica* species.

The dispersal of the pathogen by wind and rain-splash is the main mechanism for gene flow, which allows the exchange of alleles between populations (Barrett *et al.* 2008). Pathogens with the ability to spread their inoculum over long distances (e.g. wind-borne spores) tend to have increased gene flow, resulting in homogeneous population structures. Although ascospores of *P. brassicae* are wind-dispersed, Majer *et al.* (1998) calculated a moderate F_{st} (differentiation among subpopulations) value that suggested the absence of long-distance movement. This implies that the pathogen is likely to form subpopulations between geographical regions of the UK (Majer *et al.* 1998). However, the cropping area of oilseed rape in the UK has increased by almost ~200 000 ha since 1998 and it can be argued that availability of suitable host plants has led to a decrease in diversity among pathogen populations due to selection for specific races (Barrett *et al.* 2008). With the increase in oilseed rape production, problems with light leaf spot have increased throughout the UK, whereas it was previously considered a major problem only in Scotland. According to Majer *et al.* (1998), there was no difference in the populations of *P. brassicae* between Scotland and England, perhaps because there had been spread of the pathogen from Scotland southwards. However, that study was based on neutral DNA (AFLP) markers, which may not fully represent pathogen variations associated with pathogenicity determinants. In addition, pathogen populations change over time. Therefore, there may still be differences between populations in terms of virulence and gene-for-gene interactions between pathogen strains and host cultivars. The UK AHDB Cereals and Oilseeds levy board recommends use of different cultivars for the north region v. the east–west region of the UK (AHDB Cereals and Oilseeds 2016).

Light leaf spot also occurs on different types of *B. oleracea* and other related *Brassica* species or subspecies. These include Brussels sprouts (*B. oleracea* var. *gemmifera*), cabbage (*B. oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*), kale (*B. oleracea* var. *acephala*), turnip (*B. rapa* ssp. *rapa*), swede (*B. rapa* ssp. *rapifera*), Chinese cabbage (*B. rapa* ssp. *pekinensis*) and black mustard (*B. nigra*) (Maddock and Ingram 1981; Simons and Skidmore 1988; Boys 2009; Karolewski 2010). Spread of light leaf spot between different *Brassica* host species has been suggested (Wafford *et al.* 1986) but there has been little work on this. *Pyrenopeziza brassicae* isolates originating from Brussels sprouts and cauliflower were able to cross-infect oilseed rape, Brussels sprouts and cauliflower (Maddock *et al.* 1981). No significant differences were observed in virulence

of isolates between different host species. Simons and Skidmore (1988) also reported the cross-infectivity of *P. brassicae* isolates that originated from oilseed rape, white cabbage or Brussels sprouts. These findings are supported by recent studies on cross-infectivity of *P. brassicae* between different *Brassica* host species; for example, isolates from oilseed rape can cause light leaf spot on cabbage or Brussels sprouts and *vice versa* (C. A. Klöppel, unpubl. data). Nevertheless, reported studies included a limited number of isolates, and more research is needed to investigate differences in *P. brassicae* virulence towards the host of origin and related species. The availability of different host species in a geographical region can increase the genetic diversity of a pathogen population (Woolhouse *et al.* 2001). Areas with both vegetable *Brassica* and oilseed rape production may have greater *P. brassicae* population diversity and thus a greater risk of severe epidemics than other areas.

The centre of origin of *P. brassicae* has not yet been identified but this finding would help to identify new sources of resistance because the host and pathogen may have co-evolved there for a long period of time (McDonald 2015). Oilseed rape production has increased greatly in the UK and problems with light leaf spot have increased in the last decade; therefore, new studies of the *P. brassicae* population structure are warranted.

Control of light leaf spot

Cultural practices

Control of light leaf spot is difficult to achieve. Plant debris from harvested *Brassica* crops that is infested with light leaf spot acts as a source of inoculum for newly emerging crops (Gilles *et al.* 2001b). Plant debris remaining after harvest can be ploughed under to reduce the initial inoculum of the pathogen. However, farmers are now using minimum tillage regimes to minimise production costs, and intensification of agriculture has led to shorter crop rotations. Figueroa *et al.* (1994) observed a substantial increase in severity of light leaf spot in oilseed rape crops when oilseed rape was grown in two successive cropping seasons. Delaying the sowing date of the oilseed rape crop by up to 14 days can decrease incidence and severity of light leaf spot because the majority of ascospores may have already been released, but this may cause problems with phoma stem canker (causal agents *Leptosphaeria maculans* and *L. biglobosa*) (Welham *et al.* 2004). Cultivation practices can reduce disease severity but do not control epidemics sufficiently well when used alone.

