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Understanding risks of severe phoma stem canker caused by *Leptosphaeria biglobosa* on winter oilseed rape in the UK

Yongju Huang, Asna Javaid, Bruce D.L. Fitt

University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB

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CONTENTS

1. Abstract

Phoma stem canker is an economically important disease of oilseed rape in the UK. It is associated with annual yield losses of about £100M and caused by two closely related pathogens: *Leptosphaeria biglobosa* and *L. maculans.* Associated with stem-base cankers, *L. maculans* is considered to be more damaging and is the focus of control. Although generally associated with upper stem lesions, recent UK studies suggest that *L. biglobosa* can also cause stem-base cankers. Furthermore, *L. biglobosa* is less sensitive to some triazole fungicides than *L. maculans*.

This project aimed to understand stem canker epidemics caused by *L. biglobosa* and to improve control of phoma stem canker by targeting both causal pathogens. It had four objectives:
1. Determine regional differences in proportions of *L. biglobosa* in pathogen populations
2. Investigate cultivar resistance against *L. biglobosa*
3. Investigate effects of fungicides on control of *L. biglobosa* compared to *L. maculans*
4. Make recommendations for phoma stem canker control targeted at *L. biglobosa* and *L. maculans* (knowledge transfer)

Regional differences

Analysis of air samples, collected from four sites in three cropping seasons, indicated that there were differences between sites and seasons in the timing and patterns of ascospore release. Ascospores were released considerably later at Impington than at the other three sites (Bayfordbury, Rothwell and Eye) in all the three seasons. Ascospores were released later in 2016, when it was hot and dry in the summer, than in 2015 and 2017 at all four sites. There were differences between sites and seasons in the proportions and timings of *L. biglobosa* and *L. maculans* ascospores release. In general, there were more *L. biglobosa* ascospores than *L. maculans* ascospores in 2016/17 for all sites, whereas there were more *L. maculans* ascospores than *L. biglobosa* ascospores in 2015/16 and 2017/18. Both *L. maculans* and *L. biglobosa* ascospores were released at similar times at three sites, while *L. maculans* ascospores were released earlier (mainly in autumn/winter) than *L. biglobosa* ascospores (mainly in spring) at Rothwell in all three seasons.

Cultivar resistance

Field experiments, using six cultivars with different AHDB Recommended Lists (RL) stem canker disease resistance ratings, showed that there were significant differences between cultivars in the severity of phoma leaf spot and phoma stem canker over the 2015/16, 2016/17 and 2017/18 seasons. In general, resistant cultivars (resistance rating $\geq$8) had fewer *L. maculans* phoma leaf spots and more *L. biglobosa* phoma leaf spots and less severe phoma stem canker than susceptible cultivars (resistance rating $\leq$ 4). For the two cultivars with the resistance gene *Rlm7* (effective against *L. maculans*), Fencer had fewer *L. maculans* and *L. biglobosa* phoma leaf spots, and less severe phoma stem canker than Harper in two out of the three seasons. This suggests that background

quantitative resistance affects resistance against both *L. maculans* and *L. biglobosa*. With a combination of major gene resistance and quantitative resistance, it is possible to breed cultivars with resistance against both *L. maculans* and *L. biglobosa*.

RL disease observation plot experiments, with 17 cultivars at two sites (Broughton in Hampshire and Morley in Norfolk) in the 2014/15 and 2015/16 seasons, showed significant differences between sites and cultivars in the numbers of *L. maculans* and *L. biglobosa* phoma leaf spots, severity of phoma stem canker, and amounts of *L. maculans* and *L. biglobosa* DNA in stem base cankers. Susceptible cultivars (resistance rating ≤4) had greater numbers of *L. maculans* and *L. biglobosa* phoma leaf spots, more severe phoma stem canker and greater amounts of *L. maculans* DNA in stem cankers than resistant cultivars (resistance rating ≥8). Resistant cultivars had less *L. maculans* phoma leaf spots, less severe phoma stem canker, smaller amounts of *L. maculans* DNA but greater amounts of *L. biglobosa* DNA in stem cankers than cultivars with resistant rating <8. Among the resistant cultivars, one of them showed resistance against both *L. maculans* and *L. biglobosa.* Results from controlled environment experiments showed that both *L. maculans* and *L. biglobosa* caused severe phoma leaf spots on susceptible cultivars, whereas *Rlm7* cultivars (resistant cultivars) showed resistance to *L. maculans* and variation in susceptibility to *L. biglobosa.*

Fungicide effects

Field fungicide experiments, in three cropping seasons, showed that Proline (prothioconazole-desthio) and Refinzar (penthiopyrad + picoxystrobin) were equally effective in reducing the numbers of *L. maculans* or *L. biglobosa* phoma leaf spots and severity of phoma stem canker on susceptible cultivars, with little or no effect on resistant cultivars. Results of *in vitro* fungicide sensitivity tests (using individual active ingredients of Proline and Refinzar) with 39 isolates showed that *L. biglobosa* isolates were less sensitive to prothioconazole-desthio and more sensitive to penthiopyrad than *L. maculans* isolates. *L. maculans* isolates collected from Proline-treated plants were less sensitive to prothioconazole-desthio and penthiopyrad than *L. maculans* isolates collected from untreated plants. There were no differences between *L. biglobosa* and *L. maculans* isolates in sensitivity to picoxystrobin.

Knowledge transfer

Project results were exploited directly in the agricultural industry during the course of the project, through AHDB and industry partners (five oilseed rape breeders, one agrochemical company and one agricultural service provider) of two BBSRC/Innovate UK projects. New findings from this and associated projects were also communicated in six research papers in international journals and as presentations at six international and six national conferences. In addition, results were presented at farm open days and industry events.

2. Introduction

Phoma stem canker is an economically important disease of oilseed rape causing annual yield losses of £80-100M in the UK, despite use of fungicides (Fitt et al., 2011). These losses will increase with the loss of effective fungicides (e.g. Punch C and Refinzar), and with predicted global warming. Effective control of this disease is crucial for maintaining oilseed rape production in the UK. The disease is caused by two closely related pathogens: *Leptosphaeria biglobosa* and *L. maculans*. The two pathogens have similar life cycles but occupy different niches, so they are able to co-exist on their host (Fitt et al., 2006a). Recent studies showed that these two related species have different strategies to suppress host resistance (Lowe et al., 2014); suggesting that host resistance against *L. maculans* may be different from resistance against *L. biglobosa. L. maculans* is considered more damaging than *L. biglobosa* because *L. maculans* generally causes damaging stem base cankers, whereas *L. biglobosa* ordinarily produces less harmful upper stem lesions (West et al., 1999; Williams & Fitt 1999; Eckert et al., 2010; Fitt et al., 2006b; Huang et al., 2011). These characteristics have directed research to improve understanding of *L. maculans*, with little research being done on *L. biglobosa*. There is a need to understand host resistance against *L. biglobosa*.

Results from HGCA funded projects (RD-2009-3676, an associated bursary project and RD-2004-3081) showed that *L. biglobosa* can cause both basal stem cankers and upper stem lesions, leading to yield losses. For example, in the 2011/2012 cropping season, severe stem base canker and upper stem lesions were observed and results from quantification of pathogen DNA using qPCR showed that *L. biglobosa* was the major cause of both stem base cankers and upper stem lesions on oilseed rape in that cropping season (Huang et al., 2014). The severe upper stem lesions caused by *L. biglobosa* led to the breakage of stems (Fig. 2.1), which suggests that *L. biglobosa* can sometimes cause considerable yield loss in the UK. This is supported by previous work suggesting that there are differences in regional distribution between the two pathogens, with *L. biglobosa* causing damaging cankers in the north of the UK and *L. maculans* causing disease in the south (RD-2004-3081; Stonard et al., 2010).

As *L. biglobosa* and *L. maculans* have co-existing life cycles, they compete with each other for resources. If, by some means, growth of one pathogen is decreased, the other pathogen has the opportunity to expand. This decrease in growth can be affected by many factors, but the two most important are host resistance and fungicide sensitivity. As *L. maculans* has been commonly targeted for control by both *R* gene-mediated host resistance and fungicides, knowledge regarding responses of *L. biglobosa* to current control measures is limited.

