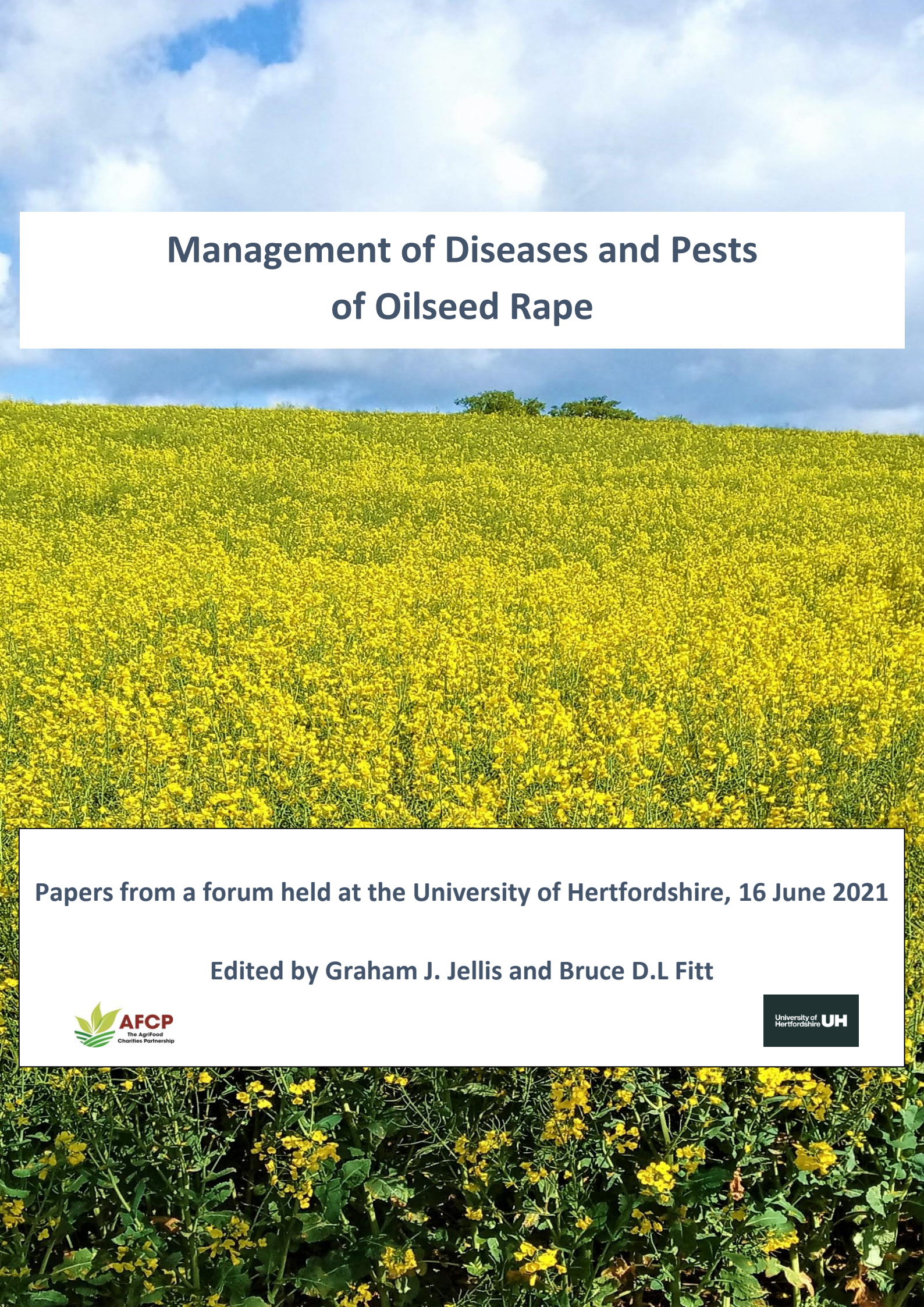

Management of Diseases and Pests of Oilseed Rape

Papers from a forum held at the University of Hertfordshire, 16 June 2021

Edited by Graham J. Jellis and Bruce D.L Fitt

AFCP
The AgriFood Charities Partnership

University of Hertfordshire UH

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Management of Diseases and Pests of Oilseed Rape

Edited by Graham J. Jellis and Bruce D.L. Fitt

Papers from a Forum held at
the University of Hertfordshire
16th June 2021

Contents

Preface

This publication is a compilation of papers derived from presentations and posters delivered at the University of Hertfordshire on June 16th, 2021. The forum was originally conceived by the AgriFood Charities Partnership* to highlight research studentships related to management of diseases and pests of oilseed rape, part-funded by some of its members and much of it at the University of Hertfordshire+. Working closely with the University, the programme was broadened to include other research in this area.

The forum was initially planned for spring 2020 but the COVID-19 pandemic led to postponement for over a year and eventually to the development of a hybrid event, with nearly all the speakers and a small number of delegates present at the University and the majority joining online. Although this had disadvantages, it did mean that we were able to welcome delegates from across the globe.

The papers are broadly grouped by disease/pest although a number relate to more than one of these. Some of the research is still on-going and so only a summary is available at present. However, email contact details are provided for each paper if readers want to learn more or seek collaboration.

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*The mission of the AgriFood Charities Partnership (AFCP) is to create opportunities to increase knowledge and expertise in the agri-food sector through collaboration and innovation via a network of charities and other funders**.** AFCP believes that charities can achieve greater effectiveness by appropriate collaborations, either with other charities or organisations such as universities, colleges, schools, research and environmental organisations. The papers presented here include research funded by five charities over the past two decades: Chadacre Agricultural Trust, Clan Trust, Felix Thornley Cobbold Agricultural Trust, Perry Foundation, and the Morley Agricultural Trust. More information on AFCP and the charities involved can be found on the website https://www.afcp.org.uk/.

+With its heritage in the UK's pioneering aeronautical industry, the University of Hertfordshire has been an innovative force in education and research since the early 1950s. Today, it hosts a thriving community of more than 25,000 students. Academics are experts in their field, delivering research which is having a meaningful economic, environmental and social impact in the UK and across the globe. From work on food security and public health, to using a pioneering laser facility to deliver critical insights into climate, staff drive forward cutting-edge research. A particular strength has been research on oilseed rape diseases in the Crop Protection and Climate Change group. https://www.herts.ac.uk/research/groups-and-units/Agriculture-food-and-veterinary-sciences/crop-protection-and-climate-change/

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

By YONG-JU HUANG, CHINTHANI KARANDENI DEWAGE and BRUCE D L FITT

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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.

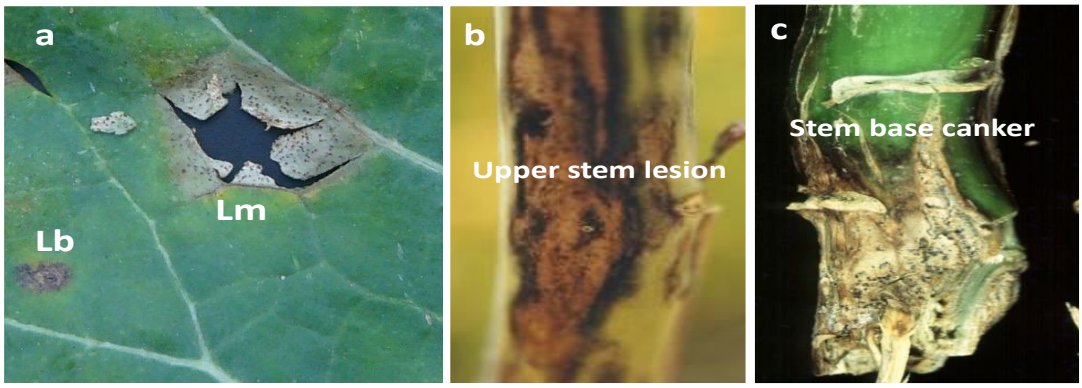

Fig. 1. Symptoms of phoma leaf spot caused by *L. maculans* (Lm) or *L. biglobosa* (Lb) (a) and symptoms of phoma stem canker on upper stem (b) and stem base (c).

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).

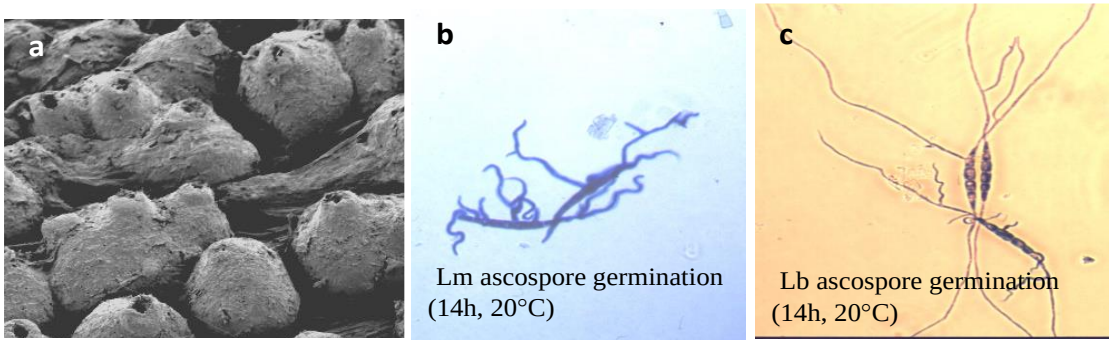

Fig. 2. Mature pseudothecia on stem debris (the ostioles are open after release of ascospores) (a), germinated *L. maculans* (Lm) (b) or *L. biglobosa* (Lb) (c) ascospores (photos are adapted from Huang *et al*., 2001 and Toscano-Underwood *et al*., 2003).

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.

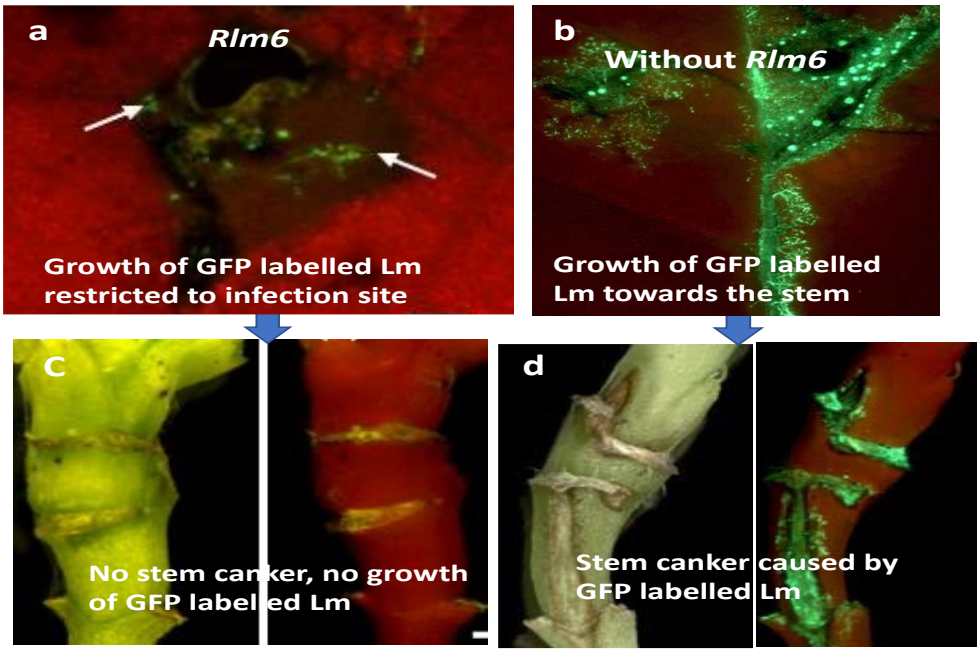

Fig. 3. Cultivar DarmorMX carrying the resistance gene *Rlm6* preventing the growth of GFP labelled *L. maculans* (Lm) carrying the corresponding effector gene *AvrLm6* from leaf lesion (a) along the leaf petiole to the stem, so no stem canker developed (c); GFP labelled Lm growing from leaf lesion along 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).

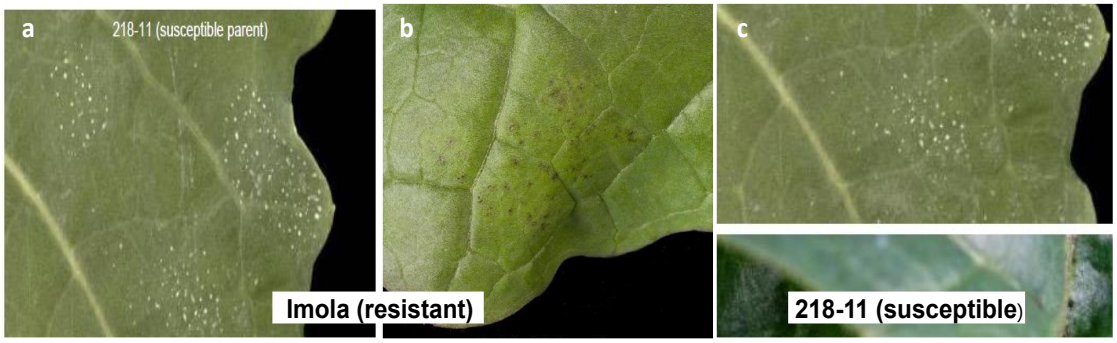

Fig. 5. Black necrotic flecking symptoms along the leaf vein (a) or on leaf lamina (b) of cultivar Imola carrying a major resistance gene against *P. brassicae* (a,b) and sporulation without black flecking on leaf lamina of susceptible line 218-1 (c) (photos are adapted from Boys *et al.*, 2007; Karandeni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

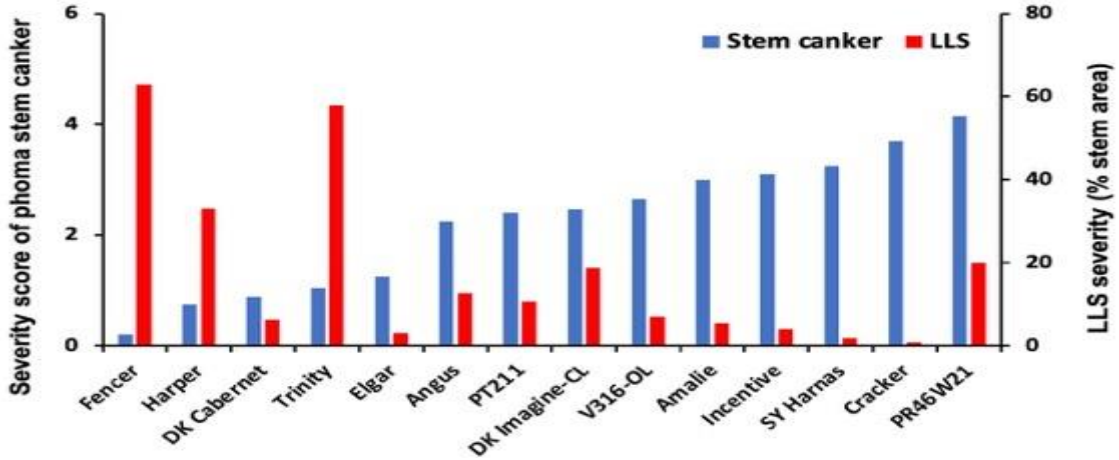

Fig. 6. Comparison of stem canker and light leaf spot severities on different cultivars in a field experiment in 2016 at Morley, Norfolk, UK.

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.

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.

Acknowledgements

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Climate change and oilseed rape diseases; impacts, adaptation and mitigation

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Summary

This review describes three aspects of interactions between climate change and arable crop diseases, for oilseed rape. 1. Impacts of climate change on incidence of diseases and their effects on yields. Use of crop-disease-climate models can produce recommendations to guide government and industry strategies for climate change adaptation. 2. Adaptation of disease management strategies to decrease losses related to climate change requires action by farmers, oilseed rape breeders and government to enable food security in future. 3. Climate change mitigation; crop management strategies to control diseases and increase yields can decrease greenhouse gas emissions per tonne of crop and contribute to climate change mitigation targets.

Key words: Climate change projections, climate change adaptation strategies, crop-disease climate models, greenhouse gas emissions

Introduction

Climate change and arable crop diseases both threaten global food security in a world where more than one billion people do not have enough to eat (FAO, 2009). Increasing concentrations of greenhouse gases are leading to increases in global temperatures, as illustrated by the increase in temperature at Rothamsted, Hertfordshire, UK over the last thirty years (Fig. 1). There, the annual mean temperature had remained stable over the period 1879-1990 but has increased by 2^{o}C from 1990 to 2020. Agricultural productivity and food security are especially vulnerable to climate change where crops are grown in marginal areas, such as sub-Saharan Africa. Thus, areas of the world where agricultural productivity may benefit from climate change, such as northern Europe (Butterworth *et al*., 2010) need to produce more food (Fitt *et al*., 2016) but produce it in a way that decreases emissions of greenhouse gases to contribute to climate change mitigation (Hughes *et al.,* 2011).

Arable crop diseases directly threaten food production because they cause losses in yield, estimated globally at 16%, despite efforts to control them (Oerke, 2006) and losses in quality. These yield losses are illustrated by considering their effects on four crops of global importance, namely rice, maize, wheat and potatoes (Table 1). Potential losses, assuming that there was no crop protection against pests and diseases, are estimated at £358 billion, whereas actual losses, despite use of crop protection strategies, are estimated at £207 billion. Losses from crop diseases may be exacerbated by climate change. This review describes three aspects of interactions between climate change and arable crop diseases, using as an example oilseed rape (*Brassica napus*), the second most important oilseed crop in the world (Friedt *et al*., 2018):

- Impacts of climate change on incidence of diseases and their effects on yields.
- Adaptation of disease management strategies to decrease losses related to climate change.
- Climate change mitigation; consequences for greenhouse gas (GHG) emissions of crop management strategies to control diseases.