Chemical control

Yield losses due to light leaf spot can be decreased by the use of fungicides. The timing of fungicide applications is crucial for effective disease control (Fitt *et al.* 1998a). A suggested fungicide regime included three spray applications against light leaf spot during the cropping season in the UK (Fitt *et al.* 1998a). The crop should receive the first fungicide application during the symptomless phase of pathogen growth in autumn, followed by a second spray in late winter that decreases the secondary spread of the pathogen (Fitt *et al.* 1998a). A third spray in spring post-flowering should control the pod infections that can lead to pod shatter but it is rarely necessary and may increase losses through mechanical damage from equipment.

The autumn spray is very important to substantially decrease light leaf spot incidence (Figueroa *et al.* 1994; Gilles *et al.* 2000), but accurate timing of the first spray is very difficult because the farmer is not able to see the disease in the crop at that time. Therefore, forecasting schemes have been developed to support farmers in their spray decisions (Gilles *et al.* 2000; Welham *et al.* 2004; Rothamsted Research 2016). In autumn, this forecasting scheme predicts light leaf spot severity in the next spring on the basis of observed deviation from 30-year mean summer temperature together with 30-year mean regional rainfall and CropMonitor survey data for pod disease incidence at the end of the previous cropping season. The risk prediction is updated in spring for the final forecast to allow for the deviation of observed winter rainfall from the 30-year mean (Welham *et al.* 2004).

Nevertheless, despite accurate timing, fungicide applications may still be ineffective because of reduced sensitivity of *P. brassicae* strains to certain fungicide groups. Reduced sensitivity to methyl benzimidazole carbamate (MBC) and azole fungicides, including imidazoles and triazoles, has been reported (Carter *et al.* 2013, 2014). Reduced sensitivity in *P. brassicae* isolates to MBCs was conferred by a single major gene with three different alleles at this locus (target β -tubulin locus) resulting in sensitivity, moderate insensitivity or insensitivity. The *P. brassicae* populations selected in this study (Scotland and England) showed no variation in the frequency of resistance alleles (Carter *et al.* 2014). When effectiveness of host-resistance activators and primers such as acibenzolar-S-methyl, *cis*-jasmonate and β -aminobutyric acid was compared with that of triazole fungicides for controlling light leaf spot on winter oilseed rape, primers and resistance activators gave better control than fungicide treatments at some stages of the crop growth (Oxley and Walters 2012). Ineffective fungicide control strategies make it necessary to improve understanding of the pathogen population and the host resistance against *P. brassicae*.

Deployment of cultivar resistance

The use of resistant cultivars is usually the most efficient, cost-effective and environmentally friendly strategy for controlling crop diseases. Farmers in the UK have the opportunity to choose cultivars from the AHDB Cereals & Oilseeds recommended list, which includes information about different crop traits such as average seed yield, agronomic traits, seed quality and score for resistance against *P. brassicae* (AHDB Cereals and Oilseeds 2016). Disease-resistance ratings are given on a scale from 1 to 9, with the higher numbers indicating better resistance. No currently recommended cultivar (2016–17 cropping season) has a resistance score >7 for light leaf spot. Moreover, there is a limited understanding of the genetic resistance mechanisms operating in different commercial oilseed rape cultivars, and the information is largely unknown to growers. Therefore, it is likely that cultivars with a similar type of resistance may be grown in the same area for a long period, exerting a strong selection on the local pathogen populations. Ultimately, this can lead to a breakdown of host resistance. Resistance breakdown of some cultivars with good resistance ratings has been reported, from which recent light leaf spot epidemics resulted. Often, this phenomenon is associated with major gene-mediated resistance

(McDonald and Linde 2002). There is a need for a better understanding of the mechanisms of resistance against *P. brassicae* to inform the search for novel sources of resistance in oilseed rape and other *Brassicaceae*, where cultivar resistance against *P. brassicae* has been poorly documented.

Analysis of infection and colonisation stages of pathogen life cycles is useful to identify possible resistance mechanisms operating in the host against that particular pathogen. The potential mechanisms of *B. napus* resistance in relation to the *P. brassicae* life cycle have been reviewed by Boys *et al.* (2007). Subcuticular colonisation by the pathogen during its asymptomatic growth phase can be a key trigger for host resistance, which may operate to delay the accumulation of pathogen biomass and prevent production of asexual spores (Boys *et al.* 2007, 2012). There have been several studies on the operation of both major-gene-mediated and quantitative resistance against *P. brassicae* in *B. napus* (Table 1). A resistant phenotype associated with the formation of black necrotic flecking on leaves of infected plants (Fig. 2) has been described and the locus for resistance has subsequently been mapped (Bradburne *et al.* 1999; Boys *et al.* 2012). However, host resistance against *P. brassicae* may not always be associated with this phenotype (Bradburne *et al.* 1999).