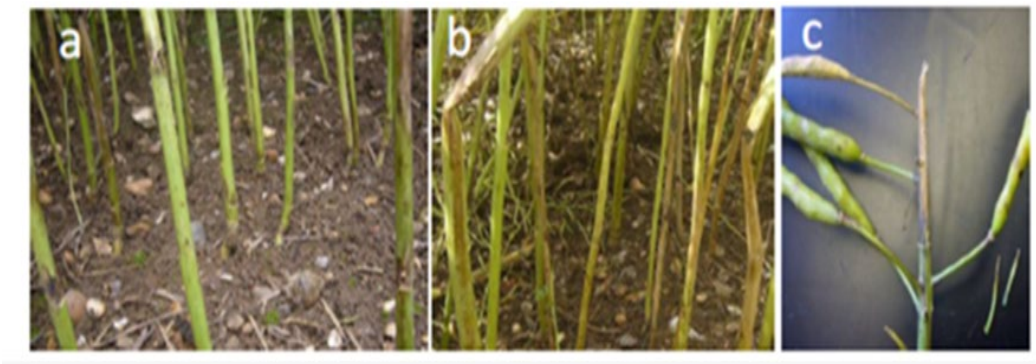

Fig. 2.1: Symptoms on upper stems (a, b) and racemes (c) in a winter rape experiment in July 2012 at Rothamsted; qPCR results showed that these were associated with *L. biglobosa.*

In relation to cultivar resistance (*R* gene-mediated), results from a recent LINK project (RD-2009-3676) have suggested that cultivars resistant to *L. maculans* may be more susceptible to *L. biglobosa* (Fig. 2.2). Recent studies suggested that *L. biglobosa* and *L. maculans* trigger different defence pathways (Lowe et al., 2014). This suggests that resistance against *L. biglobosa* is not the same as resistance against *L. maculans*. Currently, stem canker assessments for the AHDB Recommended List (RL) do not distinguish between the two causal pathogens; thus they do not assess whether the epidemics are caused by *L. biglobosa* or *L. maculans.*

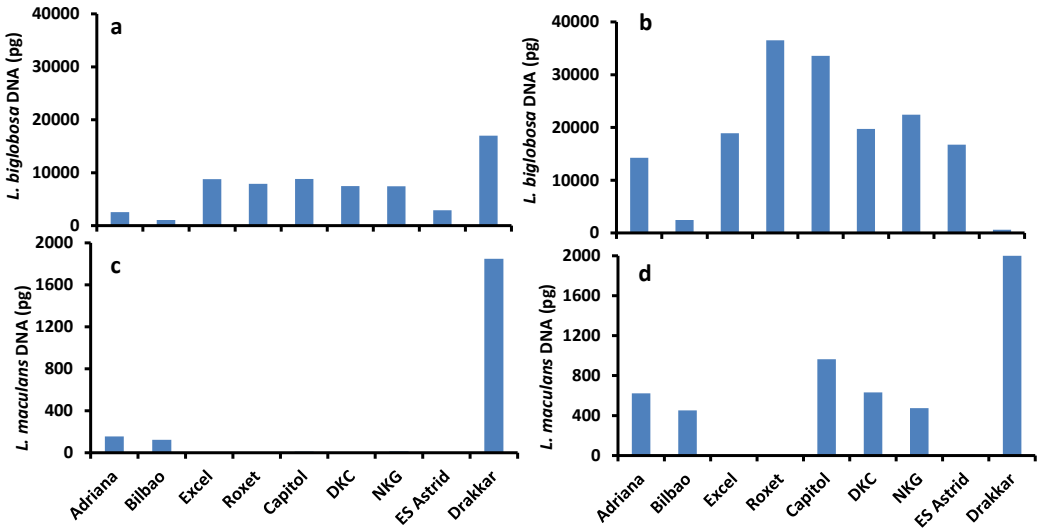

Fig. 2.2: The amounts of *L. biglobosa* (a, b) and *L. maculans* (c, d) DNA detected by qPCR in diseased upper stem (a, c) and stem base samples (b, d) from nine oilseed rape cultivars. Samples were taken in July 2012.

Research has shown that *L. biglobosa* has a decreased sensitivity to triazole fungicides, which are commonly used against phoma stem canker in crops (Huang et al., 2011; Eckert et al., 2010). This difference in fungicide sensitivity between the two pathogens (Fig. 2.3) could be selecting for *L. biglobosa*. Combining this fact with the understanding that *L. biglobosa* can cause damaging base stem cankers, it is clear that selection for *L. biglobosa* may be a threat to UK oilseed rape crops.

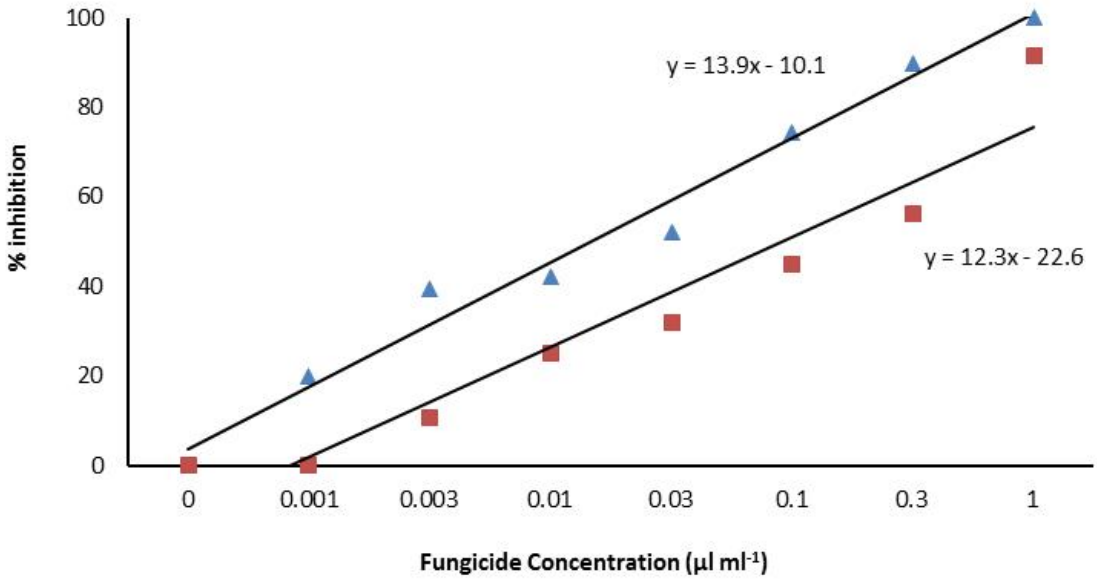

Fig. 2.3: Percentage inhibition of growth of *L. maculans* ▲ and *L. biglobosa* ■ isolates by Sanction (containing 250 µg ml^{-1} flusilazole). Both pathogens show a linear response to an increase in fungicide concentration, with *L. biglobosa* having a smaller response than *L. maculans.* (Eckert et al., 2010).

Furthermore, recent work on other causal agents of oilseed rape diseases has shown patterns of decreasing efficacy of triazole fungicides. This has been due to genetic changes in the azole target site 14 α-demethylase. The gene (CYP51) that codes for this enzyme has shown significant changes over recent years; these changes have been identified as the reason for this reduction in sensitivity (Carter et al., 2014). However, there is little work on understanding the fungicide sensitivities of *L. biglobosa* and *L. maculans*. With the recent removal of effective triazole fungicides, there is a need to investigate other commercially available fungicides for control of the two pathogens causing phoma stem canker

The overall aim of this project is to understand phoma stem canker epidemics caused by *L. biglobosa* and to improve control of phoma stem canker by targeting both causal pathogens (*L. maculans* and *L. biglobosa*). To achieve the aim, there are four related objectives.

Objective 1. To determine regional differences in proportions of *L. biglobosa* in pathogen populations.
Objective 2. To investigate cultivar resistance against *L. biglobosa*.
Objective 3. To investigate effects of fungicides on control of *L. biglobosa* compared to *L. maculans*.
Objective 4. To make new recommendations to farmers for better control of phoma stem canker by targeting both *L. biglobosa* and *L. maculans* (knowledge transfer).

3. Materials and methods

3.1. Regional differences in proportions of *L. biglobosa* in pathogen populations

To investigate the threat of *L. biglobosa* to oilseed rape production in the UK, differences between regions in proportions of *L. biglobosa* in local pathogen populations were investigated by monitoring ascospore release and identifying the pathogen species of the ascospores released.

3.1.1. Monitoring the timing of ascospore release

Phoma stem canker epidemics are initiated by air-borne ascospores. To monitor the timing and abundance of ascospores released in the air, air samples were collected using Burkard 7-day recording air samplers (Fig. 3.1a). The air samplers were operated from September to February/March in each season (2015/16, 206/17, 2017/18) at four sites (Bayfordbury in Hertfordshire, Rothwell in Lincolnshire, Impington in Cambridgeshire and Eye in Suffolk) (Table 3.1). Oilseed rape stems affected by phoma stem canker from the previous season were collected from the area where the sampler was set up and placed around the Burkard samplers (Huang et al., 2005). The spore tapes were removed from the air sampler drums and processed (Lacey & West, 2006). At 7-day intervals, each exposed tape was removed from the sampler drum, and cut into pieces 48 mm long (each representing 24 h). Each 48 mm long piece of tape was cut in half lengthwise (Fig. 3.1b). One half was mounted onto a microscope slide for counting ascospores (Huang et al. 2005; Lacey & West, 2006); the other half was placed in a 1.5 µL Eppendorf tube and stored at -20°C for DNA extraction (Huang et al., 2011) and detection of *L. biglobosa* and *L. maculans* ascospores released in the air using the quantitative polymerase chain reaction (qPCR) (Huang et al., 2011).