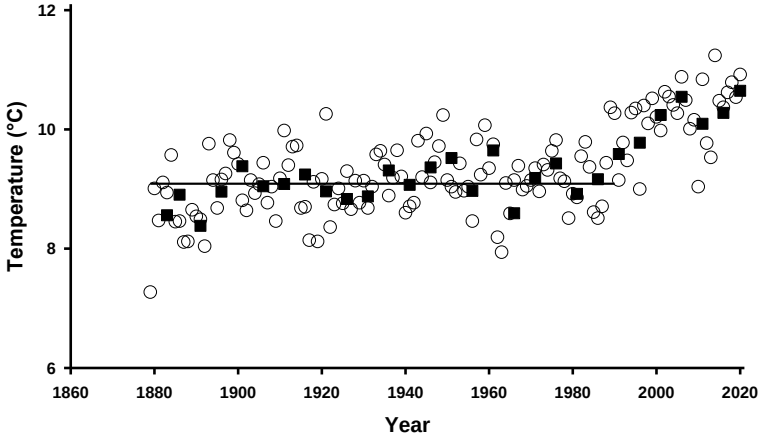

Fig. 1. Change in annual mean (○) and 5-year mean (■) air temperatures at Rothamsted, Hertfordshire, UK, in the period 1879 to 2020. The horizontal line indicates the mean air temperature for the period 1879 to 1990 (data from Rothamsted Research http://www.rothamsted.ac.uk/)

Table 1. *Crop protection in relation to food security worldwide, illustrated by rice, wheat, maize and potato crops. A comparison between actual estimated losses for these crops and potential losses if no crop protection measures were used to control pests and diseases.*

	Actual crop losses (with crop protection)		Potential crop losses (without crop protection)	
	%	£bn[a]	%	£bn[a]
Rice	37	84	77	174
Wheat	28	34	50	61
Maize	31	55	40	71
Potato	40	28	75	52

[a] Estimates of losses obtained by multiplying percentage crop losses to pests and diseases (expressed as proportions) obtained by Oerke (2006) by 2018 worldwide values of production of these crops estimated by FAO (http://faostat.fao.org/)

Impacts of climate change

It is necessary to assess impacts of climate change on crop diseases in order to produce recommendations to guide government and industry forward planning strategies for adaptation to climate change. Whilst much of the early work to assess impacts of climate change on crop diseases was qualitative and relied on experimental work done with artificial pathogen growth media rather than plants, there has now been more work done that uses quantitative combined

crop-disease-climate models based on data collected from experiments with diseased crops (Fitt *et al*., 2011). For example, work has been done with UK winter oilseed rape crops and the two main diseases there, namely light leaf spot (*Pyrenopeziza brassicae*) and phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*). Generally, light leaf spot is more important in Scotland and northern England, whilst phoma stem canker is more important in southern England (Boys *et al*., 2007; Butterworth *et al*., 2010). However, it is projected that climate change will increase the range of phoma stem canker northwards (Evans *et al*., 2008; Fig. 2a,b), whilst light leaf spot has been spreading southwards, partly because it has developed insensitivity to azole fungicides used to control it (Carter *et al*., 2014). Work to project impacts of climate change on phoma stem canker was done by inputting UK temperature and rainfall values under high and low CO_2 emissions for the 2020s and 2050s into weather-based models for forecasting severity of epidemics. Similar work projected that climate change will decrease the severity of light leaf spot, even in Scotland (Evans *et al*., 2010; Fig. 2c,d). Furthermore, work with a crop growth model projected that if diseases were controlled, climate change will increase yields of oilseed rape, especially in Scotland (Butterworth *et al*., 2010; Fig. 2e,f). Such projections illustrate the contrasting impacts of climate change on different diseases and the urgent need to do similar work on other diseases, especially since it can take 10-15 years to breed a new crop cultivar or develop a new fungicide (Fitt *et al*., 2016).

Nevertheless, it is important to realise that there is uncertainty in projected estimates of impacts of climate change on crop diseases (Newbery *et al.*, 2016, 2020). For example, it is essential to base models on observed data collected over a wide range of weather conditions over different growing seasons and locations and not to project future scenarios that are outside the range of observed data. Furthermore, Newbery *et al.* (2020) illustrate the risks associated with basing climate change projections on data collected in artificial conditions; the optimum temperature for growth of *Leptosphaeria maculans* in plants was considerably less than the optimum temperature for growth on artificial media (Fig. 3).

Adaptation to climate change

As part of strategies for adaptation to climate change, there are different types of crop protection actions available to farmers (Barnes *et al*., 2010; Fig. 4). For example, it may be possible for farmers to use more effective fungicide regimes by using web-based disease forecasts for light leaf spot or phoma stem canker, developed at Rothamsted (Welham *et al.*, 2004; Evans *et al*., 2008) and now available on the AHDB web-site (https://ahdb.org.uk/light-leaf-spot-forecast; https://ahdb.org.uk/phoma-leaf-spot-forecast). It may also be possible for farmers to use cultural control strategies, such as extending the intervals in crop rotations between planting oilseed rape crops to allow levels of pathogen inoculum to decrease further. If farmers are facing disease-associated crop losses, it gives more incentive for oilseed rape breeders to select cultivars that have improved resistance against the pathogens that cause diseases such as light leaf spot and phoma stem canker. Furthermore, in anticipation of warmer climates, breeders can start to test their new breeding material in areas where the current climate is similar to that expected for the UK in the future. In addition, The AHDB can give a higher priority to resistance against these pathogens in the criteria for selecting cultivars to go onto the Recommended Lists (https://ahdb.org.uk/knowledge-library/recommended-lists-for-cereals-and-oilseeds-rl). Aspects of the work to prepare for adaptation to climate change are likely to require investment from government, especially where the outputs will not lead to short-term increases in the profitability of the industry.

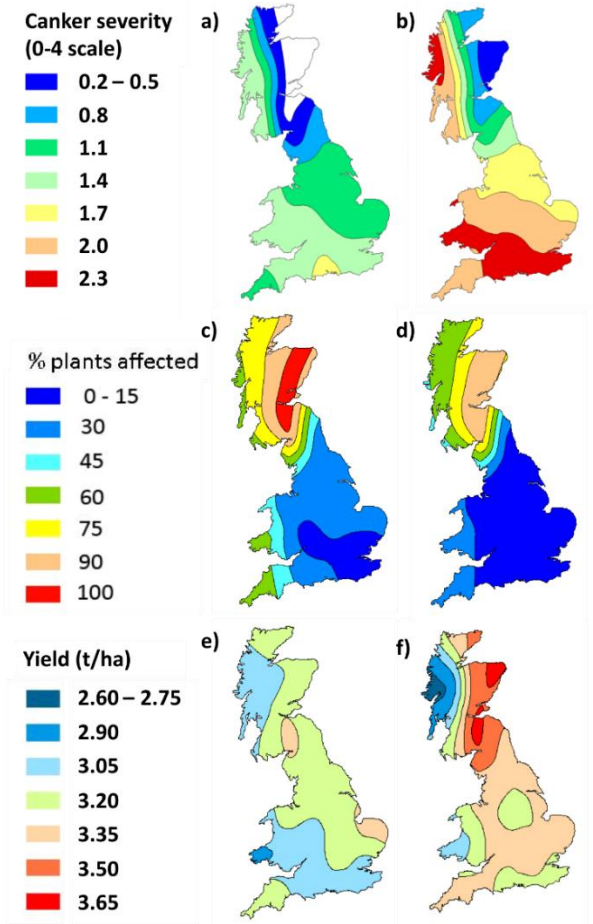

Fig. 2. Impacts of climate change on severity of phoma stem canker, incidence of light leaf spot and yield of oilseed rape treated with fungicide to control diseases. Predicted severity of phoma stem canker (*Leptosphaeria maculans*) at harvest of winter oilseed rape crops (mean of resistant and susceptible cultivars) for (a) baseline 1961-1990, (b) 2050s climates (mean of low and high emission scenarios); stem canker severity on a 0-4 scale (0, no disease; 4, plant dead); areas where crops are unaffected by the stem canker disease are marked white. Predicted incidence (% plants affected) of light leaf spot (*Pyrenopeziza brassicae*) at green flower bud (GS 3,3) of UK winter oilseed rape crops (mean of resistant and susceptible cultivars) for (c) baseline 1961-1990 and (d) 2050s high emissions climate scenarios. Predicted yield (t/ha) of winter oilseed rape (treated with fungicide to control diseases) for (e) baseline 1961-1990, and (f) 2050s high emissions climate scenarios using the STICS crop growth model. Predicted values are interpolated from predictions for 14 sites across the UK. Winter oilseed rape crops are generally grown in the eastern halves of England and Scotland; less fertile and mountainous areas in the west are unsuitable for arable crops. This figure is adapted from figures in Evans *et al.*, (2008, 2010) and Butterworth *et al.*, (2010).

Mitigation of climate change

Both the UK Climate Change Commission, which advises the Government (https://www.theccc.org.uk/publication/sixth-carbon-budget/) and the National Farmers Union (https://www.nfuonline.com/nfu-online/business/regulation/achieving-net-zero-farmings-2040-goal) have recently set targets for decreasing greenhouse gas emissions from agriculture, which account for 10% of total UK greenhouse gas emissions. This has prompted a debate about how to produce food at an affordable price for the most vulnerable in our society and for

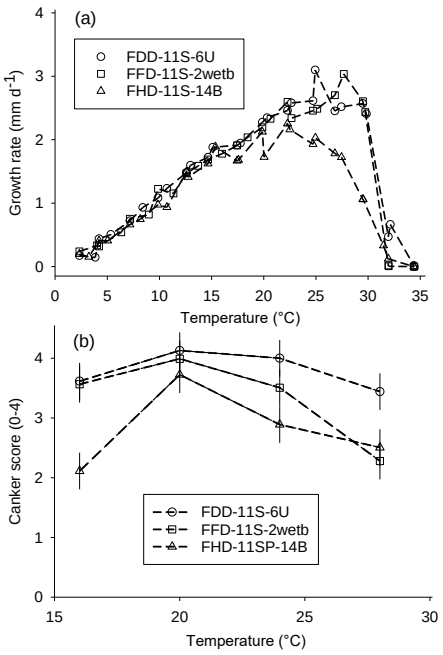

Fig. 3. Response to temperature of growth of three isolates of *Leptosphaeria maculans* (cause of phoma stem canker) when grown (a) on artificial medium (V8 agar, growth assessed as mm/day) or (b) in oilseed rape plants (*Brassica napus*, cultivar Drakkar, with no known resistance genes) inoculated in their leaf petioles to allow pathogen growth to the stem (growth assessed as a canker severity score on the Aubertot 0-4 scale). Data from Newbery *et al.* (2020)

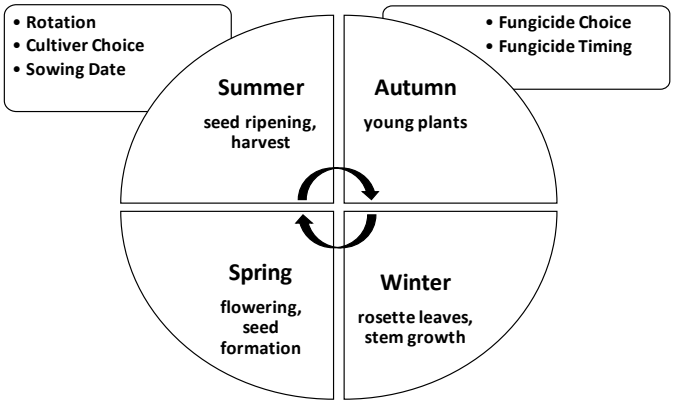

Fig. 4. Seasonal development of winter oilseed rape in the UK in relation to farmer-led autonomous adaptation strategies. Seeds are sown in late summer, rosette leaves develop in late autumn and stem extension occurs in late winter, followed by flowering in spring and harvest in the summer. Farmer-led adaptation strategies include crop rotation, cultivar choice (based on AHDB Recommended Lists) and sowing date to optimise yield. Farmers also optimise the timing and frequency of fungicide sprays based on forecasting (https://ahdb.org.uk/phoma-leaf-spot-forecast). Adapted from Barnes *et al*., (2010).

those areas of the world suffering food shortages due to climate change whilst decreasing greenhouse gas emissions from UK agriculture. Mahmuti *et al.* (2009) calculated the greenhouse gas emissions associated with production of 1 tonne of winter oilseed rape seed (Fig. 5). Most of the greenhouse gas emissions were associated with the manufacture and use of the nitrogen fertilizer; unused fertilizer is broken down by soil bacteria to produce nitrous oxide, a very potent greenhouse gas. They used data from field experiments with plots in which light leaf spot and phoma stem canker were controlled by fungicides by contrast with unsprayed plots in which they were not controlled; they observed that the greenhouse gas emissions per tonne of seed increased as disease control and yield decreased. In a series of experiments, the disease-induced yield loss was associated with a net increase in greenhouse gas emissions of

100 kg CO_2 equivalent per tonne of oilseed rape. Similar work done for winter and spring barley and winter wheat was summarised by Hughes *et al.* (2011), who estimated that disease control with fungicides decreased greenhouse gas emissions by *c*. 1.6Mt CO_2 equivalent each year from 2005 to 2009 (Fig. 6). Furthermore, if these crop yields were to decrease in future, more arable land would be required to maintain food production and there would be less land directly available for wildlife.

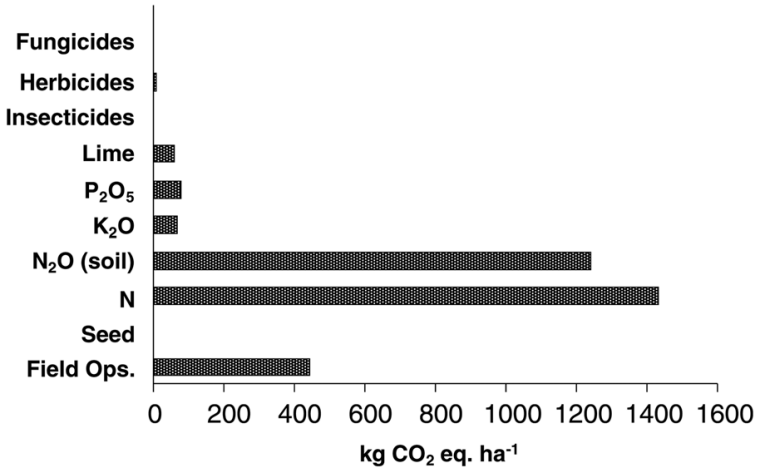

Fig. 5. GHG emissions related to different inputs in the production of 1 ha of winter oilseed rape in the UK. These inputs include nitrogen, potash (69 kg CO_2 eq./ ha) and phosphate fertilizers (78 kg CO_2 eq. /ha). For nitrogen fertilizers, GHG are released when they are manufactured (1433 kg CO_2 eq. /ha) and when they are applied (1242 kg CO_2 eq. /ha). The other inputs are the emissions from seed production (2 kg CO_2 eq. /ha), field operations such as tractor use (443 kg CO_2 eq. / ha) and the manufacture of fungicides (1.37 kg CO_2 eq. /ha), herbicides (7.89 kg CO_2 eq. /ha), insecticides (0.15 kg CO_2 eq. /ha) and lime (60 kg CO_2 eq. /ha). Adapted from Mahmuti *et al.* (2009).

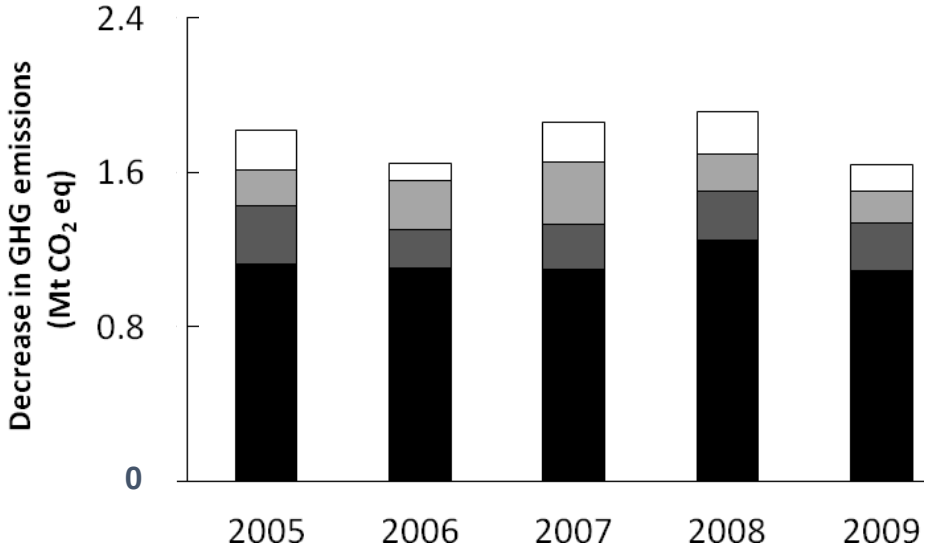

Fig. 6. Estimated decrease in GHG emissions (Mt CO_2 eq.) through use of fungicides to control diseases and increase yields in winter wheat (■), winter oil seed rape (■) winter barley (■) and spring barley (□) for the United Kingdom in harvest years 2005–2009. Total decreases in GHG emissions are 15% (2005), 14% (2006), 15% (2007), 14% (2008) and 13% (2009) of the estimated total GHG emissions (Mt CO_2 eq.) if these four crops were grown without fungicide treatment. Adapted from Hughes *et al.* (2011).

Conclusions

This work emphasises

- The need for more information on projected impacts of climate change on arable crop diseases, including those of oilseed rape, to guide government and industry strategies for adaptation to climate change.
- The need for accurate assessments to show how improved crop disease control can contribute to climate change mitigation to decrease greenhouse gas emissions from agriculture, whilst producing food at a price affordable for the most vulnerable people.
- The need for vigilance to maintain and improve crop disease control despite changing pathogen populations and the changing climate.

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Changing grower priorities for deployment of varietal resistance to pests and diseases in winter oilseed rape

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Summary

When deciding which varieties to grow, a farmer will consider many traits including yield, potential markets and resistance to pests and diseases. Resistance to pests and diseases is an important trait and crop breeders have made significant improvements in the resistance of oilseed rape (*Brassica napus*) to phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) and light leaf spot (*Pyrenopeziza brassicae*) in the past 20 years, in addition to introducing resistance to turnip yellows virus into elite varieties. However, evidence suggests that farmers are not taking full advantage of the newest most resistant varieties. Part of this may be a consequence of the withdrawal of neonicotinoid seed treatments from the market in the UK, which has resulted in widespread and frequent crop losses to cabbage stem flea beetle (*Psylliodes chrysocephala*) (CSFB). This has resulted in growers seeking vigorous varieties that are potentially more tolerant to CSFB and has forced some growers into a low risk/low cost strategy for growing the crop, using cheaper, often farm-saved seed, rather than the latest varieties. If solutions to the CSFB problem are developed, then farmers are likely to focus more on resistance to other pests and diseases and may then choose newer varieties.