Major-gene-mediated resistance described in these studies appears to limit subcuticular colonisation and/or the asexual sporulation of *P. brassicae*, but with no effect on subsequent sexual sporulation of the pathogen (Boys *et al.* 2007, 2012). Further characterisation of the genetic basis of the resistance loci identified can provide useful information to search for new sources of resistance.

Involvement of gene-for-gene interactions between *P. brassicae* and *B. oleracea* was reported by Simons and Skidmore (1988). Their experiment on F₁ hybrid lines of cabbage and Brussels sprouts showed differential interactions with *P. brassicae* isolates tested. In addition to cultivar-specific resistance, pre-existing structural host defence mechanisms such as cuticle thickness and composition may provide resistance. Increased susceptibility to *P. brassicae* has been reported after application of herbicides such as dalapon (2,2-dichloropropionoc acid) that alter the epicuticular wax structure (Rawlinson *et al.* 1978a). Plant tolerance and disease escape can also play an important part in minimising yield loss by restricting pathogen penetration and the amount of inoculum. For example, delayed senescence in oilseed rape leaves can reduce the ascospore inoculum for new infections later in the cropping season (Boys *et al.* 2007).

Table 1. Research on identification and mapping of resistance against *Pyrenopeziza brassicae* in doubled-haploid populations of oilseed rape

Study	Method of assessment	Type of resistance	Resistant phenotype	QTLs and corresponding chromosomes identified
Pilet <i>et al.</i> 1998	Plots assessed for disease severity on leaves and stems using 11-point scale (1, healthy appearance of plots; 11, severely damaged plants)	Quantitative resistance		Ten (six environmentally stable) QTLs identified
Bradburne <i>et al.</i> 1999	Cotyledons scored for presence/absence of <i>P. brassicae</i> asexual sporulation and presence or absence of black flecking	Major gene-mediated	No sporulation Black flecking	<i>PBR1</i> , chromosome A1 <i>PBR2</i> , chromosome C6
Boys <i>et al.</i> 2012	9-point scale (1, most severe; 9, no symptoms) and % leaf area covered with <i>P. brassicae</i> asexual sporulation	Major gene-mediated	Black flecking ^A	Chromosome A1

^APrevents asexual reproduction; allows sexual reproduction.

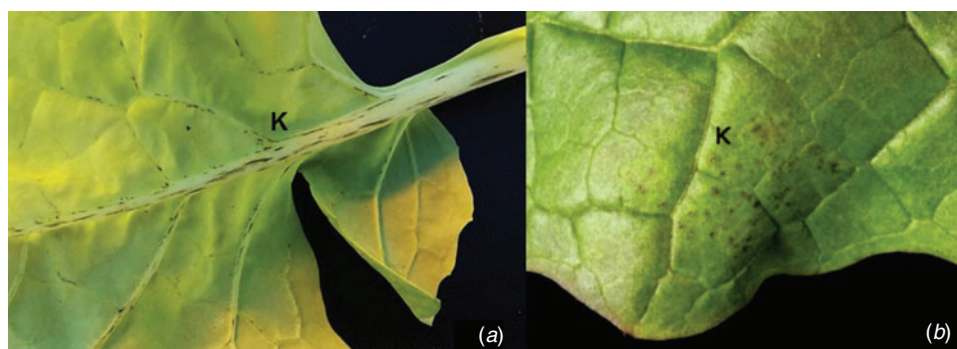


Fig. 2. Black necrotic flecking (K) on *Brassica napus* cv. Imola, which has a major gene for resistance against *Pyrenopeziza brassicae*: (a) along the leaf veins at 23 days post-inoculation (leaves were spray-inoculated with a mixture of *P. brassicae* populations collected from diseased oilseed rape leaves from winter oilseed rape crops); (b) on the leaf lamina at 28 days after point-inoculation with a suspension of *P. brassicae* conidia (Boys 2009).