3.1.2. Identification of *L. maculans* and *L. biglobosa* ascospores

To determine the differences between the four regions in pathogen population structures (i.e. the proportions of *L. biglobosa* and *L. maculans*), air samples were assessed using molecular techniques. Because it is difficult to distinguish *L. biglobosa* ascospores from *L. maculans* ascospores under a microscope, the presence and abundance of *L. biglobosa* and *L. maculans* ascospores in the air samples were analysed using qPCR. The DNA from the spore tapes was extracted using the CTAB (hexadecyltrimethylammoniumbromide) protocol (Kaczmarek et al., 2009) with some minor modifications. The amounts of *L. biglobosa* DNA and *L. maculans* DNA on each spore tape were quantified using species-specific qPCR (Huang et al., 2011).

Table 3.1 Locations for Burkard spore samplers in the 2015/2016, 2016/2017 and 2017/2018 growing seasons for collecting ascospore samples

Organisation	Location
Limagrain	Rothwell, Market Rasen, Lincolnshire, LN7 6DT
Grainseed	Unit 3 Airfield Industrial Park, Langton Green, Eye, Suffolk, IP23 7HN
LS Plant Breeding	North Barn, Manor Farm, Milton Road, Impington, Cambridge, CB24 9NF
University of Hertfordshire	Bayfordbury, Lower Hatfield Road, Hertford, Hertfordshire, SG13 8LD

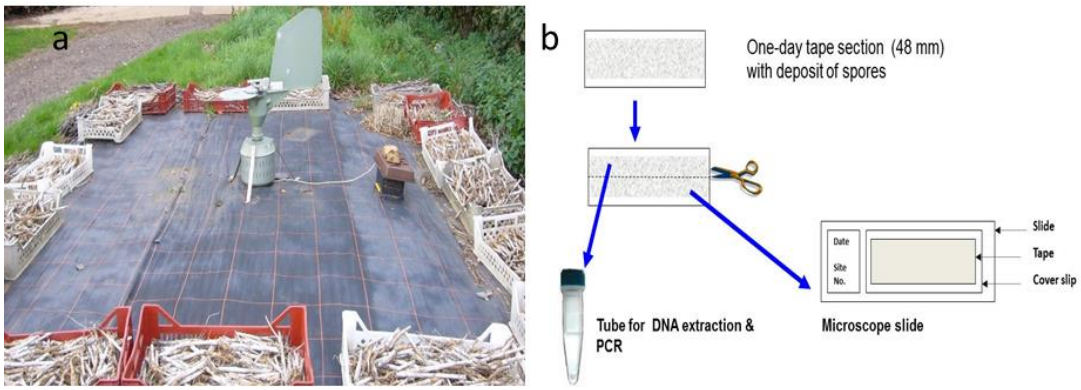

Fig. 3.1 A Burkard air sampler surrounded by oilseed rape stem debris used to sample ascospores of *L. maculans* and *L. biglobosa* released into the air (a) and the processing of air samples (b).

3.1.3. Effects of environmental factors on ascospore release

Temperature and rainfall are the two main factors affecting maturation of ascospores and development of phoma leaf lesions (Toscano-Underwood et al., 2001; Huang et al., 2007). To investigate effects of weather factors on the timing of ascospore release and the severity of stem canker epidemics caused by *L. biglobosa*, for each season the data for daily temperature and rainfall near the air sampling sites were obtained from the AHDB weather station network for three sites (Impington in Cambridgeshire, Eye in Suffolk and Rothwell in Lincolnshire) and from an on-site weather station for Bayfordbury in Hertfordshire. The daily weather data (rainfall and temperature) were collected from August to the following July in the 2015/2016, 2016/2017 and 2017/2018 growing seasons.

3.2. Investigation of cultivar resistance against *L. biglobosa*

To investigate differences between cultivars in resistance against *L. biglobosa*, cultivars with different levels of 'field' phoma stem canker resistance (AHDB RL resistance rating) were selected from the AHDB Recommend List (RL) for use in field experiments and controlled environmental experiments. In addition, cultivars from AHDB RL Disease Observation Plots (DOP) at two sites in the 2014/2015 and 2015/2016 growing seasons were used for assessing cultivar resistance.

3.2.1. Cultivar resistance in field experiments

Six cultivars were used in field experiments in three growing seasons. A total of 17 cultivars from AHDB RL DOP in two growing seasons were used for assessing severity of phoma leaf spot and phoma stem canker caused by *L. maculans* and *L. biglobosa*.

3.2.1.1. Field experiments with six cultivars

Six cultivars with different levels of 'field' phoma stem canker resistance (Table 3.2) were used in field experiments in three growing seasons (2015/2016, 2016/2017 and 2017/2018). The field experiments were set up in a randomised block design with three replicates. In the 2015/2016 season, the experiment was set up at Boxworth in Cambridgeshire. Due to severe cabbage stem flea beetle damage, the field experiment in the 2015/2016 season was very patchy, so the experiments were set up at Terrington, Norfolk in the next two seasons (2016/2017 and 2017/2018). In each season in the autumn, the severity of phoma leaf spot caused by *L. biglobosa* or *L. maculans* was assessed by counting the numbers of *L. maculans* and *L. biglobosa* types of leaf spot lesions on each cultivar according to the appearance of the symptoms: *L. maculans* type leaf lesions are big, pale lesions with many pycnidia, while *L. biglobosa* type lesions are small, dark lesions with no or few pycnidia (Fig. 3.2). Ten plants were randomly sampled from each of the three replicate plots of each cultivar. The numbers of *L. maculans* and *L. biglobosa* types of leaf spot lesions on each cultivar were counted. In the summer before harvest, twenty plants were randomly sampled from each of the three replicated plots. The plants were uprooted and cut near the stem base. The severity of phoma stem canker was assessed, based on the percentage of necrosis on the stem cross-sections on a 0-7 scale (0, no canker; 1, <5% stem area affected; 2, 5-25% stem area affected; 3, 26-50% stem area affected; 4, 51-75% stem area affected; 5, 76-99% stem area affected; 6, 100% stem area affected and 7, complete necrosis of stem). The 0-7 scale is modified from the 1-6 scale of Lô-Pelzer et al. (2009). To investigate whether the stem base cankers were caused by *L. maculans* or *L. biglobosa,* the necrotic tissues were sampled from basal stem cankers, placed in 2.0 ml tubes for freeze-drying and DNA extraction. The amounts of *L. biglobosa* DNA and *L. maculans* DNA in each stem sample were assessed using species-specific qPCR (Liu et al., 2006; Huang et al., 2009). The relationships between severity of stem canker and the amount of *L. biglobosa* DNA or *L. maculans* DNA were analysed.

Table 3.2: List of cultivars used in winter oilseed rape field experiments in three growing seasons (2015/2016, 2016/2017 and 2017/2018).

Cultivar	Resistance genes	RL cultivar resistance rating
DK-Cabernet (DKC)	Quantitative resistance; *Rlm1*	6
Fencer	*Rlm7*[*]	8
Harper	*Rlm7*[*]	8
Incentive	No *R* genes	4
PR46W21	No *R* genes	3
Quartz	Quantitative resistance (with *Rlm7*?)	9

[*] The resistance genes *Rlm1* and *Rlm7* are for resistance against *L. maculans.*

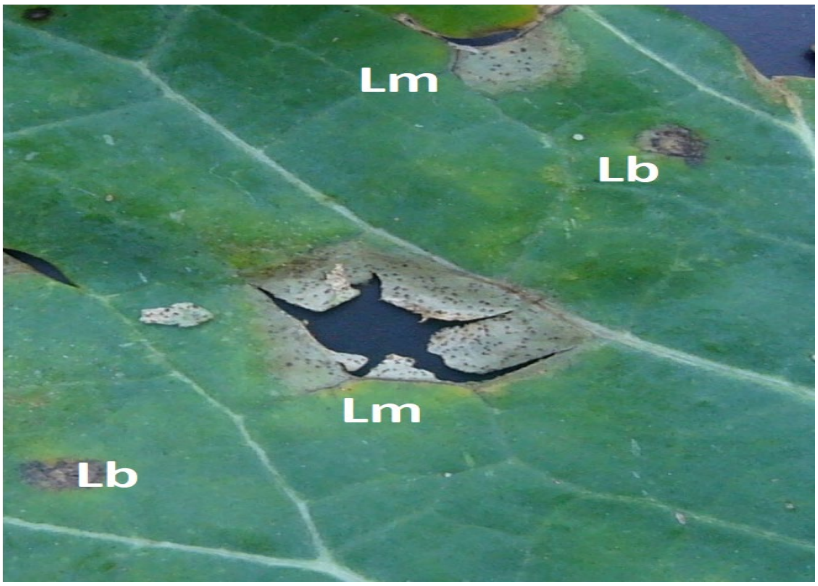

Fig. 3.2: Appearance of *L. maculans* and *L. biglobosa* leaf spot lesions on winter oilseed rape. *L. maculans* (Lm) is generally associated with large, pale lesions with pycnidia, while *L. biglobosa* (Lb) is generally associated with small, dark lesions with no or few pycnidia.