Key words: resistance, varieties, cabbage stem flea beetle

When farmers are choosing a variety to grow, they are faced with a huge number of potential candidates. At the end May 2021, there were 288 varieties of winter oilseed rape (WOSR) (*Brassica napus*) on the UK National List of varieties that may be sold in the UK (Defra, 2021). Independent data on the characteristics and performance of these varieties are not always easy to obtain, making the task even more difficult. In order to provide some information, the Agriculture and Horticulture Development Board (AHDB), a statutory levy board that is funded by UK farmers, growers and others in the supply chain, conducts trials on new varieties to establish their characteristics. This includes national and regional yields, agronomic characteristics, such as earliness of maturity, and resistance to key pests and diseases. Results of individual trials are reported annually and multiyear datasets are analysed to provide multiyear means. The new varieties with the best combination of yield, agronomic and disease resistance characteristics are added to a 'Recommended List' of varieties to grow (AHDB, 2021).

Rapid progress in breeding over the last decade has resulted in a notable increase in the disease resistance of WOSR varieties and this is reflected in the disease resistance scores of varieties on the AHDB Recommended List (Fig. 1). In addition to increases in phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) and light leaf spot (*Pyrenopeziza brassicae*) resistance, breeders have incorporated resistance to turnip yellow virus (TuYV) into elite oilseed rape varieties over this period, with 37% of varieties on the 2021/2022 Recommended List having this trait (AHDB, 2021). This improvement in varietal resistance to pests and diseases has not come with a yield penalty.

Despite these improvements in varieties, evidence from the AHDB planting survey (AHDB, 2020) indicates that growers of oilseed rape in the UK are not taking up these improved varieties, but are growing older lower yielding, more pest and disease susceptible varieties (Table 1).

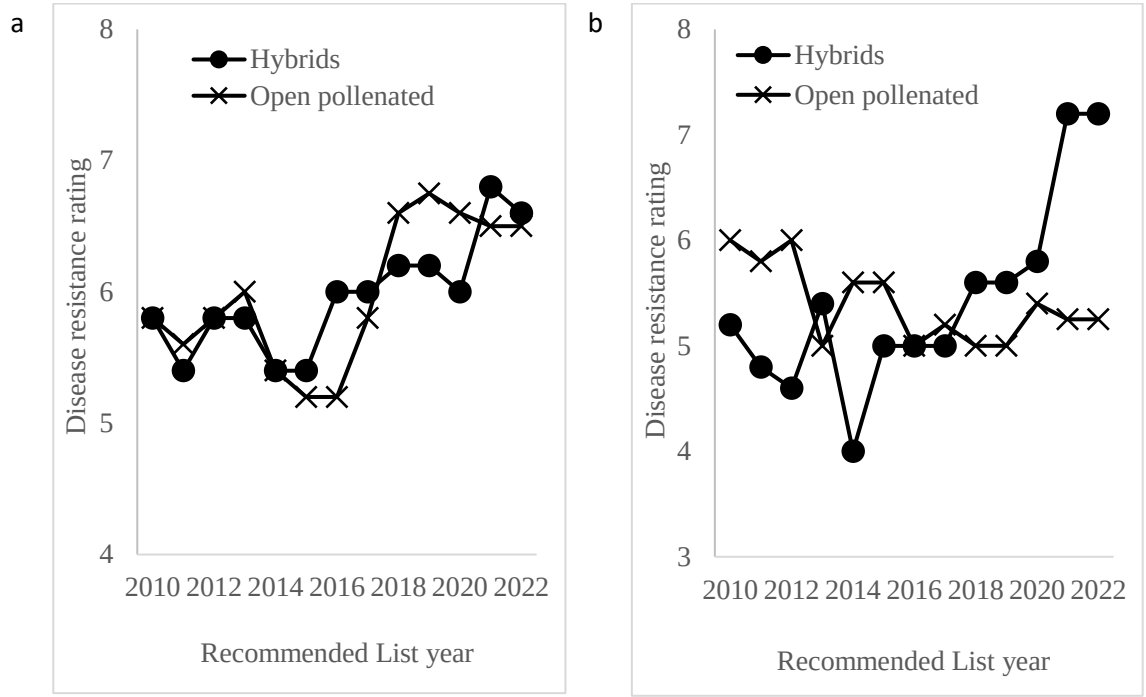

Fig. 1. Change in AHDB Recommended List disease resistance ratings between 2010 and 2022 for a) phoma stem canker and b) light leaf spot, of the top five yielding varieties on the Recommended List. Higher ratings indicate a higher level of disease resistance.

The variety Campus was first added to the Recommended List in 2015 and is below the average disease resistance rating of the highest yielding varieties (Fig. 1).The variety Elgar was added in 2016 and though having a high light leaf spot resistance rating, is less than than the average for phoma stem canker resistance (Fig. 1) and lacks TuYV resistance. Both varieties are several percent less than the newest varieties in terms of yield.

There may be a number of reasons that growers are not choosing the newest most resistant varieties, such as reluctance to change from a known variety to an unknown one and seed availability. However, a key factor in the choice of variety for UK growers has become cabbage stem flea beetle (*Psylliodes chrysocephala*) (CSFB).

Since the withdrawal of seed dressings containing neonicotinoid insecticides, including imidacloprid, clothianidin and thiamethoxam, on bee attractive crops in December 2013, growing WOSR in southern, central and eastern England has become challenging. With no effective foliar applied insecticides or cultural options to control the CSFB, total crop losses have become common. The result has been a significant decline in the area of oilseed rape in the UK, from a maximum of 756,000 ha in 2012 to just 388,000 ha in 2020 (Defra, 2020).

Table 1. *The three most popular winter oilseed rape varieties by area grown as reported for harvest years 2018-2021 in the AHDB planting survey (AHDB, 2020). Variety type (O) open pollinated (H) hybrid.*

	Disease resistance rating		Resistance trait
	Light Leaf Spot	Phoma Stem Canker	TuYV
2021			
Campus (O)	6	5	-
Aspire (O)	7	6	+
Elgar (O)	7	6	-
2020			
Campus (O)	6	5	-
Elgar (O)	7	6	-
DK Exalte (H)	8	8	-
2019			
Elgar (O)	7	6	-
Campus (O)	6	5	-
DK Extrovert (H)	7	8	-
2018			
Elgar (O)	7	6	-
DK Extrovert (H)	7	8	-
Campus (O)	6	5	-

The main threat to WOSR from CSFB comes in the crop establishment phase, when migrating beetles enter the crop to feed on the young plants and lay eggs. Under heavy infestations total plant and crop loss is possible, and even if the plant recovers it may succumb to damage from larval feeding. Many growers who have continued to grow the crop have sought to reduce risks and minimise financial outlay on the crop in this phase, through a number of actions.

One way to reduce cost is to use seed of the previous crop, so called farm-saved seed, rather than purchase new certified seed. Another is to grow open pollinated varieties, as seed for these varieties is generally cheaper. Anecdotal evidence suggests this is happening and it is notable that the most popular varieties reported in the planting survey are generally open pollinated (Table 1). These could be from farm-saved-seed or bought-in seed.

Another factor anecdotally affecting variety choice is vigour, both at establishment and in the spring. Vigour is not reported in the Recommended List and is poorly defined. However, many growers believe that vigorous varieties are more resilient to CSFB damage and varieties are in some cases marketed on their vigour. With such challenging growing conditions in the key WOSR growing regions of the UK, it is perhaps not surprising that growers are focusing not

on disease resistance and yield, but on a trait that can increase the chances of a successful crop, whether this effect is real or perceived.

Conclusions

The lack of effective insecticides or cultural control measures for CSFB has driven variety choice for WOSR in England down an unusual path in the last five years, at least for a section of growers. Nevertheless, the breeding companies continue to improve resistance to traditionally important diseases and improve yields, with new traits such as tolerance to TuYV being introduced to elite varieties. It remains to be seen if, when effective measures to control CSFB are developed through new insecticides, a better understanding of cultural control or resistant varieties, growers that have switched to a low investment, low risk model of growing the crop using older varieties, will more readily adopt the newer trait-laden crops or will retain their new model of growing the crop.

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Chemical warfare: the fungal quest to conquer oilseed rape

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Key words: Phoma stem canker, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, sirodesmin PL, interspecific interactions, phomamide

Introduction

Phoma stem canker, caused by *Leptosphaeria maculans* and *L. biglobosa*, causes an average yield loss of > £70M annually in UK oilseed rape (www.cropmonitor.co.uk) (Zhang *et al*., 2014). Previous studies had shown that *L. biglobosa* ascospores were released later than those of *L. maculans* (Huang *et al*., 2011). However, more recent investigations that have used qPCR analysis have reported that ascospores of both species are more frequently released at similar times (Javaid *et al*., 2018). *L. maculans* produces sirodesmin PL, a non-host selective epipolythiodioxopiperazine; *L. biglobosa* does not (Pedras & Yu, 2009). Sirodesmin PL has an inhibitory effect on *L. biglobosa* (Elliott *et al*., 2007). There has been limited work investigating the interaction between *L. maculans* and *L. biglobosa* at key stages of their life cycles. Therefore, this study aims to provide a better understanding of the unknown interactions between *L. maculans* and *L. biglobosa* and investigate the changes in phytotoxin production as a result of increased interspecific competition.

Materials and methods

L. maculans and *L. biglobosa* were cultured in liquid culture, either individually or dual cultured with a competing pathogen. After 14 days, a secondary metabolites ethyl acetate extraction was done for each treatment, to investigate the effect of secondary metabolites on the colony growth of *L. maculans* and *L. biglobosa*. Fungal plugs (8mm diameter) of *L. maculans* or *L. biglobosa* were inoculated onto clarified V8 juice agar plates. Each fungal plug was inoculated with the corresponding secondary metabolite extract from each treatment or ethyl acetate. Each treatment was replicated five times; the ethyl acetate control was replicated three times. Colony diameters for *L. maculans* and *L. biglobosa* were recorded at 7 days post inoculation and converted to colony areas. To investigate the changes in phytotoxin production, the secondary metabolites extracted from each treatment were analysed to identify differences in composition using HPLC and LC-MS.

Results

Analysis of interspecific interactions between the pathogens *in vitro* confirmed that different mechanisms of interspecific competition were used to out-compete each other. The secondary metabolites produced by *L. maculans* inhibited *L. biglobosa* colony growth. This inhibition was not observed when *L. biglobosa* was inoculated with secondary metabolites extracted from

the co-culture of *L. maculans* and *L. biglobosa*. There were three unique maxima found only in the secondary metabolite extracts that inhibited *L. biglobosa* colony growth. Using HPLC and LC-MS, these maxima were identified as sirodesmin PL precursors deacetylsirodesmin PL and phomamide, sirodesmin PL and an unknown compound. When *L. maculans* and *L. biglobosa* were co-inoculated, sirodesmin PL and its precursors were not produced. Additional maxima on the HPLC chromatograph were not found. Results of this study suggest that *L. biglobosa* must inhibit the formation of sirodesmin-precursor. Due to sirodesmin having an antagonistic effect on *L. biglobosa*, it is thought that this interference must happen very early in *L. maculans-L. biglobosa* interactions, before the production of sirodesmin. Considering application of the results for control of phoma stem canker in field conditions, if *L. maculans* and *L. biglobosa* ascospores are released at the same time, phoma leaf spot lesions may appear later or be smaller, allowing fungicides to be applied later.

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Mycovirus induced hypervirulence of *Leptosphaeria biglobosa* enhances systemic acquired resistance to *Leptosphaeria maculans* in *Brassica napus*

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Phoma stem canker (blackleg) is one of the most important diseases of winter oilseed rape (*Brassica napus*) worldwide and is caused by a complex that comprises at least two species: *Leptosphaeria maculans* and *L. biglobosa*. Screening a panel of field *Leptosphaeria* isolates from *B. napus* for the presence of mycoviruses revealed the presence of a novel double-stranded RNA quadrivirus in *L. biglobosa* and no viruses in *L. maculans*. Following elimination of the mycovirus, virus-infected and virus-free isogenic lines of *L. biglobosa* were produced. A direct comparison of the growth and virulence of these isogenic lines illustrated that virus infection caused hypervirulence and resulted in induced systemic resistance towards *L. maculans* in *B. napus* following lower leaf pre-inoculation with the virus-infected isolate. Analysis of the plant transcriptome suggests that the presence of the virus leads to subtle alterations in metabolism and plant defences. For instance, transcripts involved in carbohydrate and amino acid metabolism are enriched in plants treated with the virus-infected isolate, while pathogenesis-related proteins, chitinases and WRKY transcription factors are differentially expressed. These results illustrate the potential for deliberate inoculation of plants with hypervirulent *L. biglobosa* to decrease the severity of phoma stem canker later in the growing season.

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The author(s) declare no conflict of interest.

Regional differences in the proportions of *Leptosphaeria maculans* and *L. biglobosa* (the cause of phoma stem canker on oilseed rape) in Eastern England

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Phoma stem canker, a globally important disease of oilseed rape caused by fungal pathogens *Leptosphaeria maculans* (Lm) and *Leptosphaeria biglobosa* (Lb), leads to annual yield losses of £50-90M in England. To monitor the regional differences in proportions of *Leptosphaeria* spp. in the air in Eastern England, Burkard air samplers were set up at four regional locations (Bayfordbury, Hertfordshire; Eye, Suffolk; Impington, Cambridgeshire and Rothwell, Lincolnshire) and daily amounts of Lm DNA and Lb DNA in the air were quantified from September to March in 2015-2016, 2016-2017 and 2017-2018 cropping seasons using quantitative PCR (qPCR). There were differences between seasons and locations in the relative amounts of *Leptosphaeria* spp. DNA detected. In 2015-2016, there was more Lm DNA than Lb DNA at all sites except Bayfordbury. In 2016-2017, there was more Lb DNA than Lm DNA at all sites except Rothwell. In 2017-2018, all sites had more Lm DNA than Lb DNA. The results also showed that there were differences between seasons and locations in the timing of Lm and Lb ascospore release measured by the amounts of Lm DNA and Lb DNA. For all three seasons, both Lm DNA and Lb DNA were detected at similar timings at all sites except Rothwell, where Lm DNA was detected earlier than Lb DNA. Comparison between locations showed that both Lm DNA and Lb DNA were detected later at Impington than at other locations in all three seasons. These variations in the amounts of Lm DNA and Lb DNA indicated that there were different proportions of Lm and Lb ascospores in the air in different seasons, with some seasons having more Lb ascospores than Lm ascospores. These differences may have been due to differences in weather conditions, local crop cultivars or fungicide efficacy at the different locations in different seasons. The timing and proportions of ascospore release can be used to guide the timing and choice of fungicide sprays in local areas.

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Molecular mechanisms of mutation to virulence in *Leptosphaeria maculans* populations in the UK

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Leptosphaeria maculans, the cause of phoma stem canker of oilseed rape, develops gene-for-gene interactions with its host plant resistance genes. Pathogens may evolve to overcome recognition by host resistance proteins (resistance gene products) to render the host resistance ineffective. In this study, the regional distribution of the *L. maculans* races in the UK was monitored and the molecular mechanisms of mutation to virulence were investigated. Field experiment sites were set up at different locations in the UK: from leaf spot lesions on Drakkar (susceptible cultivar, trap crop) and other cultivars (with *Rlm7* resistance gene), 64 and 88 *L. maculans* isolates were obtained in the 2015/2016 and 2016/2017 cropping seasons, respectively. Changes in frequencies of avirulent *AvrLm1*, *AvrLm4* or *AvrLm7* alleles were investigated by testing isolates on cotyledons of a differential set of cultivars. Isolates virulent towards *Rlm1*, *Rlm4* or *Rlm7* were investigated for molecular events of mutations. There were variations in the frequencies of avirulent *AvrLm1* and *AvrLm4* alleles between cropping seasons. All the isolates from different sites were avirulent against *Rlm7* in the 2015/2016 season. In the 2016/2017 season, 6.8% of isolates were virulent towards *Rlm7*. The molecular mechanism of mutation to virulence in *AvrLm1* was observed to be whole gene deletion in 86% of isolates. Another 13% of isolates were sequenced and the molecular events of mutations will be investigated. Whole gene deletion was observed in 6% or 50% of isolates carrying the virulent alleles of *AvrLm4* or *AvrLm7* respectively. The others need to be sequenced for further investigation.

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Rapid detection strategy for pathogens causing phoma stem canker of *Brassica napus*

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The fungus *Leptosphaeria maculans,* the pathogen causing phoma stem canker (blackleg) of *Brassica napus* (oilseed rape, canola), produces the phytotoxin sirodesmin PL. The disease is responsible for major yield losses of oilseed rape worldwide. Due to the importance of the disease in global trade, rapid detection of *L. maculans* (Lm) at both the ports and in crops in China is essential to controlling the spread of this disease and guaranteeing the quality of oilseed rape seeds.

In our study, we have developed three rapid detection methods based on an isothermal amplification technique to detect both the aggressive species *L. maculans* and the related species *L. biglobosa* (Lb). Recombinase polymerase amplification (RPA), done at a fixed temperature between 37-42°C, was used to develop a rapid and portable detection method for testing suspected fungi in plant tissues with phoma stem canker (blackleg) symptoms, or the fungi with white mycelium cultured from the infected tissue that was cultured on PDA (Fig. 1). To achieve the required rapid and *on-site* diagnostic test, each step of the assay, including DNA extraction, primer-probe pair design, RPA condition optimisation and amplicon fluorescence quantification, were optimised with the portable devices and appropriate methods. DNA adsorption or extraction and separation with magnetic beads do not require high-speed centrifugation; thus this is a better choice for *on-site* DNA extraction. As shown in Fig. 1B, the total time for DNA isolation was about 30 min. The eluted DNA was mixed with primers, probes and rehydration buffer, and the mixture was added to the freeze-dried powder containing all the enzymes and reagents necessary for DNA amplification. Finally, $MgAc_2$ was added to initiate the amplification reaction at temperature 37~42˚C.

A duplex approach, including two probes modified with two fluorescent groups with different emission spectra, was constructed for detection of *L. maculans* and *L. biglobosa* in one sample. The results showed that the Lm-eF2/Lm-eR1/Lb-probe and Lb-eF1/Lb-eR1/Lm-probe (Table 1) can be used to specifically amplify *L. maculans* and *L. biglobosa*, respectively, and there is no cross-reaction between the primers and probes. About 440 copies of Lm genomic DNA can be detected using this fluorescence RPA method. The spiked experiments indicated that when 0.54 ng of *L. maculans* genomic DNA was mixed with 37 ng of oilseed rape genomic DNA, 1.4% *L. maculans* genomic DNA could still be clearly differentiated from oilseed rape genomic DNA. With the development of portable devices with a rechargeable battery and a magnetic mixer in the unit (e.g. T8), these RPA techniques could provide a useful and fast screening method for *L. maculans* infected *Brassica napus* seedlings or seeds directly in crops, thus enabling management decisions to be made immediately. Furthermore, simultaneous assay of *L. maculans* and *L. biglobosa* in one RPA reaction enables fast identification of fungi in *Brassica napus* with phoma stem canker (blackleg) symptoms.