Different components of resistance may contribute differently in minimising yield losses (Boys *et al.* 2007). *R*-gene-mediated resistance is favoured by most plant breeders, because it can completely prevent the disease. Moreover, the selection of such resistance is much more straightforward than selection for quantitative resistance, because of its Mendelian inheritance. Nevertheless, the durability of *R*-gene-mediated resistance can be short, because the selection exerted on the pathogen population selects for virulent pathogen races. Therefore, it is important to consider genetic variation in *P. brassicae* populations to detect the presence of effector genes. Pyramiding several *R* genes in elite cultivars can provide better resistance because it requires several mutations in the pathogen genome to overcome host resistance (McDonald and Linde 2002). Nevertheless, this does not eliminate the risk of selection for virulent pathogen races over time. Rotation of cultivars that contain different *R* genes or growing them together as multilines decreases the rate of selection for virulent alleles (McDonald and Linde 2002). Addition of *R*-gene-mediated resistance into a quantitative resistance background could enable cultivar resistance to last longer (Brun *et al.* 2010).

Novel genomic approaches for rapid identification of *R* genes and pathogenicity determinants

Successful disease management strategies require a thorough understanding of the underpinning molecular mechanisms and the genetic basis of host–pathogen interactions (Burdon *et al.* 2016). Rapid expansion of genomic approaches has enabled significant improvements in control of crop diseases. Improved efficiency and cost-effectiveness of next generation sequencing (NGS) technologies have allowed whole-genome sequences of numerous crop and pathogen species to be generated. Increasing availability of *Brassica* genomic information offers new possibilities for the identification of host resistance and new opportunities to provide molecular tools to assist in breeding for disease resistance.

The genomes of five *Brassica* species have been sequenced. The first *Brassica* genome sequence was obtained from *B. rapa* (Wang *et al.* 2011). Genome sequences of *B. oleracea* (Liu *et al.* 2014) and *B. napus* (Chalhoub *et al.* 2014) followed; *B. napus* is an allotetraploid species that contains A and C sub-genomes from its ancestors, *B. rapa* and *B. oleracea*, respectively. Recently, the genomes of allotetraploid *B. juncea* and its B genome progenitor *B. nigra* were sequenced (Yang *et al.* 2016). The implications of genome-enabled technologies for the breeding of crops have been reviewed (Snowdon and Iniguez Luy 2012). Single-nucleotide polymorphism (SNP) markers and transcriptome sequencing (mRNA-Seq) have been used for genome-wide association studies (GWAS) to identify individual genes that contribute to important agronomic traits (Harper *et al.* 2012). Since then, a *Brassica* 60k SNP array has been used in combination with large association panels by several research teams to analyse the genetic basis of traits, including resistance against pathogens (Li *et al.* 2014; Hatzig *et al.* 2015; Wu *et al.* 2016). Resequencing of 52 diverse natural and synthetic *B. napus* accessions has resulted in identification of >4 million SNPs, which are being exploited for breeding using primary and secondary gene pools (Schmutzer *et al.* 2015).

Transcriptome sequencing has been used to analyse the interaction between *B. napus* and *L. maculans* (Lowe *et al.* 2014; Haddadi *et al.* 2016); both studies have used susceptible cultivars to determine pathogen and host gene expression. Such studies are useful to determine potential pathogenicity (e.g. effector) and resistance genes. Transcriptome analysis can provide valuable information related to quantitative resistance of the host against particular pathogens (Joshi *et al.* 2016; Wu *et al.* 2016).

Pyrenopeziza brassicae is an apoplastic fungal pathogen; *R*-gene-mediated resistance against it is likely to involve receptor-like proteins (RLP), which contribute to recognition of pathogen effectors that are secreted into the extracellular environment of the host (effector-triggered defence, ETD) (Stotz *et al.* 2014). This resistance is different from that involving *R* genes operating against appressorium-forming, cell-penetrating fungal pathogens that cause diseases such as rusts and mildews, which recognise pathogen effectors that are delivered into the cytoplasm of the host cell (effector-triggered immunity, ETI) (Jones and Dangl 2006). The different categories of *R* genes have recently been reviewed (Sekhwal *et al.* 2015). *R*-gene-specific sequence information has recently been exploited for resistance gene enrichment and sequencing (RenSeq) to identify previously unknown *R* genes (Jupe *et al.* 2013). Such approaches, in combination with advanced genome information, hold the promise of rapidly identifying the genetic basis of several resistance traits, including major resistance quantitative trait loci operating against *P. brassicae*.