Table 3.3: List of cultivars sampled from AHDB RL DOP trials at Broughton and Morley in the 2014/2015 and 2015/2016 cropping seasons.

Cultivar[1]	Resistance rating for cultivars used in 2014/2015 season[2]	Resistance rating for cultivars used in 2015/2016 season[2]
RLDOP1	3	-
RLDOP2	4	-
RLDOP3	4	4
RLDOP4	6	6
RLDOP5	5	5
RLDOP6	8	8
RLDOP7	4	4
RLDOP8	3	3
RLDOP9	3	3
RLDOP10	3	3
RLDOP11	5	5
RLDOP12	9	-
RLDOP13	5	-
RLDOP14	6	6
RLDOP15	6	6
RLDOP16	4	4
RLDOP17	6	6
RLDOP18	-	5
RLDOP19	-	8
RLDOP20	-	6
RLDOP21	-	8

[1] Due to the Confidentiality Agreement with AHDB on use of these RL cultivars for this study, the names of these RL cultivars are coded. [2] Cultivar resistance rating was based on AHDB RL for winter oilseed rape for 2015/2016 or 2016-2017 in the East/West Region. Resistance rating range is on a 0-9 scale; 0= very susceptible, 9= very resistant.

3.2.1.1. AHDB RL DOP field experiments

Seventeen cultivars from the AHDB RL DOPs in the 2014/2015 and 2015/2016 growing seasons were selected. Due to the Confidentiality Agreement with AHDB on use of these RL cultivars for this

study, names of the RL cultivars were coded as RLDOP 1, RLDOP2, … RLDOP21 (Table 3.3). The selection of cultivars was based on the AHDB RL resistance rating so that cultivars with different resistance ratings were included (Table 3.3). The RL DOP field trials at Broughton in Hampshire and Morley in Norfolk were used for assessing severity of phoma leaf spotting and phoma stem canker caused by *L. maculans* and *L. biglobosa*. The RL DOP field trials at Broughton and Morley were set up with one replicate for each cultivar. In each season in the autumn, ten plants were randomly sampled from each plot. The numbers of *L. maculans* and *L. biglobosa* phoma leaf spots were counted on each plant as described in 3.2.1.1. Then the average number of *L. maculans* and *L. biglobosa* phoma leaf spots per plant was calculated for each cultivar. In summer before harvest, twenty plants were randomly sampled from each plot. The severity of phoma stem base canker was assessed on a 0-7 scale as described in 3.2.1.1. To investigate whether the stem base cankers were caused by *L. maculans* or *L. biglobosa,* the necrotic tissues were sampled from basal stem cankers for DNA extraction and qPCR using species-specific qPCR (Liu et al., 2006; Huang et al., 2009). The relationship between severity of stem canker and the amount of *L. biglobosa* DNA or *L. maculans* DNA in the stem for each cultivar were analysed.

3.2.2. Cultivar resistance in controlled environment experiments

To investigate the differences between different cultivars in resistance/susceptibility to different *L. maculans* and *L. biglobosa* isolates, controlled environment experiments were done with different cultivars by cotyledon inoculation.

3.2.2.1. Collection of *L. maculans* and *L. biglobosa isolates* from field experiments for inoculation

In the 2015/2016 cropping season, following phoma leaf spot assessment, ten leaves with phoma leaf spots were collected from each of the Broughton and Morley field sites. Single pycnidial isolates of *L. maculans* and *L. biglobosa* were collected from these leaf lesions using methods described by Huang et al. (2018). The isolates were sub-cultured onto V8 agar for pycnidial production. Conidial suspensions of the sub-cultured isolates were prepared, adjusted to 10^7 spores/ml and stored at –20°C ready for cotyledon inoculation (Huang et al., 2018). All isolates collected were identified as *L. maculans* or *L. biglobosa* based on colony morphology and then isolate species were reconfirmed using species-specific PCR.

3.2.2.1. Inoculation and disease assessment

Six cultivars with different RL field resistance ratings and different resistance genes (i.e. against *L. maculans*) were selected (Table 3.4) to assess the differences between cultivars in resistance against *L. maculans* or *L. biglobosa*. To compare the pathogenicity of UK *L. biglobosa* isolates with Chinese *L. biglobosa* isolates, a Chinese *L. biglobosa* isolate was used. Cotyledons of two-week old

seedlings were wounded using a sterile needle and a 10µl drop of conidial suspension was placed over each wound. Each isolate was inoculated onto eight plants of each cultivar. Inoculated plants were covered with a plastic propagator lid for 72 h to maintain high humidity and incubated at 20°C with alternating 12h light (light intensity 210µmol $m^{-2}s^{-1}$) and 12h darkness. At 16 days post inoculation (dpi), disease symptoms on each plant were scored on a 0-9 scale (0, no darkening around the wound; 9, large pale grey lesion with pycnidia) (Huang et al., 2018). Cultivars with a mean disease score <3.5 were considered resistant, while cultivars with a mean score ≥3.5 were considered susceptible. In addition, at 16 dpi, the sizes of the lesions (i.e. the maximum length and width of each lesion) on each plant were measured. A total of 20 isolates (6 *L. maculans* and 14 *L. biglobosa* isolates) were tested on cotyledons of the six oilseed rape cultivars with 12 isolates tested in Experiment 1 and 8 isolates tested in Experiment 2. Of the 20 isolates, 19 were collected in 2015/2016 cropping season from Broughton (11 isolates) and Morley (8 isolates) and one was from China.

Table 3.4: List of oilseed rape cultivars used in controlled environment experiments for testing cultivar resistance against *Leptosphaeria maculans* and *Leptosphaeria biglobosa*. Drakkar and Topas are spring oilseed rape cultivars with no AHDB RL resistance rating, they were used as controls.

Cultivar	Resistance genes	Cultivar resistance rating
DK-Cabernet (DKC)	Quantitative resistance and *Rlm1*[*]	6
Drakkar	No resistance genes	N/A
Excel	*Rlm7*[*]	9
Fencer	*Rlm7*[*]	8
Harper	*Rlm7*[*]	8
Topas	No resistance genes	N/A

[*] The resistance genes *Rlm1* and *Rlm7* are for resistance against *L. maculans.*

3.2.3. Statistical analysis

The statistical analysis of the data from both field experiments and controlled environment experiments were done using statistical software package GenStat (General Statistics) (Payne et al., 2011). To determine the differences between sites, cultivars and interactions between site and cultivar in the numbers of *L. maculans* or *L. biglobosa* phoma leaf spots, severity of phoma stem canker and amounts of *L. maculans* or *L. biglobosa* DNA in stem cankers in field experiments, Analysis of variance (ANOVA) was done for the field data. *P* values ≤0.05 were considered

significantly different and *P* values >0.05 were not considered significantly different. Where there were significant differences, 'Fisher's least significant difference' (LSD) test was done to compare the pair-wise differences. For the controlled environment experiments, ANOVA was done to analyse the differences between isolates, cultivars and the interactions between isolate and cultivar. Where there were significant differences, pairwise comparisons were done using the LSD.

3.3. Effects of fungicides on control of *L. biglobosa* compared to *L. maculans*

To investigate effects of fungicides on control of phoma stem canker caused by *L. maculans* and *L. biglobosa*, field experiments with six cultivars treated with two different fungicides and controlled environment experiments with different isolates tested for fungicide sensitivity were done.

3.3.1. Effects of fungicides on control of phoma stem canker on different cultivars in field experiments

The six cultivars with different 'field' phoma stem canker resistance ratings (Table 3.2) used in 3.3.1 were used for fungicide field experiments in three growing seasons (2015/2016, 2016/2017 and 2017/2018).

Table 3.5: Timing and rate of fungicide application for field experiments in 2015/2016, 2016/2017 and 2017/2018 growing seasons.