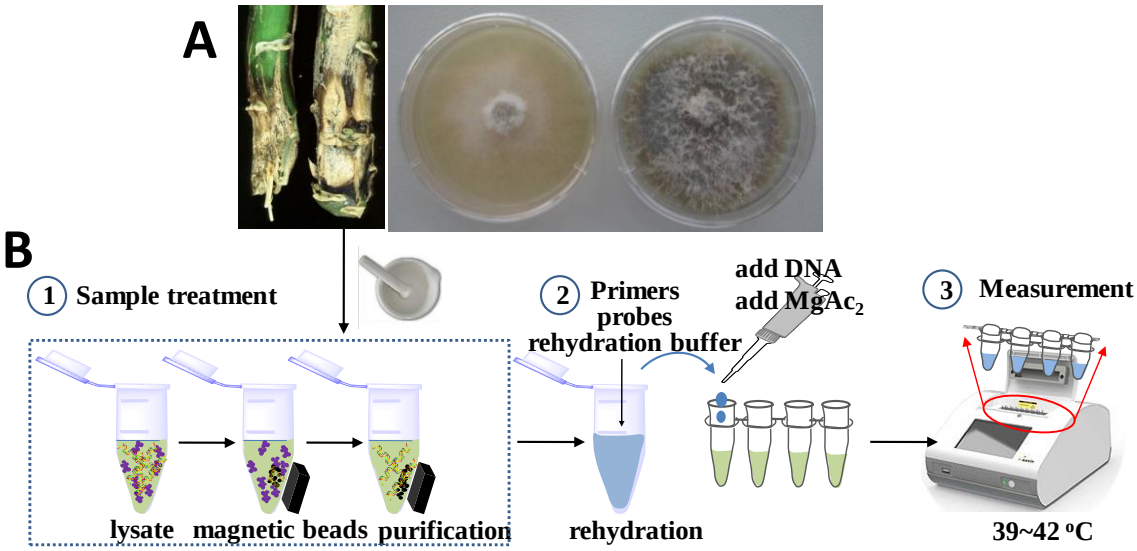

Fig. 1. Symptoms on *Brassica napus* infected with *L. maculans* (A, left), mycelium of cultured *L. maculans* (A, right) .The technique for rapidly identifying genomic DNA (B).

Table 1. *Sequences of primers and probes for the construction of exo RPA assays for L. maculans and L. biglobosa.*

Name	Sequences 5'-3'
Lm-eF1	AAGCACTGCCGCCTCGATCAGTGGCGGC
Lm-eF2	ACTGCCGCCTCGATCAGTGGCGGCAGTCTAC
Lm-eF3	CACTGCCGCCTCGATCAGTGGCGGCAGTC
Lm-eR1	TTGCAAGTGGTTTTAGGGGATCCAATTGGTG
Lm-eR2	AATTGCAAGTGGTTTTAGGGGATCCAATTGGTGGG
Lm-eR3	CAATTGCAAGTGGTTTTAGGGGATCCAATTGGTGGGC
Lm-P	ACTGCCGCCTCGATCAGTGGCGGCAGTCTAC(FAM-dT)(THF)(BHQ1-dT)GATTCTGCCCATGT-C3 spacer
Lb-eF1	CCCTTCTATCAGGGGATTGGTGTCAGCATTTCGG
Lb-eR1	TTACAAGTGGTTTGAATTGTCCTTTTGGCAGGC
Lb-eR2	CAATTACAAGTGGTTTGAATTGTCCTTTTGGCAG
Lb-P	TCAGCATTTCGGCCTTTGGCTTACTTTCTGGCCC(ROX-dT)(THF)(BHQ1-dT)CCTTTCTGATTCTA-C3 spacer

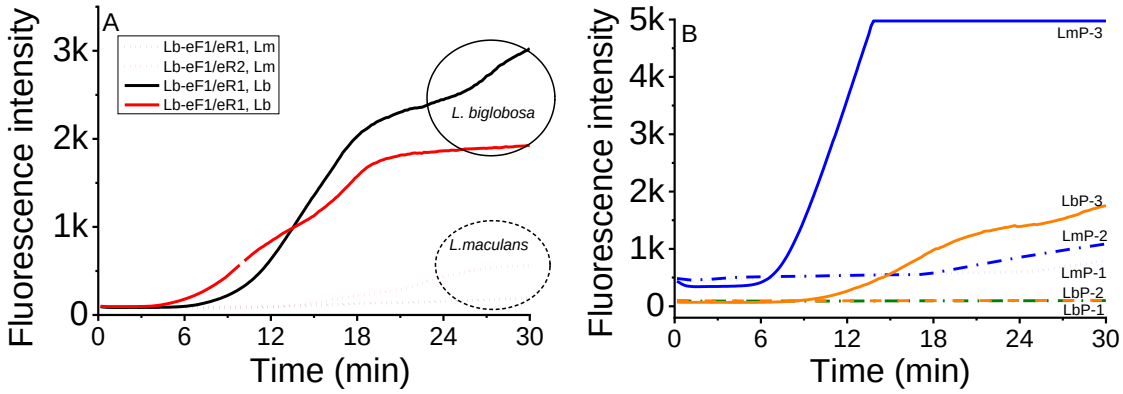

Fig. 2. A: Real-time fluorescence curves of the RPA assay using different *L. biglobosa* primer combinations (black line, Lb-eF1/eR1; red line, Lb-eF1/eR2) for amplification of *L. biglobosa* DNA (solid lines) and *L. maculans* DNA (dashed lines). B: The real-time fluorescence curves of an RPA assay using the *L. biglobosa* probe and *L. maculans* primers to amplify water (LbP-1), *L. maculans* DNA (LbP-2), or using the *L. maculans* probe and *L. biglobosa* primers to amplify water (LmP-1) or *L. biglobosa* DNA (LmP-2). LbP-3 shows that the *L. biglobosa* probe and *L. biglobosa* primers will amplify *L. biglobosa* DNA. LmP-3 shows that the *L. maculans* probe and *L. maculans* primers will amplify *L. maculans* DNA. All the primer combinations are listed in Table 1.

However, the limit of quantification (LOQ) of 21.6 pg (4.43×10^2 DNA copy) in the fluorescence RPA assay was found to be insufficient for the screening of infected seeds from a large sample of healthy seeds (Lei *et al*., 2019). To overcome the limitation of low sensitivity of this method for field applications, we combined microcantilever (MCL) biosensing and the RPA technique to develop an ultrasensitive detection method. MCL has emerged as a viable biosensor because of its outstanding features, such as high sensitivity detection (Lang, 2008). Therefore we developed a strategy by combining the rapid RPA technique with gold nanoparticle (AuNP)-enhanced MCL for *L. maculans* sensing nucleic acid screening. The results indicated that the sensitivity of the RPA-MCL assay is greater than that of the fluorescence RPA assay, with the detection limit at only one copy of *L. maculan*s DNA. In the practical assay, the newly developed RPA-MCL method was found to detect 57 ppm *L. maculans* genomic DNA in the oilseed rape seeds genomic DNA sample. Considering the high sensitivity and specificity of this strategy, we envisage that the proposed RPA-MCL assay could have wide applications in nucleic acid diagnostics for plant pathogen detection and species identification (Lei *et al.*, 2021).

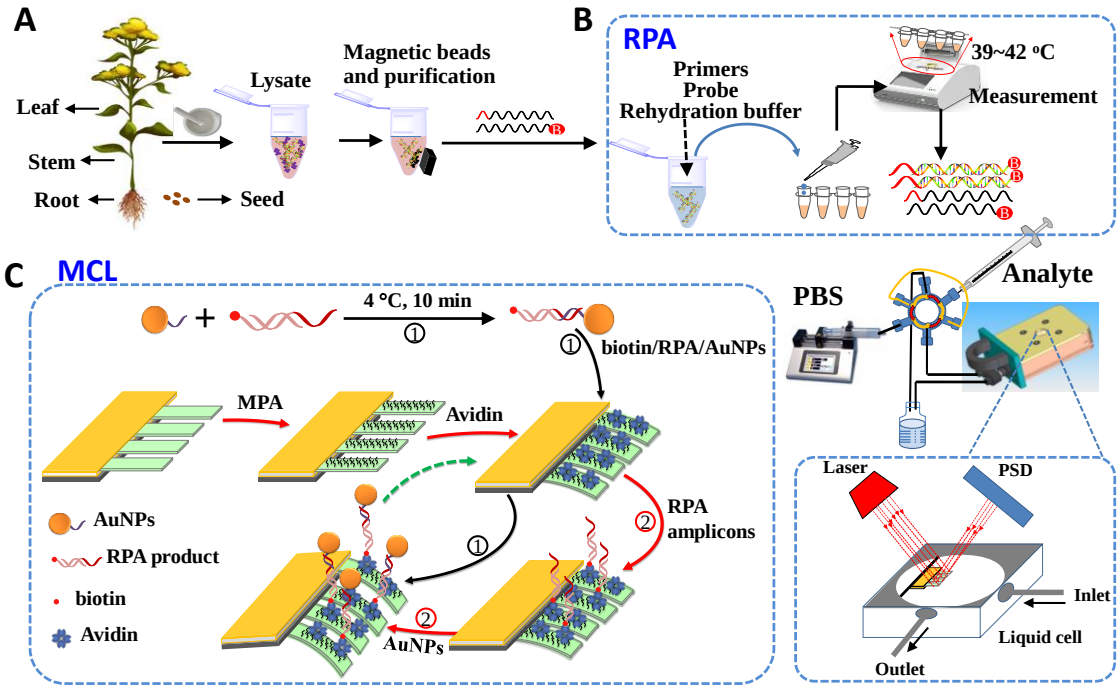

Fig. 3. Schematic illustration of the combined RPA and microcantilever (MCL) assay strategy.

Paper-based lateral flow detection techniques do not need instruments and can be used in remote areas. To rapidly screen for *L. maculans* in oilseed rape plants with phoma stem canker symptoms, we developed a paper-based lateral flow technique to detect RPA products with FAM and biotin groups. The results showed that this method can specifically detect *L. maculans* genomic DNA but not *L. biglobosa* DNA. Using this strategy, the total assay time is less than 60 min, and only a portable incubator and pipettes are required. All the equipment and materials are portable.

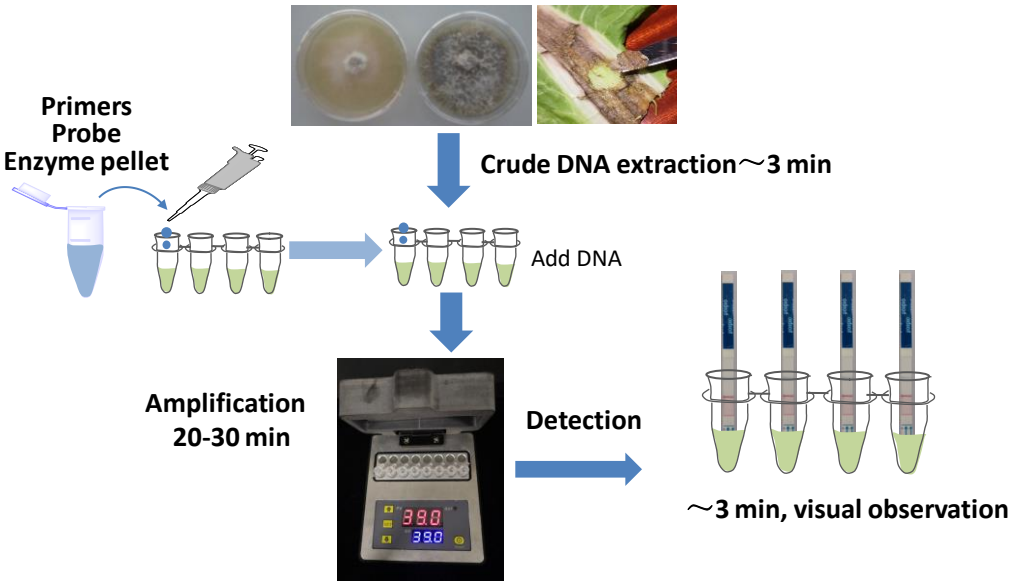

Fig. 4. Schematic illustration of the combined RPA and paper-based lateral flow strips assay strategy.

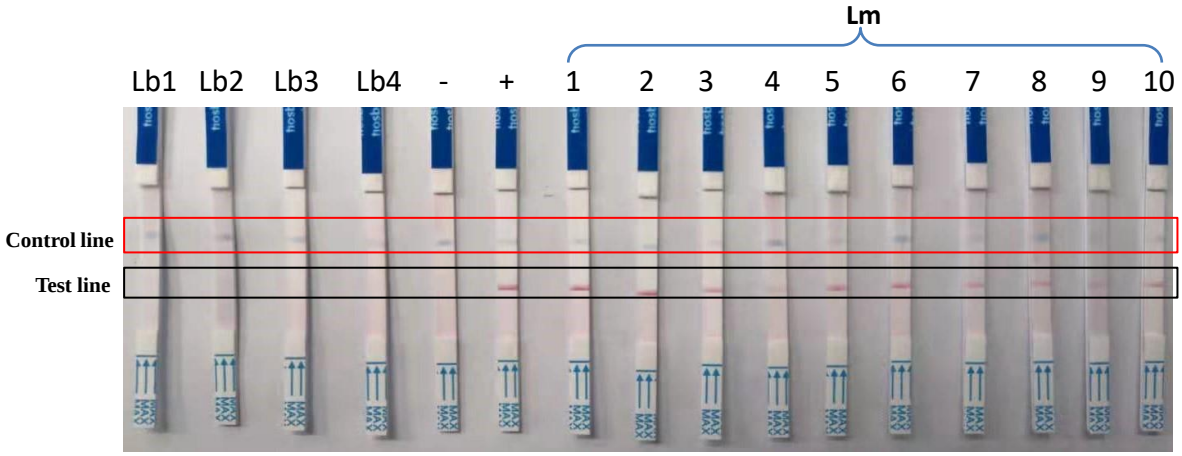

Fig. 5. Detection results using paper-based lateral flow strips for *L. maculans* and *L. biglobosa* genomic DNA.

Conclusion

Three methods based on recombinase polymerase amplification (RPA) have been developed to detect the fungi responsible for phoma stem canker (blackleg) of *Brassica*. Real-time RPA with duplex probes can detect *L. maculans* and *L. biglobosa* in one reaction in 30 minutes. Detection of RPA products using lateral flow strips can distinguish *L. maculans* from *L. biglobosa*, and no complex equipment is required. To assay the small amount of *L. maculans* in oilseed rape seed, an ultrasensitive detection method combining a microcantilever sensor and RPA (RPA-MCL) was developed. In general, the established rapid assay strategy is simple, fast, portable and feasible for field monitoring and detection of pathogenic fungi.

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Disease management in oilseed rape: insights into the *Brassica napus*-*Pyrenopeziza brassicae* pathosystem

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Introduction

Light leaf spot, caused by the fungal pathogen *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 (www.cropmonitor.co.uk) 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 (Gilles *et al*. 2001). Splash-dispersed conidia produced on diseased leaves are the main component of the secondary inoculum (Evans *et al*., 2003). Currently, the management of light leaf spot relies on fungicide applications, recommendations for resistant cultivars and cultural practices (AHDB, 2021). However, considering the frequent occurrence of light leaf spot epidemics in recent years, it appears that current control strategies are not sufficient to achieve successful control of light leaf spot. Even though the occurrence of severe epidemics of light leaf spot has been reported in the UK since 1970s, the knowledge about this pathosystem, especially in relation to the operation of host resistance, is very limited compared to that for other important pathosystems of oilseed rape such as phoma stem canker (*Leptosphaeria maculans*) (Boys *et al*., 2007; Karandeni Dewage *et al*., 2018). Therefore, this study focused on investigating interactions between *P. brassicae* and *B. napus* using single-spore isolates to provide better understanding of the different sources of resistance against *P. brassicae*.

Materials and methods

Single-spore isolates of *P. brassicae* were obtained from diseased leaf samples collected from oilseed rape crops and sub-cultured onto malt extract agar (MEA) plates. Pathogen inoculum was prepared from conidia produced by sub-cultured isolates using sterilised distilled water. The inoculum concentration was adjusted to 10^5 spores/ml and it was stored at -20ºC until needed. Oilseed rape genotypes that included commercial cultivars and breeding lines were selected, based on resistance ratings from the AHDB Recommended List (RL) trials and to represent diverse genetic backgrounds. Selected genotypes were grown under glasshouse conditions until they reached growth stage 1,4-1,5 (four-five leaves unfolded) and spray-inoculated with conidial suspensions prepared from single-spore isolates of *P. brassicae*. After inoculation, regular observations were made to identify the timing and appearance of different resistant/susceptible phenotypes. Final disease assessment was made at 29 days post inoculation (dpi) on plants that were destructively harvested at the stem base at 24 dpi and

incubated at 4ºC for five days in polyethylene bags. Light leaf spot severity was measured as % leaf area covered with *P. brassicae* sporulation and compared between different treatments to identify resistant/susceptible interactions.

Results and Discussion

Infected plants showed different resistant/susceptible phenotypes associated with *P. brassicae*. This included leaf deformations, necrosis and different numbers of acervuli (asexual sporulating structures of *P. brassicae*) with or without the presence of visible lesions. Leaf deformations appeared at *c.*7 dpi, indicating possible association with early stages of *P. brassicae* colonisation. Symptoms included leaf curling, leaf distortions and petiole elongations and could be seen to some extent in all the cultivars/lines tested in the experiments. Some of the cultivars/lines appeared to have necrotic responses to *P. brassicae* infection. Of these cultivars/lines, the majority had reduced numbers of *P. brassicae* acervuli, suggesting an association between these two phenotypes. Considering the light leaf spot severity measured as % leaf area with *P. brassicae* sporulation, there were significant differences ($P \leq 0.05$) between cultivars/lines and between isolates and there were significant cultivar/line-isolate interactions. Some of the pre-breeding lines showed resistance against most of the isolates tested. These lines may provide sources of resistance for oilseed rape breeding programmes. There has been little work on specific host-pathogen interactions regarding light leaf spot disease, and identification of different types of resistance available in commercial oilseed rape cultivars is important for effective deployment of cultivar resistance. This study shows the potential for studying specific cultivar-by-isolate interactions in the *B. napus* – *P. brassicae* pathosystem using single-spore isolates of *P. brassicae*. It is anticipated that this type of experimentation will help to identify different sources of resistance that can be exploited in oilseed rape breeding programmes.