In contrast to *Brassica* genomic information, little information is available about the *P. brassicae* genome. Research on *P. brassicae*–*B. napus* interactions provides a framework to understand its pathogenicity; however, the number of factors so far known to be involved in defence signalling pathways is limited. There are substantial improvements in efficiency of DNA-sequencing technologies. Whole-genome sequencing of pathogens allows for genome-wide analysis of pathogenicity-related genes (Klosterman *et al.* 2016). Comparative genomics approaches can be applied between related pathogen species to improve understanding of the pathogenicity in poorly understood pathosystems. Several phytopathogenic fungi are evolutionarily related to *P. brassicae* (Table 2). Sequence information for the internal transcribed spacer (ITS) region provided evidence for a close phylogenetic relationship between *Rhynchosporium commune* (formerly known as *R. secalis*) and two Leotiomycete genera, *Pyrenopeziza* and *Oculimacula* (formerly *Tapesia*) (Goodwin 2002).

The genome of *R. commune* has been sequenced (Penselin *et al.* 2016). Seven proteins with at least one LysM domain, which are mostly found in secreted LysM effectors of fungi, have been identified in the *Rhynchosporium* genome. LysM-domain-containing effector proteins prevent the activation of pathogen associated molecular pattern (PAMP)-triggered immunity by sequestering chitin oligosaccharides. The close phylogenetic relationship between *R. commune* and *P. brassicae* can be exploited to identify whether pathogenicity-related genes of *P. brassicae* and *R. commune* LysM-domain-containing proteins are good candidate effectors for *P. brassicae* infection. Moreover, ~330 cell-wall-degrading enzymes (CWDEs) have been identified in the *R. commune*

Table 2. Phytopathogenic fungi evolutionarily related to *Pyrenopeziza brassicae*

Leotiomycete pathogen genera *Pyrenopeziza*, *Rhynchosporium* and *Oculimacula* were considered to have a close phylogenetic relationship based on sequence information for the internal transcribed spacer (ITS) region (Goodwin 2002). Main host species are listed; diseases are categorised as polycyclic (p) or monocyclic (m)

Pathogen	Disease and the host	Mode of infection	Niche	Pathogenicity factors identified	Genome sequenced	References
<i>Pyrenopeziza brassicae</i>	Light leaf spot on oilseed rape and vegetable brassicas (p)	Cuticular penetration	Subcuticular	Extracellular cutinases, extracellular proteases, cytokinins	No	Li <i>et al.</i> 2003; Batish <i>et al.</i> 2003; Ashby 1997
<i>Rhynchosporium commune</i>	Leaf blotch on barley (p)	Cuticular penetration	Subcuticular	Necrosis-inducing proteins (NIP), LysM	Yes (Penselin <i>et al.</i> 2016)	Kirsten <i>et al.</i> 2012; Zhan <i>et al.</i> 2008
<i>Oculimacula yallundae</i> and <i>O. accuformis</i>	Eyespot on wheat, barley, rye (m)	Cuticular penetration by formation of appresoria	After germination, the pathogen produces a mycelial network on plant surfaces and later colonises leaf sheath and stem cells		No	Crous <i>et al.</i> 2003; Blein <i>et al.</i> 2009

genome, and considering their putative substrates, ~64% of these were identified to target host cell walls. Gene expression data have been analysed for the necrosis-inducing protein (NIP) and small, secreted effector proteins (Penselin *et al.* 2016). This information can be incorporated into gene expression analysis to identify candidate effector genes. Whole-genome sequencing and re-sequencing of allelic variants can be used as an effective tool for studying pathogen population variation by identifying molecular markers such as microsatellites and SNPs.

Concluding remarks

Understanding of the molecular genetic mechanisms underpinning the *B. napus*–*P. brassicae* interactions is essential for developing effective, durable disease-management strategies. Although light leaf epidemiology is well understood, substantial gaps remain in understanding of the operation of *Brassica* resistance and *P. brassicae* pathogenicity. With recent advances in *Brassica* genomics and understanding of the genetic basis of resistance against extracellular pathogens (i.e. *B. napus* resistance against *Leptosphaeria maculans*) (Larkan *et al.* 2013, 2015), rapid improvement in identifying novel sources of resistance against *P. brassicae* can be expected. Resistance genes mapped in previous studies can be further examined to characterise the genetic basis of resistance and they can be cloned. This information can be utilised to search for similar genes and to produce molecular markers to facilitate marker-assisted selection (MAS) in oilseed rape breeding programs (Collard *et al.* 2005). However, to achieve effective disease control through deployment of cultivar resistance, considerable improvements in understanding of *P. brassicae* genomics are also needed. Differences in cultivar resistance between different regions in the UK indicate the presence of pathogen population variation, and this can also put pressure on breeding programs. It is important to study this variation by using molecular markers related to pathogenicity. This information will then need to be

considered when recommending cultivars for different regions to sustain the available sources of resistance against *P. brassicae*.

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