Early spray	2015-2016	2016-2017	2017-2018	Late spray	2015-2016	2016-2017	2017-2018
	20 Oct 2015	12 Nov 2016	25 Oct 2017		25 Nov 2015	13 Dec 2016	21 Nov 2017
	Rate (kg/ha)	Rate (kg/ha)	Rate (kg/ha)		Rate (kg/ha)	Rate (kg/ha)	Rate (kg/ha)
Proline	0.32	0.32	0.32	Proline	0.32	0.32	0.32
Refinzar	0.50	0.50	0.50	Refinzar	0.50	0.50	0.50

3.3.1.1. Fungicide treatments in field experiments

The field experiments were set up in combination with the field experiments in 3.2.1 at Boxworth in Cambridgeshire in 2015/2016 and at Terrington in Norfolk in 2016-2017 and 2017-2018. The experiments were set up in randomised block designs with three replicates. Each cultivar was treated with two different fungicides; Proline (prothioconazole-desthio) (fungicide 1; F1) and Refinzar (penthiopyrad + picoxystrobin) (fungicide 2; F2) to test the effectiveness of these two commercially available fungicides on control of phoma stem canker. The fungicide-untreated plots were used as controls (C). In total, there were 54 plots in each of the three field experiments. The cultivars were sown in late August in 2015 or early September in 2016 and 2017. The fungicide Proline was sprayed at the rate of 0.32 kilograms/hectare (kg/ha) and Refinzar at the rate of 0.50

kg/ha. The fungicides were applied as early spray and late spray. The timing of the early spray was based on the time when 10% of plants in the plots had phoma leaf spots; the later spray was one month later when the weather permitted spraying (Table 3.5).

3.3.1.2. Phoma leaf spot and phoma stem canker assessment

The severity of phoma leaf spot caused by *L. biglobosa* or *L. maculans* was assessed by counting the numbers of *L. maculans* and *L. biglobosa* types of leaf spot lesions, the severity of phoma stem canker was assessed based on percentage necrosis on the stem cross sections on an 0-7 scale as described in 3.2.1.1. To investigate whether the phoma stem cankers were caused by *L. maculans* or *L. biglobosa,* the stems with basal stem cankers were sampled for DNA extraction and species-specific qPCR (Liu et al., 2006; Huang et al., 2009). The effects of fungicides in reducing the visible severity of stem canker and reducing the amounts of *L. biglobosa* DNA or *L. maculans* DNA were analysed.

3.3.2. Effects of fungicides on control of the growth of *L. maculans* and *L. biglobosa in vitro*

To investigate efficacy of currently available fungicides for control of mycelial growth of *L. biglobosa* or *L. maculans,* the individual components of the two fungicides (Proline and Refinzar) used in field experiments were tested with different *L. biglobosa* and *L. maculans* isolates.

3.3.2.1. Preparation of *L. maculans* and *L. biglobosa* isolates

A total of 18 *L. biglobosa* and 21 *L. maculans* isolates were collected from different regions. Of the 21 *L. maculans* isolates, eight isolates were collected from untreated plants, seven were taken from plants sprayed with Proline (prothioconazole-desthio) and six isolates were from plants sprayed with Refinzar (penthiopyrad + picoxystrobin). All *L. biglobosa* isolates were taken from untreated plants because there were no *L. biglobosa* isolates recovered from fungicide-treated plants. One *L. biglobosa* isolate 'W10' (from China) used in previous studies was used as a control. All the isolates were retrieved from colonised filter discs stored at -20°C. The filter discs were placed onto freshly prepared V8 agar plates and incubated at 20°C for 5-6 days before inoculation onto fungicide amended media.

3.3.2.2. Preparation of fungicide amended media

Stock solutions of fungicides prothioconazole-desthio, penthiopyrad and picoxystrobin were prepared for fungicide-amended media for *in vitro* fungicide-sensitivity tests. The fungicide (in solid powder form) was weighed in a fume hood on a weighing balance. A stock solution of 6 mg/ml was

made for each fungicide by dissolving 6 mg of the fungicide powder in 1 ml of acetone (Sigma Aldrich, for HPLC, ≥ 99.9%). To achieve fungicide-amended media with different concentrations of the fungicide, different volumes of the stock solution were added into the V8 medium before pouring the plates. For prothioconazole-desthio, the final fungicide concentrations in the V8 media were 0, 0.05, 0.1, 0.25, 0.5 and 1 μg/ml. For penthiopyrad and picoxystrobin, the final fungicide concentrations in the V8 media were 0, 0.05, 0.1, 0.25 and 0.5 μg/ml. For the control medium, pure acetone (without fungicide) was added into the medium. To prepare agar plates, 10 ml of medium was poured into each 9 cm diameter Petri-dish using a 10 ml sterile pipette (Greiner bio-one, Frickenhausen Germany) under aseptic conditions. Both control and fungicide-amended V8 media plates were prepared one day before *in vitro* fungicide-sensitivity tests.

3.3.2.3. Fungicide sensitivity test

Plugs of fungal mycelia were taken from the outer edges of the colonies (freshly prepared on V8 agar plates and incubated at 20°C for 5-6 days) using a 0.5 mm diameter cork borer and placed in the centre of control and fungicide-amended V8 media plates. Two replicates were made for each isolate on both control plates and fungicide-amended media plates with different fungicide concentrations. The plates were incubated at 20°C in darkness and the diameters of radial mycelial growth were measured at regular intervals. The final measurements for both control and fungicide-amended media were made at 12 dpi. The fungicide sensitivity of each isolate was measured by the EC_{50} (effective concentration at which 50% of growth is inhibited). The EC_{50} values were compared between isolates and between fungicides.

3.3.3. Statistical analysis

The statistical analyses of the data were done using statistical software package GenStat (General Statistics) (Genstat, 2015, 18th Edition). To compare the effects of different fungicides on control of phoma leaf spots and phoma stem canker caused by *L. biglobosa* and *L. maculans* in field experiments, general analysis of variance (ANOVA) was done. Where there were significant differences ($P < 0.05$). 'Fisher's protected least significant difference' (LSD) test was done to compare the pair-wise differences in the severity of phoma leaf spotting, phoma stem canker and amounts of pathogen DNA on untreated and fungicide-treated plants of each cultivar.

To test the effects of fungicides on the radial mycelial growth of *L. biglobosa* and *L. maculans* isolates in *in vitro*, EC_{50} values of different isolates with different fungicides were analysed using general ANOVA.

3.4. Exploitation and technology transfer

3.4.1. Exploitation and dissemination of new knowledge

Results of this project were exploited directly in the arable sector of the agricultural industry during the course of the project through AHDB and industry partners of an Innovate UK project. This project is related to two Innovate UK projects with industry partners including oilseed rape breeders (Limagrain, LS Plant Breeding, Monsanto, KWS and Grainseed), an agrochemical company (DuPont) and an agricultural service provider (Hutchinsons). New developments were conveyed to the industry through AHDB, breeders' open days, Hutchinsons' agronomists and farmer conferences.

3.4.2. Publications and conferences

New information generated from this project was disseminated through publications and conference presentations. After publication of research papers in peer reviewed international journals, the press and other media (e.g. hand out leaflets, posters) were used during the course of the project to highlight the benefits and novelty of the research. Findings were also communicated as research papers in international journals and as presentations or publications in scientific conference proceedings and seminars.

4. Results

4.1. Regional differences in proportions of *L. biglobosa* in pathogen populations

4.1.1. Regional differences in timing and pattern of ascospore release

The air samples were collected from four sites (Bayfordbury in Hertfordshire, Rothwell in Lincolnshire, Impington in Cambridgeshire and Eye in Suffolk) in the 2015/2016, 2016/2017, 2017/2018 growing seasons from September to February/March. Results for daily release of ascospores showed that there were differences between sites and between seasons in the timing and patterns of ascospore release. Ascospores were released considerably earlier at Bayfordbury, Rothwell and Eye than at Impington in all the three seasons. In the 2015/2016 season, first major ascospore release (10% of the maximum number of ascospores) was observed on 21 September at Eye, on 5 October at Bayfordbury, on 20 October at Rothwell and on 12 November at Impington. The maximum ascospore release was on 7 November at Bayfordbury, 17 November at Eye, 27 November at Rothwell and on 23 January at Impington (Fig. 4.1). In 2016/2017, there was a similar pattern in ascospore release at all four sites as observed in the 2015/2016 season, with the maximum ascospore release on 15 November at Bayfordbury, 8 December at Rothwell, 10 December at Eye and 1 February at Impington (Fig. 4.2). In 2017/2018, ascospore release was earlier at all sites than in the previous two seasons, with the maximum ascospore release on 19 October at Bayfordbury, 20 October at Rothwell, 19 October at Eye and 21 December at Impington (Fig. 4.3).