Acknowledgments

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Effects of plant age and inoculum concentration on light leaf spot disease phenotypes on oilseed rape

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Summary

Light leaf spot is caused by the fungal pathogen *Pyrenopeziza brassicae* and is the most economically damaging disease of oilseed rape (*Brassica napus*) in the UK. Current control relies on repeated fungicide applications; however, pathogen fungicide-insensitivity development highlights the need for non-chemical controls like host resistance. A study was done to assess light leaf spot disease phenotype on the susceptible *B. napus* cultivar Charger in different treatment conditions; factors studied included plant age and inoculum concentration. Results showed that older plants grown in a controlled-environment cabinet produced the most visible symptoms. Plants that received a greater inoculum concentration (10^5 spores/ml) were significantly shorter by 5 cm than those inoculated with a smaller inoculum concentration (10^4 spores/ml), suggesting possible correlations between fungal inoculum concentration and plant growth. Additionally, > 20 *P. brassicae* field isolates were collected from leaf samples across England through single-spore isolation and will be screened for virulence.

Introduction

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is the most economically damaging disease of winter oilseed rape (*Brassica napus*) in the UK, with annual yield losses of > £100M (CropMonitor, 2021; Karandeni Dewage *et al*., 2017). Control of light leaf spot is challenging because it is a polycyclic disease, with epidemics started in autumn by ascospores released from apothecia on infected crop debris from the previous season. Subsequently, conidia produced by asexual sporulation on infected leaves cause secondary infections on all parts of the plant (Boys *et al*., 2007; Evans *et al*., 2003; Fitt *et al*, 1998). Current control relies on fungicides; however, insensitivity development in *P. brassicae* isolates highlights the need for non-chemical controls like host resistance (Carter *et al*., 2014; Huang *et al*., 2006). There is currently little information about pathogenic *P. brassicae* populations and host resistance mechanisms, highlighting a need for new research. There is a need to improve our current knowledge about host resistance of winter oilseed rape against *P.*

brassicae by studying virulent races in pathogen populations, identifying candidate resistance genes and investigating mechanisms of host resistance.

Materials and methods

Results of a preliminary experiment to produce *P. brassicae* conidial inoculum were expanded to investigate the light leaf spot disease development on the *B. napus* susceptible cultivar Charger in different treatment conditions. Plants of cultivar Charger were grown in a controlled-environment cabinet and inoculated with *P. brassicae* conidial suspensions with 10^4 or 10^5 spores/ml when the plants were 4, 5, 6 or 7 weeks old. Severity of light leaf spot and plant height on inoculated plants were assessed at 23 days post-inoculation (dpi) and compared between different treatments to identify the effects of inoculum concentration and growth stage of plants on light leaf spot severity.

To establish a collection of *P. brassicae* isolates, leaves with light leaf spot symptoms were sampled from winter oilseed rape crops and fungal isolates for further study were obtained through single-spore isolation and subculturing on malt extract agar plates (Fig. 1).

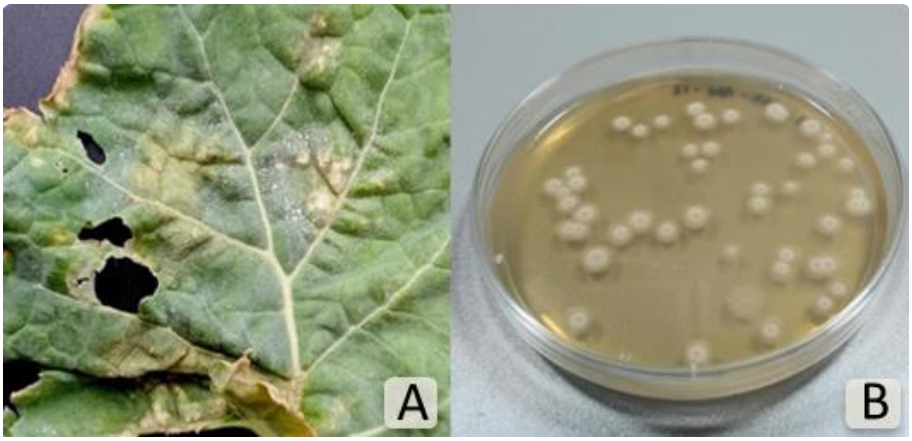

Fig. 1. Leaf of oilseed rape cv. Charger with light leaf spot (showing white *P. brassicae* acervuli containing conidia) (A). Single-conidial *P. brassicae* colonies derived from the same acervulus grown on a malt extract agar plate (B).

Results

Older plants of cv. Charger (7 weeks old at time of inoculation) grown in a controlled-environment cabinet that received the greater inoculum concentration (10^5 spores/ml) produced the most severe light leaf spot symptoms. Plants inoculated with the greater inoculum concentration were significantly shorter (by up to 5 cm) than those inoculated with the smaller inoculum concentration (10^4 spores/ml) (Figs 2 & 3).

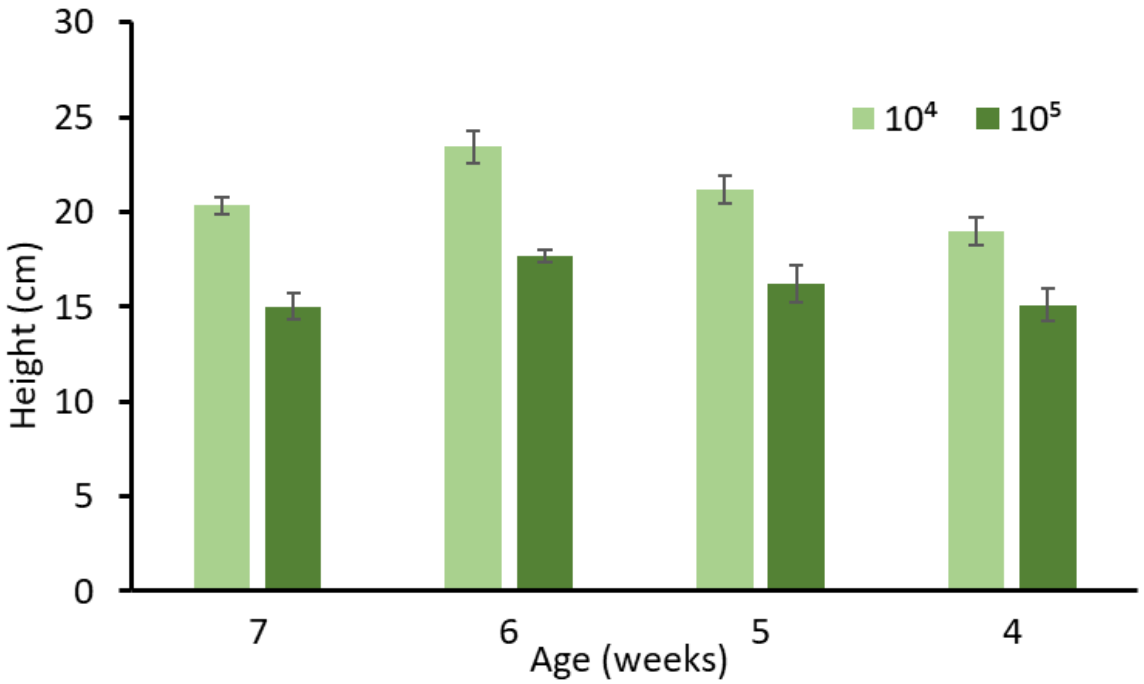

Fig. 2. Height of plants inoculated with different *P. brassicae* conidial concentrations (10^4 or 10^5 spores/ml) at 23 days post-inoculation (dpi). Error bars are standard error of the means (DF = 3).

Fig. 3. Height of plant (aged 6 weeks at time of inoculation) that received 10^4 spores/ml (1) or 10^5 spores/ml (2) of *P. brassicae* inoculum, assessed at 23 dpi.

Discussion

Greater fungal inoculum concentration (10^5 spores/ml) produced a greater disease severity and reduced height of plants, suggesting a possible correlation between inoculum concentration and plant growth. Observations of plant growth stunting on light leaf spot-infected plants have been reported previously in field trials (Karandeni Dewage *et al*., 2018), with no prior reports in cabinet-grown plants. These results will be investigated further in future experiments.

More than 20 *P. brassicae* field isolates have been collected from oilseed rape and kale cultivars across England and will be further screened for virulence.

Acknowledgements

We thank the funders, the Hertfordshire Knowledge Exchange Partnership (HKEP), the University of Hertfordshire and the Perry Foundation for their financial contributions to this work. We equally thank the industrial partner RSK ADAS for their in-kind contributions and for hosting the associate Laura Sapelli for an invaluable one-year industrial placement.

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Genetic basis of partial resistance against P*yrenopeziza brassicae* in oilseed rape

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Introduction

Light leaf spot (LLS) caused by *Pyrenopeziza brassicae* is the most damaging disease of oilseed rape (*Brassica napus*) in the UK. The disease accounts for up to £160M yield loss annually in England, despite expenditure of £20M on fungicides, and the severity of the disease is much greater in Scotland (Karandeni Dewage *et al*., 2018; Ashby, 1997). In the UK, the disease has been increasing as a national problem in recent decades, rather than just being confined to Scotland and northern England.

LLS is currently controlled by a combination of cultivar resistance, fungicide applications and cultural practices. However, resistance mechanisms of the oilseed rape plant against *P. brassicae* are not well understood. Furthermore, fungicide control is problematic as the pathogen has developed insensitivity to triazole and MBC fungicides (Carter *et al*., 2013; Carter *et al*., 2014). Thus, it is necessary to identify the genes involved in quantitative resistance of *Brassica napus* to design an improved and durable control strategy against LLS. The aim of this project is to better understand (i) the genes involved in quantitative resistance against *P. brassicae* in oilseed rape and (ii) the contribution of the host wax/cuticle to this host-pathogen interaction.

Materials and methods

A phenotypic screen for LLS susceptibility was done in glasshouse experiments using 195 accessions of *B. napus*. In collaboration with the John Innes Centre, this screen was used together with an associative transcriptomics pipeline (http://www.yorknowledgebase.info) to identify gene expression markers (GEMs). Experiments will be done using TILLING mutants to confirm the involvement of specific GEMs in quantitative resistance.

Trypan blue staining and scanning electron microscopy (SEM) were used to monitor the pathogen during the infection process and to determine time points for gene expression analysis. Wax and cutin were quantified and the components were compared to assess the role of these components in host resistance or susceptibility. *B. rapa* wax mutants and resistant and/or susceptible *B. napus* accessions were used for this purpose. Furthermore, toluidine blue staining was used to compare these accessions/mutants for cuticle permeability and host resistance. Cutinase expression will be analysed to determine whether wax/cutin components can affect the expression of this gene implicated in pathogenicity.

Results

Eight GEMs were expressed differently in accessions susceptible or resistant to *P. brassicae*; among these genes were cinnamate 4-hydroxylase, beta-adaptin and KIN10, a SNF1 kinase homolog. Genotyping and phenotyping of TILLING mutants which have been obtained (J130317a, J130819a, JI30010-b, J1320204a and JI31647b) will establish the role of these genes in resistance against *P. brassicae*. SEM showed that the pathogen germinates 1-day post-inoculation (dpi) on leaf surfaces, penetrates the cuticle by 2 dpi and colonises the subcuticular layer by 8 dpi.

Discussion

Wax and cutin analysis of wax mutants, together with cutinase expression in these mutant and wild-type plants, will provide host data corresponding to the role of cutinase in pathogenicity. Li *et al*., (2003) have shown that a cutinase-deficient mutant of *P. brassicae* was unable to penetrate the cuticle and asexually propagate on oilseed rape. Together with the GEM data, this will help crop breeders to develop oilseed rape with better protection against *P. brassicae*.

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Biocontrol of *Sclerotinia sclerotiorum* and *Leptosphaeria biglobosa* on oilseed rape with formulated bacterial powder of *Bacillus velezensis* CanL-30

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Strain CanL-30 of *Bacillus velezensis* is an endophytic bacterium isolated from a healthy plant of oilseed rape (*Brassica napus*). It is an antagonist of *Sclerotinia sclerotiorum* and *Leptosphaeria biglobosa*, the causal agents of sclerotinia stem rot and phoma stem canker (blackleg) of oilseed rape, respectively, in China (**Figs. 1, 2**). *B. velezensis* CanL-30 also promotes growth of *Arabidopsis thaliana* and *B. napus* through production of volatile organic compounds, suggesting that strain CanL-30 is a multifunctional biocontrol agent. In this study, *B. velezensis* CanL-30 was incubated in 10-L, 1-ton and 10-ton jar fermenters for characterization of bacterial growth and endospore development. A water wettable powder was prepared based on the endospores in the cultures, and the formulated bacterial powder was evaluated for its efficacy in controlling sclerotinia stem rot and blackleg of oilseed rape. CanL-30 multiplied and formed endospores in the fermenters. After incubation at 30°C for 48 h in the 10-L fermenter, for 36 h in the 1-ton fermenter and for 40 h in the 10-ton fermenter, the percentages of endospore-forming bacteria were 74%, 93% and 92%, respectively. In the 1- and 10-ton fermenters, the final endospore yields reached 1×10^{10} cfu/mL and 3.3×10^{9} cfu/mL, respectively. The resulting bacterial cultures were spray-dried, and a water wettable bacterial powder (1×10^{11} cfu/g) was prepared by amendment of the bacterial powder with additives. The formulated powder conforms to the national standard for wettable powder pesticides regarding the thermal stability (54°C), suspension and wettability.

The results from the bioactivity test on agar medium showed that the powder had antifungal activity against both mycelial growth and sclerotial formation by *S. sclerotiorum* (Fig. 3), and against conidial germination of *L. biglobosa*. The laboratory biocontrol assays on leaves of oilseed rape showed that the powder effectively suppressed infection by *S. sclerotiorum* and *L. biglobosa*. The field biocontrol assay showed that the bacterial powder suppressed severity of sclerotinia stem rot and blackleg by 55% and 42%, respectively, and the efficacy values were not significantly different ($P > 0.05$) from those in the treatment of the fungicide prochloraz (562.5 μg a.i./mL), which suppressed sclerotinia stem rot and phoma stem canker (blackleg) by 51% and 37%, respectively. The yield of seed of oilseed rape was increased by 13% compared to that in the control treatment. Formulation of the bacterial powder of strain CanL-30 provides a biocontrol method for controlling SSR and phoma stem canker of oilseed rape.

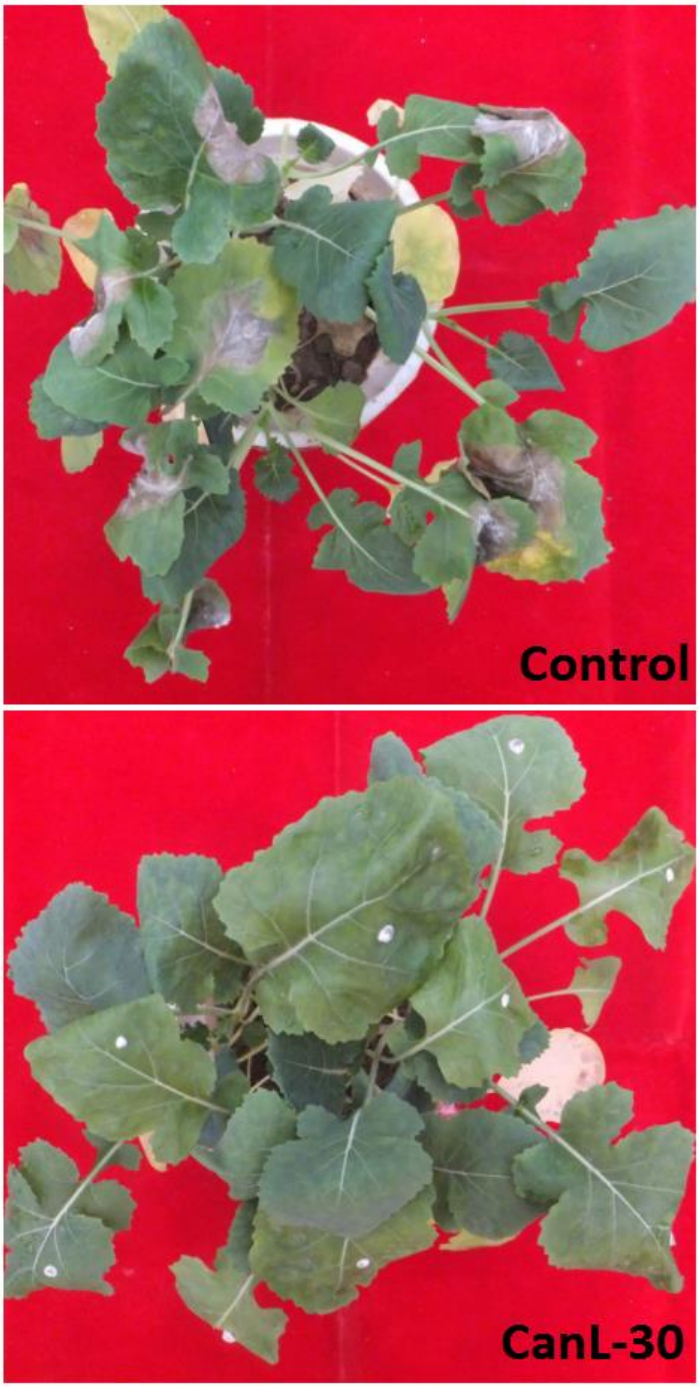

Fig. 1. Suppression of infection by *S. sclerotiorum* on leaves of oilseed rape (7 dpi)

In the control and CanL-30 treatments, leaves were sprayed with water alone and the PDB culture of CanL-30 respectively, and then the mycelial agar plugs of *S. sclerotiorum* were inoculated on the leaves. The plants were maintained under humid conditions.

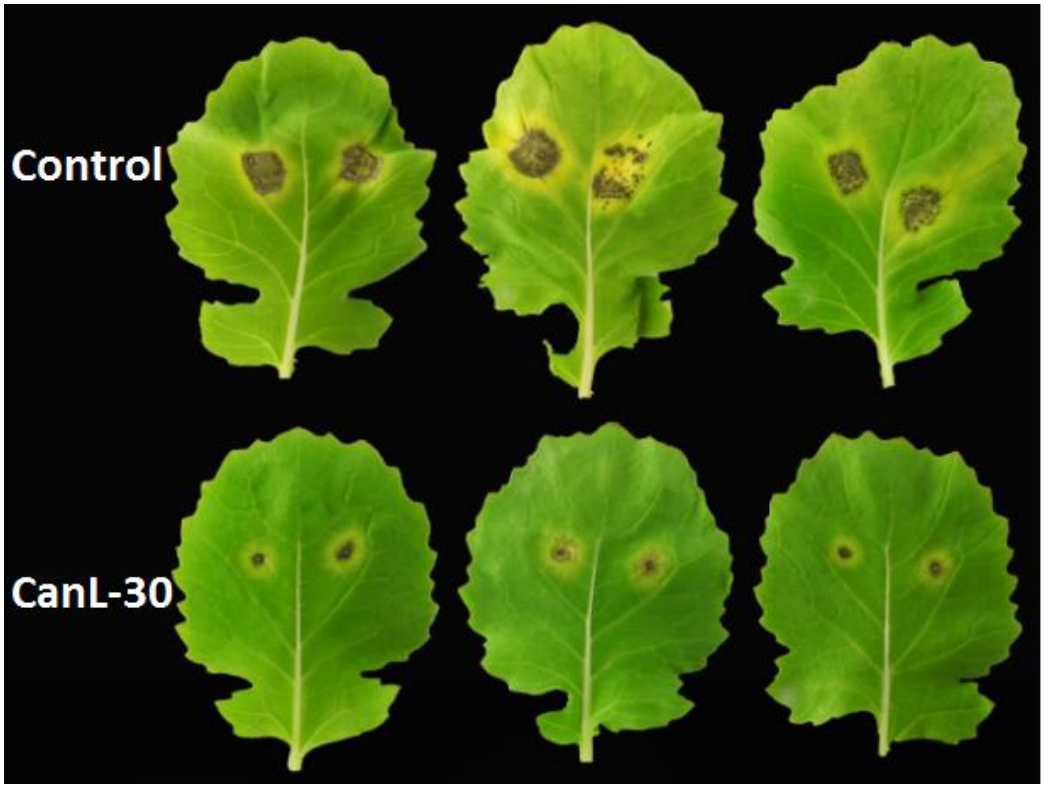

Fig. 2. Suppression of colonisation *L. biglobosa* on leaves of oilseed rape (7 dpi)

In the control and CanL-30 treatments, wounded leaves were sprayed with water alone or with the PDB culture of CanL-30, respectively, and conidia of *L. biglobosa* were inoculated on the wounded areas. The treated plants were maintained under the humid conditions.

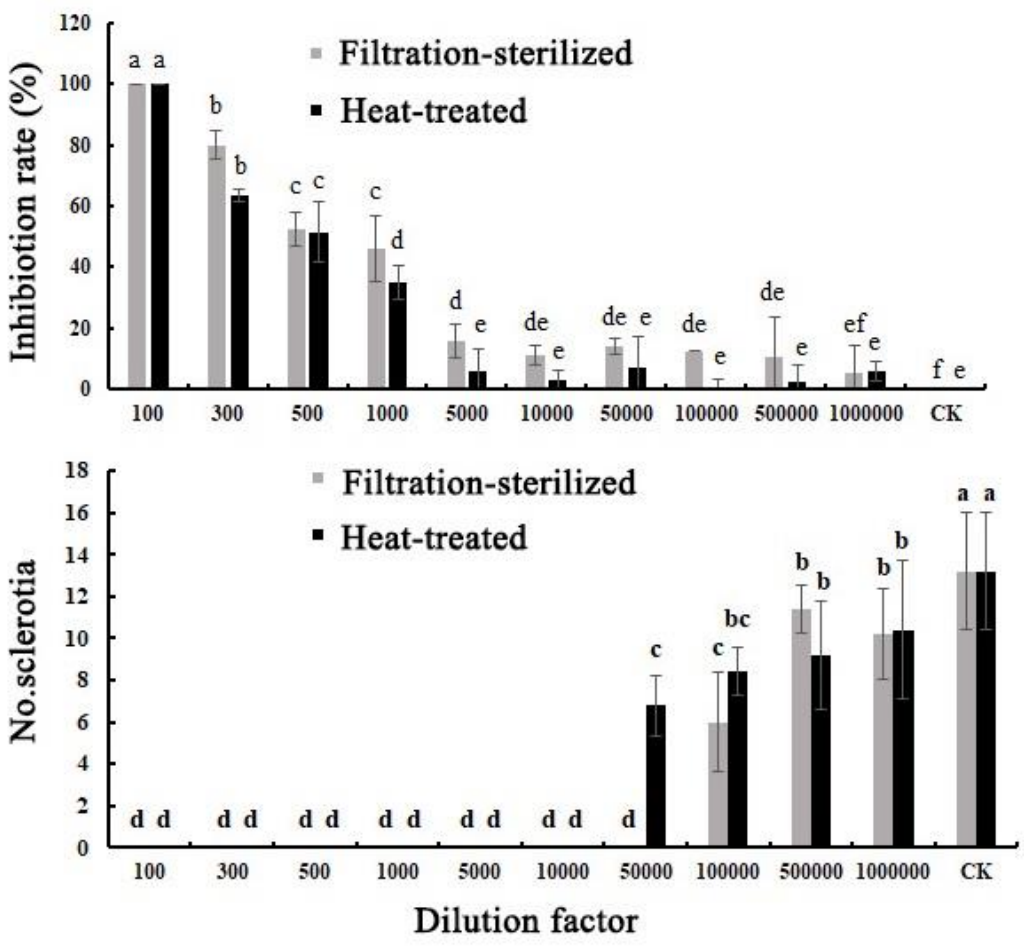

Fig. 3. Suppression of mycelial growth and sclerotial formation by *S. sclerotiorum* on potato dextrose agar

The bacterial powder of CanL-30 (1×10^{11} cfu/g) was dissolved in sterilised water, the suspension was centrifuged, the resulting supernatant was divided into two parts, one part was filter-sterilised and the other part was heat-sterilised (100°C, 30 min). The treated solutions were amended in PDA, and water was added to PDA as a control. Mycelial agar plugs of *S. sclerotiorum* were inoculated on PDA, the cultures were incubated at 20°C for 2 days and colony diameter was measured for calculation of growth inhibition rates. The cultures were further incubated for 30 days, and sclerotia formed in each culture were counted. Bars with the same letters indicated no significant differences ($P > 0.05$) according to the least significant difference test.

Potential of biopesticides and optimising the use of conventional insecticides for the control of cabbage stem flea beetle (*Psylliodes chrysocephala*)

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Since the ban by the European Union in 2013 of neonicotinoid insecticides in flowering crops, the control of the cabbage stem flea beetle in oilseed rape crops has been reliant on pyrethroid insecticides. However, populations of cabbage stem flea beetle are now largely resistant to this class of insecticides and pyrethroids are known to be harmful to naturally occurring predators and parasitoids that may help to reduce cabbage stem flea beetle populations. Alternatives are then needed to effectively control the cabbage stem flea beetle in oilseed rape crops. The aim of the current research is to identify alternatives to conventional synthetic insecticides, including the use of microorganisms such as entomopathogenic fungi and bacteria, as well as nematodes, plant extracts and physically acting products.

Several assays have been completed. The entomopathogenic bacteria *Bacillus thuringiensis* subsp. *tenebrionis*, has shown to be effective against other species of Coleoptera. Three formulations that are not commercialised yet, CEU-40770-I-WG, CEU-40780-I-WG and INBS32, were tested at field rates, each replicated 6 times, plus a control. Oilseed rape leaf discs were dipped in the solutions then offered as food to cabbage stem flea beetle adults, in batches of 10 individuals. Cumulative mortality was recorded every day until 12 days post inoculation. No more than 40% mortality was found for all formulations, so the decision was made not to test bacteria in crops in September.

A further bioassay was completed, using four nematode species: *Steinernema feltiae*, *Steinernema carpocapsae*, *Steinernema kraussei* and *Heterorhabditis bacteriophora*, at three concentrations: 4,000, 10,000 and 40,000 nematodes/ml of water. Each concentration was replicated three times, in batches of 10 adult cabbage stem flea beetles. Cumulative mortality of the cabbage stem flea beetles was assessed every two days until 6 days after they were treated with the nematodes. The results were quite variable, particularly when the smaller doses of nematodes were used. However, it is clear from these results that the nematode species *Heterorhabditis bacteriophora* was the most promising species tested, with almost 75% of the cabbage stem flea beetles dead 6 days after treatment at the smallest dose tested. The nematode species *Steinernema feltiae* also performed well at the highest dose, with c. 85% mortality recorded only 2 days post treatment. *Steinernema carpocapsae* performed well at smaller nematode doses (even at the smallest dose with almost 65% mortality) but was slower to kill the beetles. *Steinernema kraussei* showed a high variability among replicates and was the least effective of the nematode species tested, killing fewer than 75% of the beetles at any nematode dose.

A laboratory bioassay was completed to test the physically acting products FLiPPER (Bayer), which is a fatty acids-based product currently used in horticultural crops against whiteflies, aphids and mites, and another physically acting product, coded CEU-40640-I-SL and currently

being developed by Certis Europe B.V.,. These products were tested at three doses: half the field rate, field rate and double the field rate, each replicated three times on batches of 10 adult cabbages stem flea beetles. The solutions were sprayed directly on the cabbage stem flea beetles and the oilseed rape leaf that served as support and food. The cumulative mortality of the beetles was recorded 1, 2 and 5 days after treatment application. Both products showed encouraging results in this laboratory bioassay, with all doses significantly different from the water control, for which no mortality was recorded. FLiPPER was very effective, with more than 85% mortality recorded just one day after application at the field rate of 10ml/l of water. It is worth noting that these products act very fast, with no significant differences between cumulative mortality at 1, 2 or 5 days and maximum effects then reached within 24 hours. The cuticle of dead beetles was observed with a scanning electron microscope after treatment with FLiPPER, and it appears, compared to the control, that FLiPPER had disrupted the integrity of the cuticle.

Future bioassays in summer 2021 will involve commercial formulations of the fungi *Metarhizium brunneum (anisopliae)* and *Beauveria bassiana.* Some new formulations of physically acting products will also be tested, as well as a conventional pyrethroid insecticide (Hallmark Zeon) as positive control. In September, I am planning to test the biopesticides that I found promising in the lab in a field, so that it reflects as much as possible the real cropping conditions.

Acknowledgements

This project is funded jointly by the Agriculture and Horticulture Development Board, Certis Europe, and five charities which are members of the AgriFood Charities Partnership: Chadacre Agricultural Trust, Clan Trust, Felix Thornley Cobbold Agricultural Trust, Perry Foundation, and the Morley Agricultural Trust.

Comparative genomics and modelling of insect pests for understanding of their host plant selections in the UK

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Summary

Withdrawal of neonicotinoid insecticides and acquisition of pesticide insensitivity among insect pests both pose new challenges for combating invasive insect species, including cabbage stem flea beetle (*Psylliodes chrysocephala* L.) and spotted wing drosophila (*Drosophila suzukii* L.), which feed on oilseed rape (*Brassica napus* L.) and small fruit, respectively. In this paper, we outline our plan to study nine insect species from three orders based on their feeding preferences. Laboratory and analytical methods, like comparative genome and transcriptome studies, will reveal more gene functions, illuminate evolutionary mechanisms, and lay the foundation for pest dynamics modelling and new Integrated Pest Management approaches with agricultural applications.

Introduction

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* (L.) (Coleoptera: Chrysomelidae), is an increasing winter oilseed rape crop pest throughout the UK and north to central Europe. Because of the CSFB problem, English oilseed rape production in 2020 was at a 21-year low, down 39% from 2019. The cultivated area of oilseed rape decreased by 30%. (Defra, 2020)

In East Anglia, where the CSFB problem first emerged (Bromand, 1999), the Norfolk trials site recorded a rapid increase in the CSFB population in 2020. In previous years, Suffolk reported losing 5% of its crop, with 5% being re-drilled due to CSFB (Eastern Daily Press, 2020)

Since 2018, the EU has banned the three main neonicotinoid insecticides (clothianidin, imidacloprid and thiamethoxam) for all uses outdoors. Without effective chemical protection approaches, the CSFB is causing significant economic costs for farmers.

Integrated Pest Management (IPM) has attracted considerable research attention. The concept of IPM was developed in the late 1950s and was widely practised in the 1970s and 1980s (Ehler, 2006). It is a system which has been developed to control pest populations and maintain them under the economic threshold without applying disruptive chemical pesticides (Stern *et al*., 1959). In the EU, all farmers are obliged to apply IPM, as specified in Annex III of the Directive 2009/128/CE.

We now have a better understanding of CSFB, but its host-plant interaction has not been thoroughly studied. Insect olfaction plays an important role in IPM because it can attract parasitoids to the pest and distract or repel insect pests.

A new invasive pest, spotted wing drosophila (SWD, *Drosophila suzukii*), first detected in 2008 in North America, has emerged as a major problem for UK agriculture since 2012. The SWD fly has infested crops of more than sixty kinds of commercial fruits (e.g. small fruits including strawberries, cherries) (Stewart *et al*., 2014).

A significant impediment to dealing with insect pests is the limited amount of information we have about these species. The genome data for Diptera (flies) has received much research interest but much less is known about Coleoptera (beetles). The vast amount of information and resources available on drosophilids makes these flies an excellent model system to explore the evolutionary ecology of behavioural diversification related to host choice. If we understand host specialisation, IPM strategies can be improved.

Climate change impacts agricultural ecosystems and pest epidemics (Stern, 2007), including major changes in herbivory rates, altered distributions and frequency of outbreaks of key insect pests. Relationships with natural enemies are altered unpredictably and a general decrease in biodiversity has occurred (Williams and Lieb-hold, 1995; Fleming, 1996; Coley, 1998). Focusing on the pests SWD and CSFB, with existing qualitative and empirical models, we now have an opportunity to link existing knowledge, phenological data, and trait information for the pest/host to enable climate model development.

Research Objectives

For this PhD project, we have three main objectives:

1. To use comparative genomics to identify herbivore-specific traits and differentiate specialist from generalist herbivores (Table 1).

2. To analyse herbivory-related pathways, including detoxification of phytochemicals and olfaction.

3. To model SWD and CSFB pest dynamics to estimate during which period of the year crop (host) protection is critical, and guide the optimal timing for application of pesticides and IPM strategies (e.g. pheromone traps)

Table 1. *Availability of genome data for understanding feed preferences*

Order	Family	Species	Feeding preference	Host plants	Genome sequence
Diptera	Drosophilidae	*Scaptomyza flava*	Specialist herbivore	*Brassicaceae*	Yes
Diptera	Drosophilidae	*Drosophila suzukii*	Generalist pest	Fruit crops	Yes
Diptera	Drosophilidae	*Delia platura*	Generalist herbivore	Peas, beans and maize	Yes
Diptera	Culicidae	*Anopheles gambiae*	Blood	None	Yes
Lepidoptera	Plutellidae	*Plutella xylostella*	Specialist herbivore	*Brassicaceae*	Yes
Lepidoptera	Noctuidae	*Spodoptera exigua*	Polyphagous herbivore	Polyphagous*	Yes
Coleoptera	Chrysomelidae	*Phyllotreta striolata*	Specialist herbivore	*Brassicaceae*	Transcriptome
Coleoptera	Chrysomelidae	*Psylloides chrysocephala*	Specialist herbivore	*Brassicaceae*	This study
Coleoptera	Chrysomelidae	*Trilobium castaneum*	Generalist herbivore	Cereals, dry fruits, pulses	Yes

* Feeds on more than 130 host plants from more than 30 families

Implications and contributions to knowledge

- By employing novel genome data and research methodologies (e.g. machine learning), we expect the proposed work will lead to a better understanding of pest dynamics.
- This work will improve knowledge about herbivory-related pathways, e.g. detoxification of phytochemicals and olfaction, and host plant choice of insect pests.
- The results from this PhD project are expected to enable functional characterisation of key genes that are important for host selection by insects. We can also gain information about pest risks during the host growing season and treatment timing as well as the

potential impacts of climate change on them. These results will benefit future research in these areas.

- With support from industry partners like Limagrain and LS Plant Breeding, the theoretical research findings of this project will deliver practical outcomes to breeders and growers and formulate a potential solution to the imminent pest problem in the UK.
- This project will be presented at national and international conferences and results published in relevant high-impact journals to disseminate our findings and benefit further research in this field. Dissemination will also occur through the University's press office and UK arable events like Cereals.

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Parasitism of adult cabbage stem flea beetle *(Psylliodes chrysocephala*) by *Microctonus brassicae* (Hymenoptera: Braconidae) in the UK: incidence and distribution

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The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* (L.) (Coleoptera: Chrysomelidae), is one of the major pests of oilseed rape (OSR) in Europe. CSFB adults and larvae feed on the plant during autumn and winter, threatening crop establishment and affecting plant vigour; often resulting in plant death, reductions in yield or, if the pest numbers are large, total crop failure. This pest has historically been controlled using insecticides, relying almost exclusively on neonicotinoid seed dressings and pyrethroid sprays. However, since the ban on the use of neonicotinoids in 2013, pyrethroids have become the only method available for growers to control CSFB. This reliance on one control method has led to increasing incidence of resistance to this insecticide since it was first reported in 2015. In this scenario, growing OSR has become challenging, leaving growers facing complete yield loses with no reliable control methods. Such instances have highlighted the need for alternative management practices and pest control methods to reduce the use of insecticides.

Recently, a parasitoid reared from the adult stage of CSFB has been identified as *Microctonus brassicae* (Haeselbarth) (Hymenoptera: Braconidae). Studies on its life cycle have revealed that the larvae of this parasitic wasp develop inside the bodies of the CSFB adults, killing them when exiting their bodies. However, no information on the occurrence, importance and efficiency of this parasitoid in the biocontrol of CSFB is available due to sampling difficulties and inadequate knowledge on rearing and identification methods. Therefore, the aim of this project is to assess the potential for biocontrol of CSFB by the parasitic wasp *Microctonus brassicae* through: 1) exploring the extent and geographical spread of this parasitoid, 2) assessing the factors affecting *M. brassicae* distribution/parasitism, and 3) investigating the effect of *M. brassicae* on host survival, oviposition and feeding.