There were differences between sites in the numbers of ascospores released, with the largest numbers of ascospores recorded at Bayfordbury in 2015/2016 and at Eye in 2016/2017 and 2017/2018. The smallest numbers of ascospores in the air were recorded at Rothwell in all three seasons. These differences in numbers of ascospores in the air were due to the differences in the numbers of diseased stems and the severity of phoma stem canker on those stems from previous growing season's crop that were surrounding the Burkard spore samplers and the differences in weather conditions. At Rothwell, the Burkard spore sampler broke down in November 2017 and therefore no data were collected during this month (Fig. 4.3c).

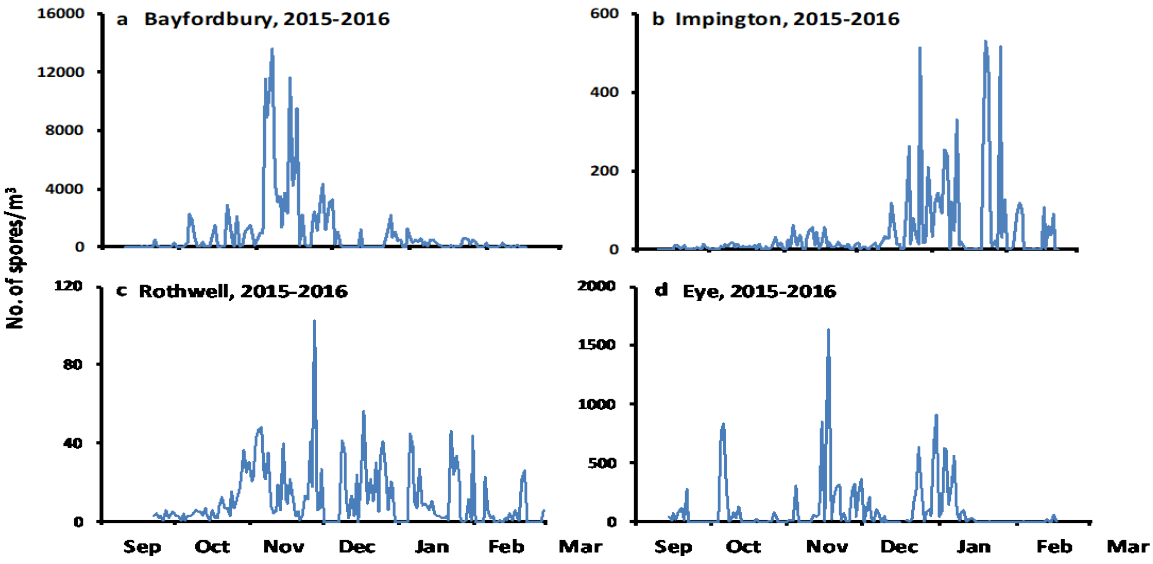

Fig. 4.1: Patterns of ascospore release in the 2015/2016 cropping season at four sites: (a) Bayfordbury in Hertfordshire; (b) Impington in Cambridgeshire; (c) Rothwell in Lincolnshire and (d) Eye in Suffolk. Using a light microscope, the daily release of ascospores was counted on the spore tapes from Burkard air samplers collected from September 2015 to March 2016.

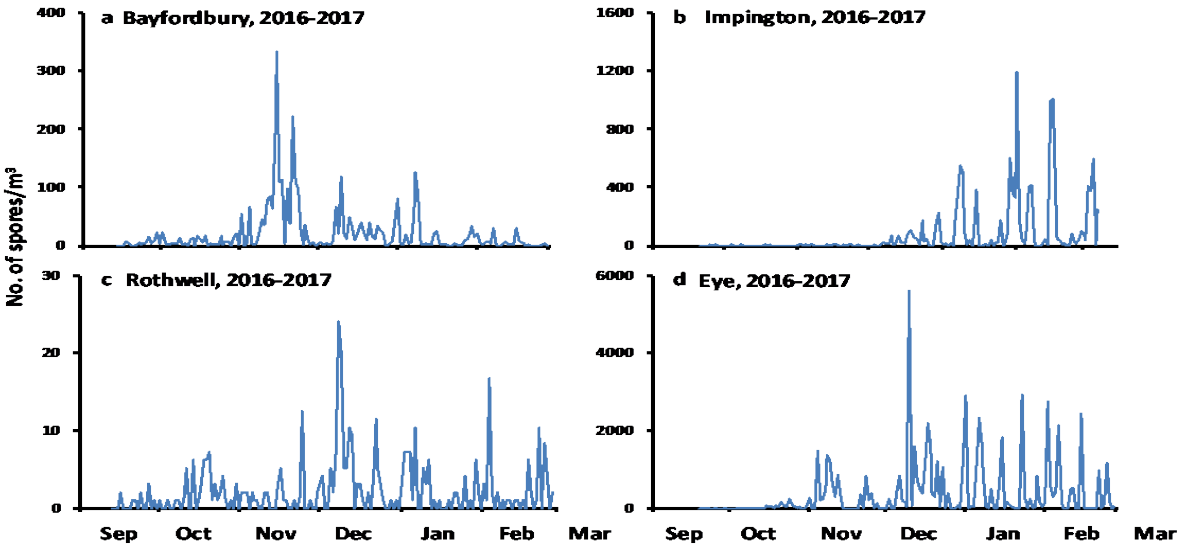

Fig.

Fig. 4.2: Patterns of ascospore release in the 2016/2017 cropping season at four sites: (a) Bayfordbury in Hertfordshire; (b) Impington in Cambridgeshire; (c) Rothwell in Lincolnshire and (d) Eye in Suffolk. Using a light microscope, the daily release of ascospores was counted on the spore tapes from Burkard air samplers collected from September 2016 to March 2017.

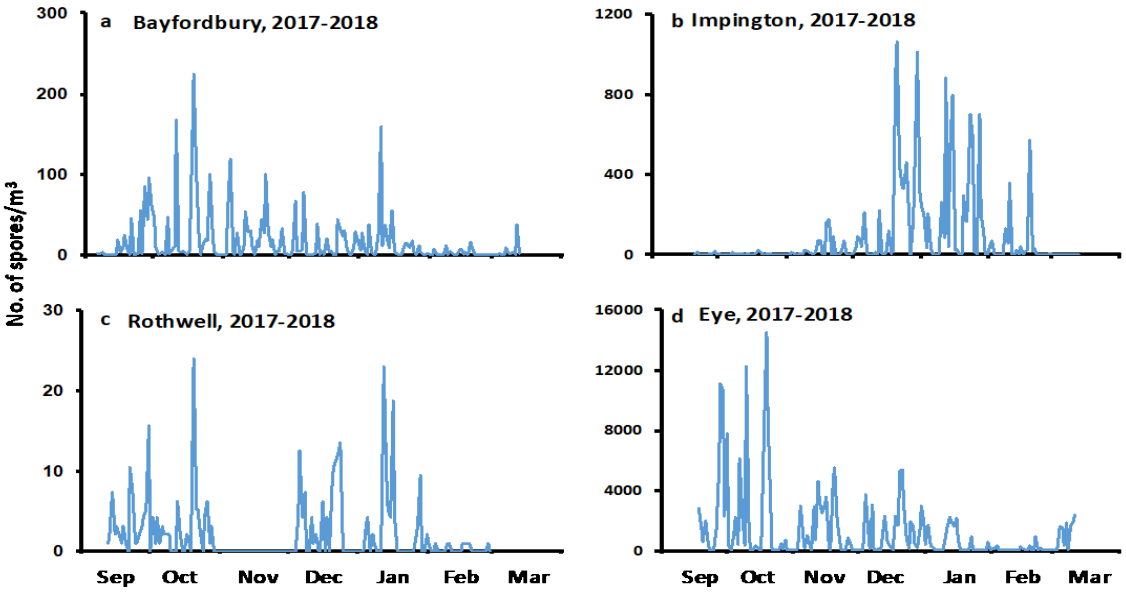

Fig. 4.3: Patterns of ascospore release in the 2017/2018 cropping season at four sites: (a) Bayfordbury in Hertfordshire; (b) Impington in Cambridgeshire; (c) Rothwell in Lincolnshire and (d) Eye in Suffolk. Using a light microscope, the daily release of ascospores was counted on the spore tapes from Burkard air samplers collected from September 2017 to March 2018. At Rothwell (c), the Burkard spore sampler broke down in Nov 2017 and therefore no data were collected during this month.

4.1.2. Proportions of *L. maculans* and *L. biglobosa* ascospores in air samples

Results of qPCR analysis of the spore tapes from the four sites in the three cropping seasons showed that there were differences between sites and between seasons in the proportions and timings of *L. biglobosa* and *L. maculans* ascospore release. At Bayfordbury, there was slightly more *L. biglobosa* DNA than *L. maculans* DNA in 2015/2016 and 2016/2017 but more *L. maculans* DNA than *L. biglobosa* DNA in 2017/2018 detected (Fig. 4.4); suggesting that there were more *L. biglobosa* ascospores than *L. maculans* ascospores in 2015/2016 and 2016/2017 but more *L. maculans* ascospores than *L. biglobosa* ascospores in 2017-2018. At Impington, there was a similar pattern in seasons for the amounts of *L. maculans* DNA and *L. biglobosa* DNA to that observed at Eye, where 2015/2016 and 2017/2018 had more *L. maculans* DNA than *L. biglobosa* DNA, but 2016/2017 had more detection of *L. biglobosa* DNA than *L. maculans* DNA (Fig. 4.5). At Rothwell, there was more *L. maculans* DNA than *L. biglobosa* DNA detected in all three seasons (Fig. 4.6), suggesting that all the three seasons had more *L. maculans* ascospores than *L. biglobosa* ascospores. At Eye, there was slightly more *L. maculans* DNA than *L. biglobosa* DNA in 2015/2016 and 2017/2018. However, in 2016/2017, most of the DNA detected was *L. biglobosa* DNA with small amounts of *L. maculans* DNA detected (Fig. 4.7).

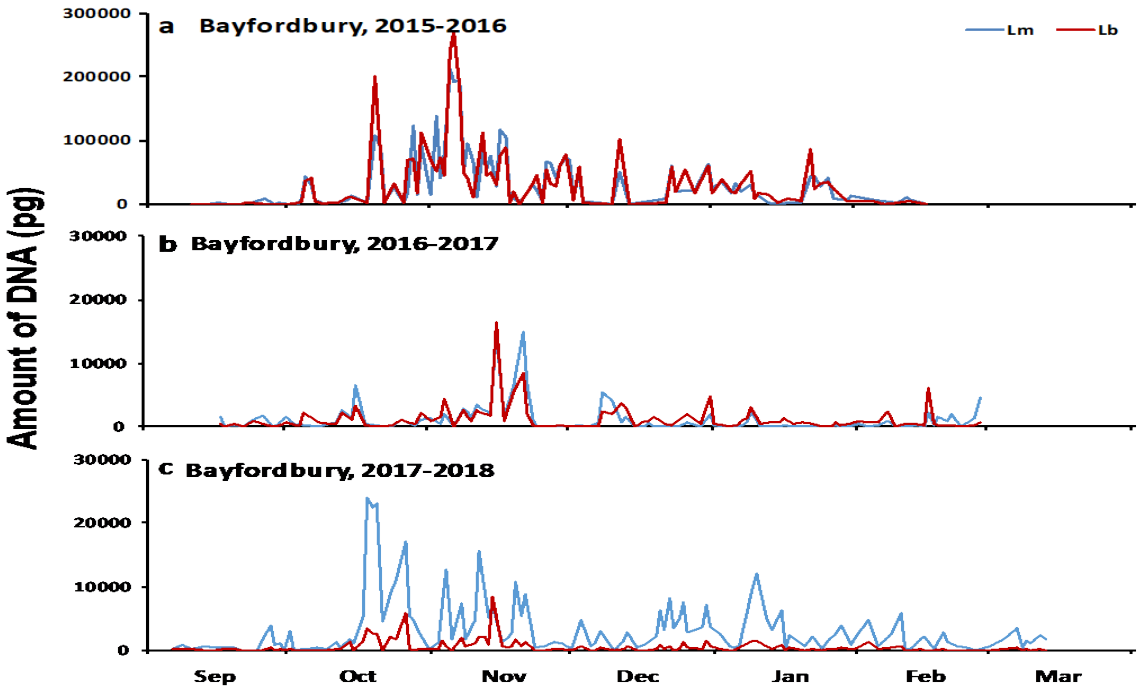

Fig. 4.4: Amounts of *L. maculans* (Lm) and *L. biglobosa* (Lb) DNA detected on spore tapes at Bayfordbury in Hertfordshire in 2015-2016 (a), 2016-2017 (b) and 2017-2018 (c). Amounts of *L. maculans* and *L. biglobosa* DNA in picograms detected on halves of spore tapes using qPCR.

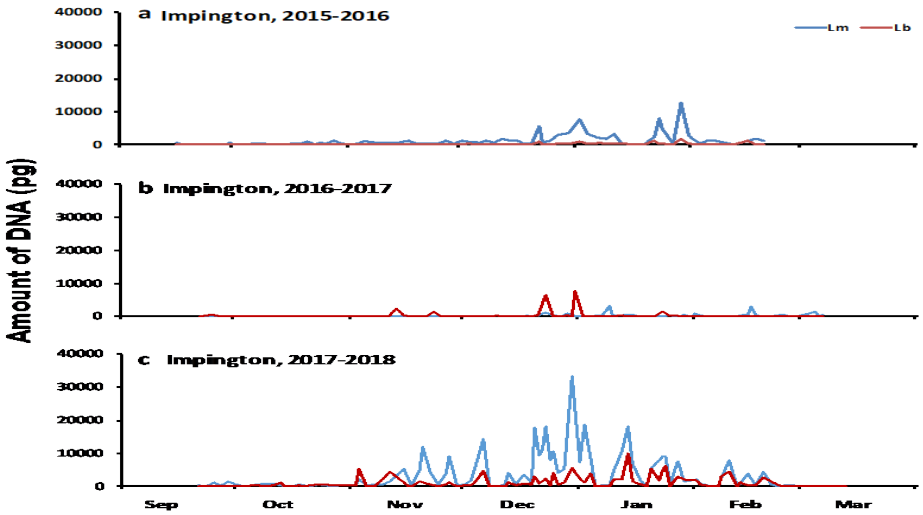

Fig. 4.5: Amounts of *L. maculans* (Lm) and *L. biglobosa* (Lb) DNA detected on spore tapes at Impington in Cambridgeshire in 2015-2016 (a), 2016-2017 (b) and 2017-2018 (c). Amounts of *L. maculans* and *L. biglobosa* DNA in picograms detected on halves of spore tapes using qPCR.

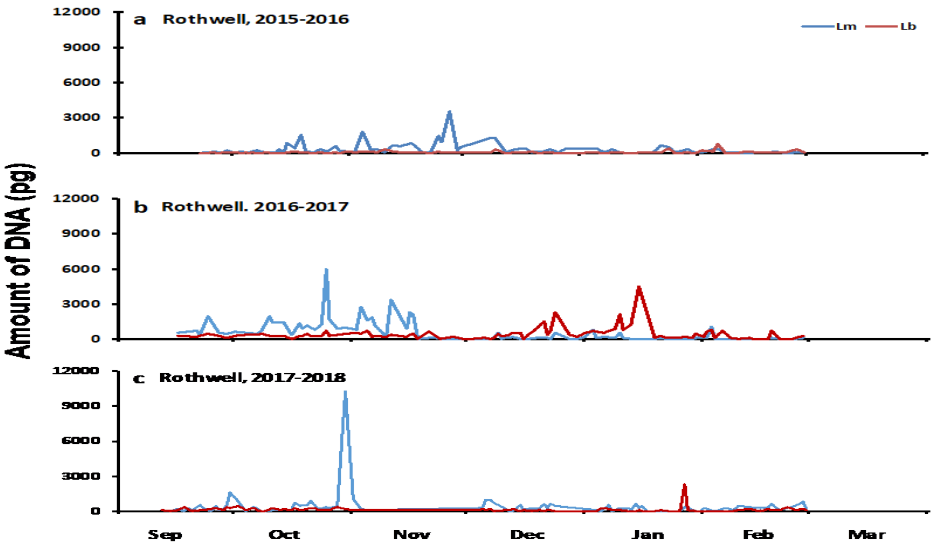

Fig. 4.6: Amounts of *L. maculans* (Lm) and *L. biglobosa* (Lb) DNA detected on spore tapes at Rothwell in Lincolnshire in 2015-2016 (a), 2016-2017 (b) and 2017-2018 (c). Amounts of *L. maculans* and *L. biglobosa* DNA in picograms detected on halves of spore tapes using qPCR.