Companion plants to reduce cabbage stem flea beetle attack on oilseed rape

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Cabbage stem flea beetles (*Psylliodes chrysocephala*) cause serious damage to oilseed rape plants in the UK by eating leaves of young plants in autumn. Since the ban on neonicotinoid seed treatments, the options available to control this pest are limited and new strategies need to be developed to reduce the impact of this insect. Companion cropping, a technique involving the growing of one or more plant species with the crop, could be an interesting way to control the pest. This approach has been shown to be an efficient way of increasing diverse ecosystem services and reducing pest damage in the crop. Growing oilseed rape with other species, especially frost sensitive legumes, is used in different countries as an effective way of controlling cabbage stem flea beetle. However, this technique is only practical if the companion crop is destroyed by frost during the winter, which is not reliably the case in the UK. Consequently, if this approach was to be implemented in the UK, non-winter hardy companion plants need to be identified. Different plant species have been reported to have been used by farmers as companion crops of oilseed rape in the UK, but their effectiveness in controlling cabbage stem flea beetle still needs to be proved.

Over three seasons, we tested the effect of different companion crops on cabbage stem flea beetle feeding attacks. These experiments were replicated in the UK and in Germany. The percentage leaf area attacked by cabbage stem flea beetles and the oilseed rape plant number and the number of larvae per plant were recorded during these experiments. Different companion plant species were identified as interesting options for reducing adult CSFB attacks. The best agronomic practices now need to be investigated to make this approach suitable for farmers.

New sources of resistance to turnip yellows virus from *Brassica oleracea* and *Brassica rapa* and their introgression into oilseed rape

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Summary

Turnip yellows virus (TuYV) causes disease in a broad range of *Brassica* crops. In the UK, TuYV can cause yield losses of up to 30% in oilseed rape (OSR) and incidences as high as 100% have been recorded in the crop. There are currently only two sources of resistance to TuYV in commercial varieties of OSR; both are quantitative. All current TuYV-resistant OSR varieties available in Europe possess the same source of quantitative resistance. Therefore, it was essential to identify new robust sources of resistance in order to manage resistance to the virus in OSR. We have identified three new sources of quantitative resistance in *B. oleracea*, two in *B. rapa* and created two resynthesised '*B. napus*' lines, each containing the new sources of resistance in both A and C genomes. Broad spectrum resistance to different TuYV clades is provided by the resistance sources in both A and C genomes. Further work is in progress to fine map the sources of the resistances, and develop breeder-friendly markers for marker-assisted selection and identify the underlying gene/s.

Key words: *Brassica napus*, turnip yellows virus, viral resistance, plant-virus interactions, oilseed rape

Introduction

Turnip yellows virus (TuYV), formally known as beet western yellows virus (BWYV), is widely spread and causes diseases in a broad range of *Brassica* crops across Europe. We have reported incidences of 8-89% (1998), 2-24% (2009) and 5-100% (2010) in different regions of England (Asare-Bediako *et al*., 2019). It also infects a wide range of weed species. Infected plants can be symptomless, or show symptoms indistinct from those caused by other stresses such as nutrient deficiency, etc. The virus is transmitted in a persistent manner by aphids; the major vector in Europe is *Myzus persicae*, with up to 70% of aphids carrying the virus. Losses of yield in OSR due to TuYV in the UK are reported to be as much as 30%, costing the industry more than £67 million a year, which equates to 9% of the total crop value (Nicholls, 2013). Losses of up to 46% of yield have been reported in other parts of the world.

There are currently only two known sources of resistance to TuYV in OSR, from the resynthesised line 'R54' (Graichen, 1994) and in the Korean Spring variety Yudal (Hackenberg *et al.,* 2020); both sources are quantitative rather than qualitative. However, all of the current TuYV-resistant OSR varieties in Europe have been developed from the resistance originating in line 'R54'. This has resulted in considerable selection on the virus to render the resistance ineffective. Therefore, it was essential to identify new, robust sources of resistance to TuYV and develop molecular markers associated with the resistances for use by breeders.

Phylogenetic analyses of TuYV isolates

Whole genomes of 179 TuYV isolates from 13 regions in the UK and five mainland European countries have been sequenced and phylogenetic analyses done (Newbert, 2016). A measure of viral diversity is important in order to assess the spectrum and potential durability of resistance to TuYV.

Identifying and utilising new sources of resistance

New sources of quantitative resistance to TuYV have been identified in both *B. oleracea* and *B. rapa*. Quantitative trait loci (QTL) have been mapped in BC_1 populations for all new sources of resistance. Additionally, two new resynthesised '*B. napus*' lines have been generated through interspecific hybridisations between the TuYV-resistant *B. rapa* and *B. oleracea* lines. Each resynthesised '*B. napus*' line contains the new sources of resistance in both A (*B. rapa*) and C (*B. oleracea*) genomes (Table 1).

The strategy employed to identify one of the new sources of TuYV resistance in *B. rapa* was to screen 14 *B. rapa* subspecies for resistance following challenge with the virus and subsequent triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA). Low absorbance values indicate that the plant has low viral titre and hence resistance. From the initial screen, it was observed that one *B. rapa* accession showed a high level of resistance to TuYV isolates from two TuYV clades. In order to map the source of resistance in this accession, a segregating BC_1 plant population was developed following a cross between a TuYV-resistant plant and a TuYV-susceptible plant. Plants segregating for resistance were genotyped on the Illumina Infinium 60K SNP array. The results enabled the identification of a major QTL. A further cross was made to create another segregating population that was phenotyped and genotyped. TuYV-resistant plants will be selfed to produce more segregating populations for further genotyping. In this way, we will develop molecular markers tightly linked to the source of TuYV resistance in *B. rapa* and the resynthesised '*B. napus'* and create pre-breeding material that can be utilised in the generation of TuYV-resistant elite cultivars.

A similar strategy was employed to map one of the TuYV resistance sources in *B. oleracea*, where a TuYV-resistant accession was crossed with a TuYV-susceptible accession to create a segregating BC_1 population. A single QTL was identified. A similar approach to that described for *B. rapa* is being pursued to develop molecular markers tightly linked to the TuYV resistance.

In order to utilise the novel sources of TuYV resistance in OSR, an interspecific cross of a TuYV-resistant *B. rapa* plant and a TuYV-resistant *B. oleracea* plant was made. Embryo rescue was necessary to produce viable progeny and, once developed, colchicine treatment and subsequent ploidy testing identified 11 of the 44 hybrids as being allotetraploid, *i.e.* AACC. Resistance to TuYV was inherited by the progeny of the interspecific cross. Crosses with OSR Westar were also made to produce resistant F_1 progeny.

Table 1. *New sources of resistance to TuYV in Brassica oleracea, Brassica rapa and resynthesised 'Brassica napus'*

Species	**Spectrum of TuYV resistance**
B. oleracea	Isolate from major clade
B. oleracea	Broad
B. oleracea	Isolate from major clade
B. rapa	Broad
B. rapa	Isolate from major clade
Resynthesised '*B. napus*'	Broad
Resynthesised '*B. napus*'	Isolate from major clade

Discussion

Most European OSR varieties contain a single source of TuYV resistance from the resynthesised '*B. napus*' line R54 (Graichen, 1994). Considering the incidence of TuYV in some crops can be as high as 100% (Asare-Bediako *et al.*, 2019), there is considerable selection for resistance-breaking strains of TuYV. We have identified a total of five new sources of resistance to TuYV, three in *B. oleracea* and two in *B. rapa*, which provide a broad spectrum of resistance to TuYV isolates from different phylogenetic clades. Additionally, we have resynthesised two new '*B. napus*' lines, each containing a source of resistance on both the A and C genomes. F_1 progeny of a cross between the resynthesised lines and OSR Westar were resistant, providing a way for introgression into elite cultivars. Utilising multiple sources of TuYV resistance reduces the likelihood of a breakdown in the resistances, enhancing the durability of resistances in both vegetable and oilseed *Brassica* crops. Incorporation of these new resistance sources into elite cultivars should facilitate increases in yield. The identity of the gene/s responsible for resistance in line 'R54' remains unclear and it may prove challenging to identify the gene/s responsible for the new sources of resistance we have identified. Once the gene/s are identified, the plant-virus protein interactions can be studied and could enable rapid identification of additional sources of TuYV resistance. Additionally, this could provide the possibility of engineering such resistance, enabling the further enhancement of the durability of resistance to TuYV in *Brassica* crops for the foreseeable future.

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Studies in *Brassica napus* (oilseed rape) and *Arabidopsis thaliana* to identify immune responses to *Rhizoctonia solani* AG2-1

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Rhizoctonia solani is a soil-borne, necrotrophic plant pathogen with a wide host range across economically important crops, including oilseed rape (OSR). *R. solani* forms distinctive Anastomosis Groups (AGs), of which AG2-1 is highly aggressive towards OSR seedlings, causing symptoms such as root and hypocotyl rot and damping-off. Control of *R. solani* is challenging, as resistant varieties have not yet been developed, so chemical and cultural controls are essential to disease management. Crop rotations and tillage are known to affect the severity of disease, and fungicidal seed treatments are used to protect the seedlings as they emerge and establish. We hypothesised that although resistance to *R. solani* has not been identified, there will be variation in the disease phenotypes of commercially available varieties of OSR. These phenotypes were explored by measuring the symptoms and total root lengths of seedlings under inoculation. The speed at which *R. solani* sclerotia germinated and formed infection plaques and cushions on different varieties was observed. To understand the variation in responses of OSR varieties with different phenotypes, differential gene expression analysis was used to measure the changes within key defence response pathways. To support this, functional analysis has taken place in *Arabidopsis thaliana* mutants, considering how the absence of specific gene activity affects the ability of plants to grow under inoculation. Previous studies have shown that some *R. solani* AGs produce phenylacetic acid (PAA), which is an auxin. The response of auxin pathways under inoculation will be investigated using $_{pro}$IAA2:GUS lines in *A. thaliana*. Our results will inform future research into *R. solani* resistance and will aid in the development of resistant OSR varieties for growers.

Acknowledgements

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ScleroScreen–A global field inoculation methodology to assess sclerotinia stem rot resistance in oilseed rape (*Brassica napus*)

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Summary

Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* is one of the most devastating diseases of oilseed rape (*Brassica napus*), leading to systematic use of fungicides. Resistant varieties would be an alternative to fungicides which are often badly timed and costly for growers and for the environment. Different mechanisms are involved in the interaction between *S. sclerotiorum* and *B. napus*, making the choice of an evaluation methodology complex. Here, we report on ScleroScreen, a method to evaluate varieties in the field for resistance to *S. sclerotiorum* . The method encompasses the production of viable inoculum to spread easily and homogeneously on microplots during field inoculation and scoring to assess resistance. Results from field trials at three locations over two years were promising, with clear symptoms to score and good repeatability of genotype ranking, regardless of environmental conditions, and consistency with results from naturally inoculated trials.

Key words: Sclerotinia stem rot, oilseed rape, varietal field evaluation, plant resistance

Introduction

Although infrequent, sclerotinia stem rot results in up to 1.5t/ha yield loss (Koch *et al*., 2007; Penaud *et al*., 2009), which leads to the systematic use of fungicide treatments. Resistant varieties represent an alternative to fungicide treatments, which are often badly timed and costly for growers and for the environment. The efficiency of fungicides decreases regularly due to resistance in pathogen populations. Different mechanisms are involved in the interaction between *Sclerotinia sclerotiorum* and oilseed rape, making the choice of an evaluation methodology very complex. Moreover, disease development is very dependent on environmental conditions, such as temperature and humidity. Managing these constraints is critical for successful infection and thus variety evaluation. Here we report on ScleroScreen, a method for evaluating varieties in the field for resistance to *Sclerotinia sclerotiorum.*

Materials and methods

To ensure homogeneous and easy inoculation, the fungus was grown and kept as live mycelium on sunflower seeds. For this, batches of 500g of sunflower seeds were cleaned with water then autoclaved. Seeds were the inoculated with plugs of mycelium and incubated at 21°C for 3 weeks. The growth of mycelium was checked regularly. The inoculum was then

dried for 24h before being spread on the oilseed rape plots. To avoid environmental effects, the plots were inoculated twice, once when the plants were between BBCH growth stages 57 and 60, and then when the plots were between stages 61 and 65 , using the plant growth stages of Lancashire *et al.* (1991).

The method was used to screen 20 contrasting winter oilseed rape genotypes at three locations in France (Verneuil, Blois and Aussonne) over two growing seasons (2017-18 and 2018-19). Trials were organized in randomized complete blocks with two treatments (Inoculated (I) and Non-Inoculated (NI)) and three replicates per treatment. Symptoms were scored as the percentage of plants affected with stem rot lesions. Scoring was done twice at 80 and 120 degree-days after the second inoculation (LEC1 and LEC2, respectively) to assess disease progression.

Results

In all six experiments, no disease was observed in the NI treatment plots, suggesting no occurrence of natural infection that could have biased results in the inoculated treatments. However, symptoms were frequently observed in the inoculated treatments, with between 5 and 90% of plants presenting symptoms on the first scoring date LEC1, (Fig. 1). Moreover, the disease clearly progressed between the two scoring dates, regardless of the location of the trial.

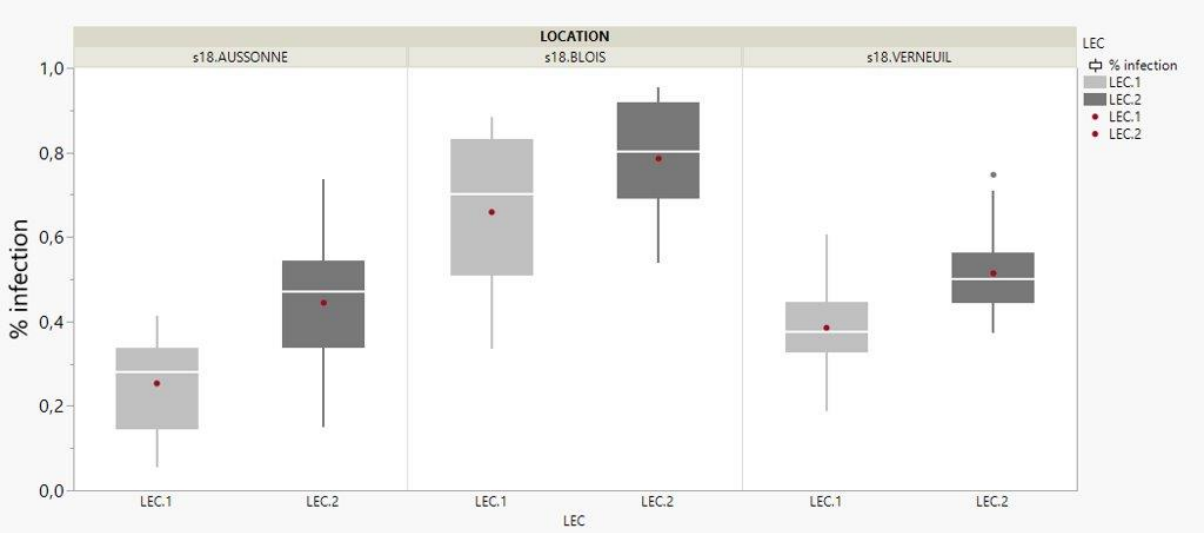

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Fig. 1. Percentage of plants with sclerotinia stem rot observed at the three locations in 2017-18 at LEC1 and LEC2

ScleroScreen provided a good indication of overall resistance to the pathogen. Indeed, amongst the 20 genotypes tested in our experiments, a subset had also been tested under natural inoculation conditions in the FSRSO « Sclerotest » project between 2011 and 2014, discriminating generally resistant varieties from generally susceptible varieties. For all experiments considered, the discrimination was consistent, either for individual or multiple experiments (Fig. 2).

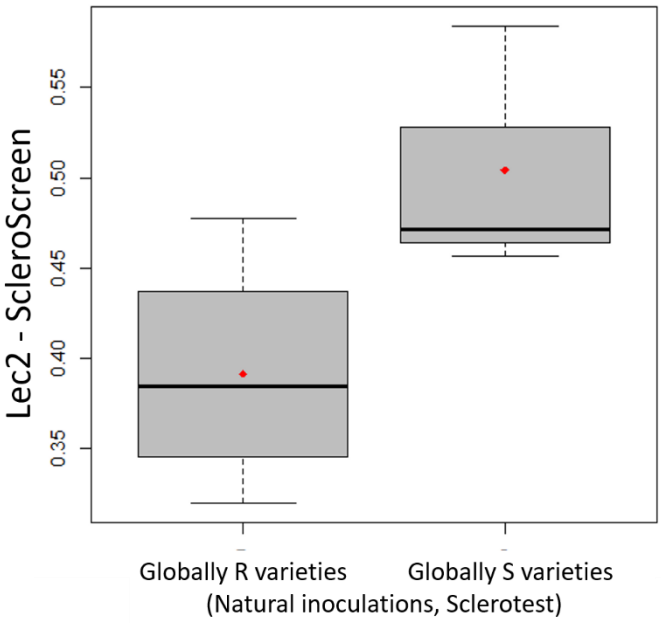

Fig. 2. Consistency in classification following natural inoculation and ScleroScreen. Resistant (R) and susceptible (S) classes were derived from natural inoculation experiments and boxplots represent the % of diseased plants in LEC2 of inoculated treatments based on a multi-year, multi-location model.

Conclusion

The ScleroScreen method allowed us to repeatably and homogeneously inoculate field trials at six locations in France, spread over two different growing seasons with minimal additional technological development. ScleroScreen generated reproducible results over locations and seasons, regardless of environmental conditions. This method has the ability to discriminate between varieties shown to be partly resistant or completely susceptible under natural conditions. Obviously, the number of genotypes analyzed in this study was very small and the method should be tested on a wider range of germplasm. However, ScleroScreen has considerable potential as it does not need to be used at many locations or under specific environmental conditions.

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We thank contributors at the experimentation sites, Luc Giton and Thomas Foubert from Lidea seeds at Blois, David Cambus and Marie Boillot from Lidea seeds at Aussonne and Gunter Leis, Thibaut Cordette, Laurent Hanneton and Jean-Eric Dheu from Limagrain at Verneuil.

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Identification, characterisation and mapping of resistance to black rot (*Xanthomonas campestris* pv. *campestris*) in *Brassica* spp.

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Black rot is the most damaging disease of vegetable brassicas (*Brassica oleracea*) worldwide and can reduce yields by >50%. The disease, caused by the bacteria *Xanthomonas campestris* pv. *campestris* (*Xcc*), can also affect other important brassica crops such as oilseed rape, swede (*Brassica napus*), mustards (*Brassica juncea*), Chinese cabbage and turnips (*Brassica rapa*), as well as vegetable brassicas. Currently, there is a lack of effective chemical treatments and varietal resistance to control *Xcc*. This project uses diverse *Xcc* isolates, including an extensive collection at the University of Warwick, to identify *Brassica* resistance to the most important *Xcc* races 1, 4, 5 and 6. *Brassica* Diversity Fixed Foundation Sets (DFFSs) of *B. napus* and *B. oleracea* were screened for resistance to these four *Xcc* races. The DFFSs have been designed to include the genetic diversity of ~6000 *Brassica* accessions in smaller subsets of homozygous lines. Once identified, these resistances will be characterised and mapped using GWAS and QTL mapping techniques, to identify resistance-linked markers that can be used to accelerate their introgression into crop types by marker-assisted selection. This work will be complemented with more fundamental research using chlorophyll and whole plant imaging techniques to visualise *Xcc* infection, investigating *Xcc* transmission routes and potential biocontrol agents. Approximately 700 *X. campestris* isolates will be sequenced with the aim to identify and functionally characterise effectors that influence the outcome of *Brassica-Xcc* interactions.

Plasmodiophora brassicae diversity in the UK and implications for clubroot management

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Summary

Clubroot disease caused by the soil-borne pathogen *Plasmodiophora brassicae* is a major threat to oilseed rape (OSR) crops worldwide with yield losses conservatively estimated to be >15% in the UK. Fungicides are not available to control the disease and agronomic practices such as liming, adjusting crop rotations and improving field drainage offer limited success. An integrated approach to managing the disease is required, which should include the use of resistant cultivars. However, the (Mendel) resistance is not effective against all pathotypes of *P. brassicae* and is being eroded where repeated deployment of resistant varieties has occurred. The objective of the study was to determine the diversity of *P. brassicae* pathotypes within the population and quantify the prevalence and distribution of Mendel-resistance breaking strains. Soil was collected from 75 individual fields which were considered high risk for clubroot. Bioassays were carried out comprising susceptible OSR cultivar Tolken, clubroot resistant OSR cultivar Mentor and Chinese cabbage susceptible controls. The plants were assessed for clubroot symptoms after 8 weeks and data were converted to a disease severity index (0-100%). The data were standardized by expressing disease severity in the resistant cultivar as a percentage of the severity in the susceptible cultivar. Pathotype determination was carried out on soil from a random sub-set of 25 fields, using differentials of the European Clubroot Differential Set (ECD). Plants were established, maintained and assessed as in the bioassays. In the bioassay tests the resistant variety remained free of clubroot disease for 18% of soils. In 17% of the soils tested, 30% -100% of the pathogen population was able to produce clubroot symptoms on the resistant variety and were thus considered to have overcome the Mendel resistance. Clubroot severity and the level of sensitivity to Mendel resistance varied from field to field but the distribution of Mendel resistance breaking strains was widespread and occurred in 15 out of 17 regions sampled throughout the UK. Twenty different pathotypes were identified using the ECD set but it was not possible to identify a single dominant pathotype from the limited number of soils tested in the project. The findings from this study highlight the need for an enhanced range of control options and sustainable integrated clubroot management strategies.

Key words: Clubroot, *Plasmodiophora brassicae*, pathotype, Mendel resistance, ECD set

Introduction

Clubroot disease caused by the soil borne root pathogen *Plasmodiophora brassicae* is one of the most intractable problems affecting oilseed rape (OSR) in the UK. Previous research has shown that on average, 0.3 t/ha of yield is lost for every 10% clubroot severity and complete crop loss can occur in severely infested fields (McGrann *et al*., 2015). Furthermore, climate change is predicted to favour disease development and its impact on yield in the future (Burnett *et al*., 2013).

There are many challenges associated with managing clubroot; the pathogen has a wide host range and is known to affect many members of the Brassicaceae family including broad acre crops such as OSR and turnip rape, vegetable brassicas, cover crops and susceptible cruciferous weeds (Dixon, 2009). Inoculum can survive in the soil for approximately 20 years with a half-life of 4 years (Wallenhammar, 1996), meaning that the disease is exacerbated by short rotations and is difficult to eradicate once present. Fungicide and bio-control options are not available and agronomic strategies such as liming to increase soil pH, improving field drainage and correcting boron deficiencies have limited success (Hwang *et al*., 2014). Good hygiene of farm machinery is essential to prevent transfer of infected soil but operational pressures mean it is not always feasible to clean machinery between fields (Faggian & Strelkov, 2009). Initial infection requires wet soils and temperatures of >16^{o}C so delaying sowing until soil temperatures fall can help avoid infection. However, late sowing is not always desirable and the potential benefit in avoiding clubroot infection needs to be balanced against the threat from diseases such as canker, which is usually more severe in late sown crops.

Varietal resistance to clubroot is currently the most effective method of control but all OSR clubroot resistant cultivars rely only on the same single resistance gene (termed 'Mendel') (Diederichsen *et al*., 2014). This resistance source is not effective against all pathotypes of the disease and in areas where resistant crops have been deployed repeatedly Mendel resistance has been eroded (Piao *et al*., 2009). *P. brassicae* is known to vary widely for pathogenicity and little is known about the pathotypes which reside in UK soils. A better understanding about the prevalence and distribution of pathotypes within the UK *P. brassicae* population would help to inform clubroot management and plant breeding decisions.

Materials and Methods

Bioassays were carried out using field soils to determine the prevalence and distribution of (Mendel) resistance breaking strains in the UK. Soil samples were collected between 2015 and 2018, from 75 fields across the UK which were identified as being high risk for clubroot. There was no formal stratification process for selecting fields but sites were chosen to represent several regions both along a north-south axis and an east-west axis. Approximately 5 kg of soil was collected from the top 30cm by walking a 'W' pattern across the field and stopping at a minimum of 50 points.

Soils were thoroughly mixed and placed in seed trays with drainage holes (20 x 14.5 x 5.5 cm). Twenty-five seedlings of Chinese cabbage cv. Granaat, the susceptible oilseed rape cultivar Tolken, and the clubroot resistant oilseed rape cultivar Mentor were sown in infected soils from each sampled field. A positive control comprising soil sampled from known infested

patches of a heavily infected site and a negative control comprising John Innes No. 2 potting compost were included in the experiment.

The seedlings were grown for 8 weeks under glasshouse conditions and were freely watered with soil temperature maintained at approximately 22^{O}C. The plants were assessed for clubroot infection using a 0-3 category scale where 0 = uninfected, 1 = slight clubbing, 2 = moderately clubbed and 3 = severely clubbed. A 0-100 severity index was calculated by weighting the incidence of plants in the three positive categories by a factor of one, two or three, respectively using the following formula:

Index = ((1* slight) + (2* moderate) + (3* severe)) * (100/3*number of plants assessed)

To account for the slight differences between sites in susceptible OSR cultivar disease severity values, the data were standardized by expressing disease severity in the resistant cultivar as a percentage of the severity expressed in the susceptible cultivar.

Pathotype determination was carried out on soil from a random sub-set of 25 fields, using differentials of the European Clubroot Differential Set (ECD) and seed was kindly provided by Prof. Geoff Dixon, from the University of Warwick. The ECD set was initially described by Buczacki *et al*. (1975), and consists of fifteen lines, split into three chromosome groups (Table 1). Each host tested within a group is assigned a Denary number, so that the hosts from each group which prove within a bioassay test to be susceptible to a particular population can be summed together, with each combination providing a unique number code which describes the population tested. Trays were sown with the 15 host species of the ECD set as outlined in Table 1. Seedlings were grown and assessed following the methodology described above.

Table 1. *European Clubroot Differential Set (ECD) host species with their denary values*

ECD genotype	ECD score	Differential	Denary value
20 chromosome group	ECD 01	Line aaBBCC	1
Brassica rapa	ECD 02	Line AAbbCC	2
	ECD 03	Line AABBcc	4
	ECD 04	Line AABBCC	8
	ECD 05	Chinese cabbage cv. Granaat	16
38 chromosome group	ECD 06	Dc101 Nevin	1
Brassica napus	ECD 07	Dc119 Giant Rape	2
	ECD 08	Dc128 selection from Dc 119	4
	ECD 09	Dc129 New Zealand resistant rape	8
	ECD 10	Dc130 Wilhelmsburger	16
18 chromosome group	ECD 11	Badger Shipper	1
Brassica oleracea	ECD 12	Bindsachsener	2
	ECD 13	Jersey Queen	4
	ECD 14	Septa	8
	ECD 15	Verheul (Kale)	16

Results

Prevalence of Mendel-resistance breaking strains

Although the selected fields were considered high risk for clubroot, symptoms failed to develop in the susceptible bioassay controls for 12% of the sampled soils (Fig. 1). The remaining 88% of soils did develop satisfactory clubroot symptoms in the susceptible controls. The resistant variety remained free of disease for 18% of soils and clubroot developed in the Mendel resistant variety in 70% of the soils. However, in 54% of the soils the proportion of the clubroot population which expressed symptoms in the Mendel resistant variety was < 30%, which is not considered significant in this study. In 17% of the soils tested, 30% -100% of the clubroot population was able to produce clubroot symptoms on the resistant variety and were thus considered to have overcome the Mendel resistance.

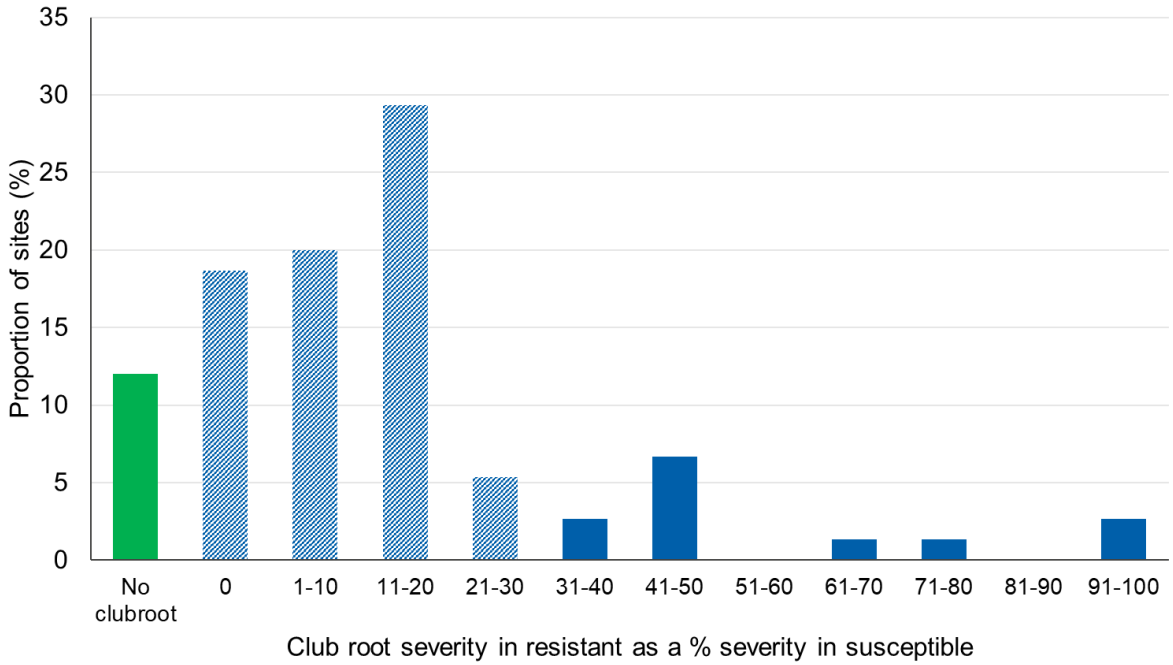

Fig. 1. The percentage of fields sampled with differing proportions of Mendel insensitive clubroot populations. The proportion of the population able to overcome the Mendel resistance is represented on the X axis, with the percentage of sites falling into each category represented on the Y-axis. Total number of fields sampled = 75.

Distribution of Mendel-resistance breaking strains

Clubroot severity and thus the level of sensitivity to Mendel resistance varied from field to field but the distribution of Mendel resistance breaking strains was widespread and occurred in 15 out of 17 regions sampled throughout the UK (Fig. 2).

Pathotype determination

Twenty different pathotypes were identified from the bioassay tests, as determined by the ECD set (Fig. 3). It was not possible to identify a single dominant pathotype from the limited

number of bioassays carried out in the project and the range of pathotypes identified suggests that a diverse clubroot population exists within the UK.

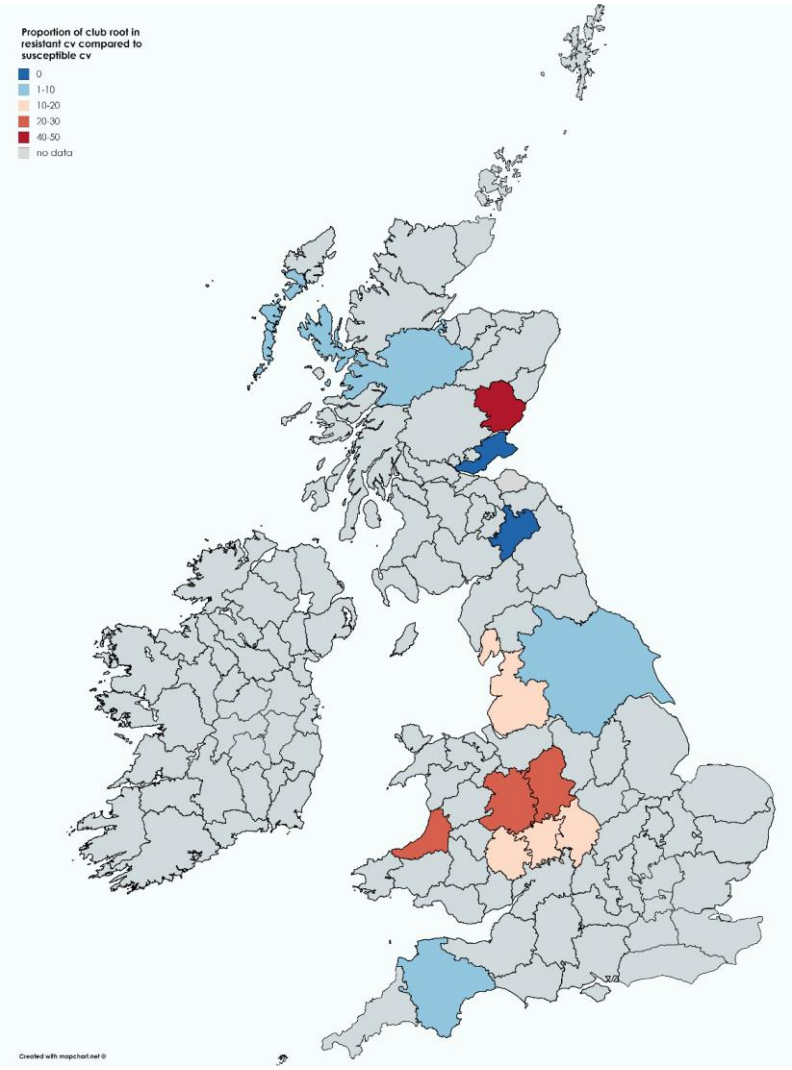

Fig. 2. Mendel-resistance breaking clubroot populations in the UK. The colour shows the average proportion of resistance breaking strain across all the fields sampled within the county. Samples were not taken from grey coloured regions.

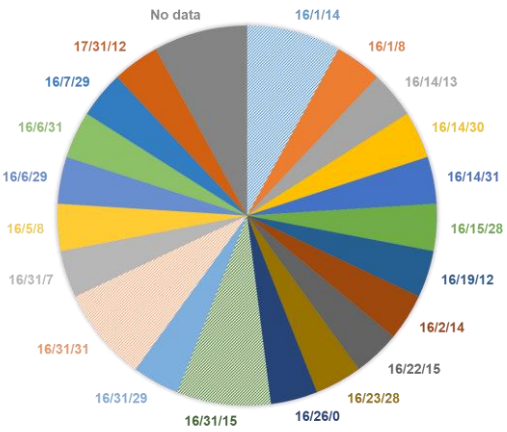

Fig. 3. Pathotypes in the UK as determined by the European Clubroot Differential (ECD) set in infected soil from 25 fields across the UK between 2015-2018.

Discussion

A global standardized methodology for conducting bioassay tests to determine clubroot infection does not exist. Řičařová *et al.* (2016) showed the number of pathotypes varied depending on the evaluation system and the threshold used to distinguish between susceptible and resistant plant reactions. Following a review of the literature and personal communication with several international clubroot researchers, a disease severity index score of 30% was used to distinguish between susceptible and resistant reactions. The threshold is considered to be robust, given that certified seed carried a 90% purity rating. The 10% infection level was deemed a reasonable tolerance if the seed used in bioassay testing was at the minimum purity standard and all 'non-Mendel' plants were susceptible. However, it is likely that a slightly different proportion of tested soils would have been classed as having overcome the Mendel resistance if a different threshold had been selected.

The frequency in occurrence and distribution of resistance-breaking strains of clubroot found in this project has significant implications in terms of clubroot management. Previous work on clubroot in the UK (McGrann *et al.*, 2015) identified varietal resistance as the most consistently effective method of management so it was inevitable it would be widely used in infected fields. Stewart (2007) followed established principles of differentiating clubroot strains by the host plants they infect, demonstrated a wide range of different strains in the UK. Not all of these strains were controlled by the Mendel resistance and at sites where resistant varieties were grown several times in the rotation, strains of clubroot had built up which were virulent on these varieties. The wide-spread occurrence of Mendel-breaking clubroot strains observed in this study illustrates the need for an enhanced range of control options and more sustainable methods of management to be developed.

A resistance mechanism that relies on a single dominant *R*-gene is vulnerable and prone to erosion by the emergence of new strains. The identification of new and novel sources of resistance is a global research priority and a greater understanding of the key strains to target would assist in tailoring breeding programmes to UK requirements.

The limited data presented in this study showed a wide diversity of clubroot pathotypes in UK soils. The diversity of clubroot pathotypes may reflect the long and varied history of susceptible brassica growing and the diversity of wild plant flora that are susceptible. This presents something of a challenge to plant breeders in developing novel host-resistance mechanisms effective against all strains and so the development of other physiological traits in breeding programmes that might preserve yield in the presence of disease is likely to be of enhanced importance. However, the sample size was very small and testing a larger set of soils may reveal the existence of a few key dominant strains, in keeping with other countries (Řičařová *et al.*, 2016). Clubroot is not uniformly distributed across fields and tends to occur in patches. Soils were sampled from across whole fields so it was not possible in this study to determine whether different pathotypes reside in each patch. A better understanding of the UK clubroot population would inform breeders and could assist in screening for effective, novel sources of genetic resistance.

Acknowledgements

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