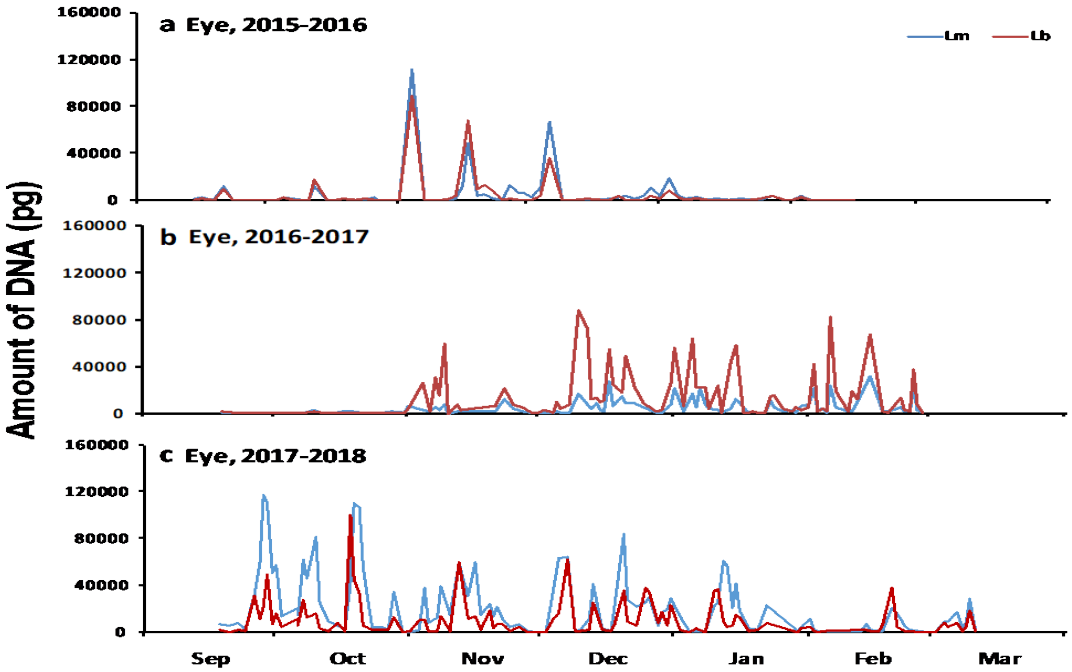

Fig. 4.7: Amounts of *L. maculans* (Lm) and *L. biglobosa* (Lb) DNA detected on spore tapes at Eye in Suffolk in 2015-2016 (a), 2016-2017 (b) and 2017-2018 (c). Amounts of *L. maculans* and *L. biglobosa* DNA in picograms detected on halves of spore tapes using qPCR.

For the timing of ascospore release at Bayfordbury, Impington and Eye, *L. maculans* DNA and *L. biglobosa* DNA were detected at the same time on most of the days in all the three seasons (Figs. 4.4 - 4.5, 4.7), suggesting that *L. maculans* and *L. biglobosa* ascospores were released at the same time. However, at Rothwell, although both *L. maculans* DNA and *L. biglobosa* DNA were detected at similar times throughout all the seasons, greater amounts of *L. maculans* DNA were detected earlier in the seasons followed by later detection of greater amounts of *L. biglobosa* DNA, especially in 2016/2017 and 2017/2018; suggesting that the ascospores released earlier were mainly *L. maculans* and ascospores released later were mainly *L. biglobosa* (Fig. 4.6).

4.1.3. Effects of environmental factors on ascospore release

At Bayfordbury, the daily temperature and rainfall in August-March varied between the three cropping seasons (Fig. 4.8). Temperature and rainfall in August-September are the main factors that affect the maturation of pseudothecia and the timing of ascospore release. August and September were hot and dry in 2016 by comparison with 2015 and 2017. The mean temperature for August and September was greater in 2016 (17.05°C) than in 2015 (14.49°C) and 2017 (14.78°C). The number of days with rainfall >0.5 mm and the total rainfall in 2016 (20 days, 100.46 mm) were less than those in 2015 (21 days, 128.68 mm) and 2017 (29 days, 153.30 mm). The hot and dry weather in the summer of 2016 led to later release of ascospores in 2016 than in 2015 and 2017 (Fig. 4.8). In 2015/2016, early-mid October was dry with intermittent days of heavy rainfall. The following months (late October to March) had regular rainfall (2-8 mm) with occasional dry days and five days with

heavy rain. The total rainfall was recorded as 148.7 mm in October-November, 174.1 mm in December-January and 131.2 mm in February-March (Fig.4.8a). The ascospore release was observed between late October and early March on days after rainfall. Few ascospores were released when temperature was ≤2°C. In 2016-2017, the average daily temperature ranged from a maximum of 23.3°C in August to a minimum of -1.2°C in December (Fig. 4.8b). The weather was mostly dry in August, September and October. November was a wet month with several days of rainfall >5 mm followed by a dry period from late November to early December. The following months (mid December-March) had constant rainfall with eight days with >5 mm rain. The spore release reached its maximum in November and continued until mid-February, after which there was little or no spore release (Fig. 4.8b). In 2017-2018, the average daily temperature ranged from a maximum of *c.* 20°C on 28 August to a minimum of -4.4°C on 28 February (Fig. 4.8c). Rainfall started earlier this season with five days with >10 mm rainfall in August and September. The following months from October-March had regular rainfall. The ascospores were released earlier than in the other two seasons (Fig. 4.8).

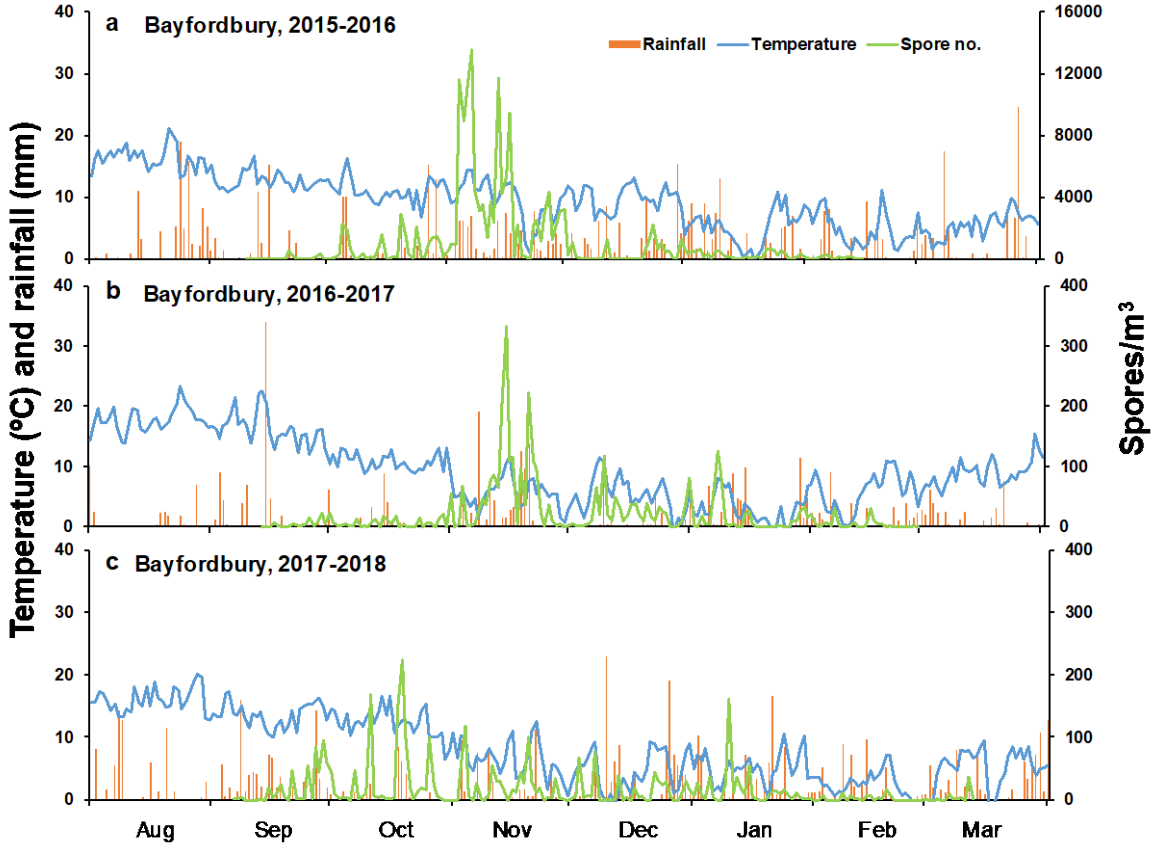

Fig. 4.8: Daily ascospore release in relation to daily temperature (°C) and rainfall (mm) in 2015-2016 (a), 2016-2017 (b) and 2017-2018 (c) at Bayfordbury. A Burkard spore sampler was set up at Bayfordbury, Hertfordshire surrounded by crop debris from the previous growing season and ascospore release was monitored from September to March in each season. The daily temperature and rainfall were monitored by an on-site weather station.

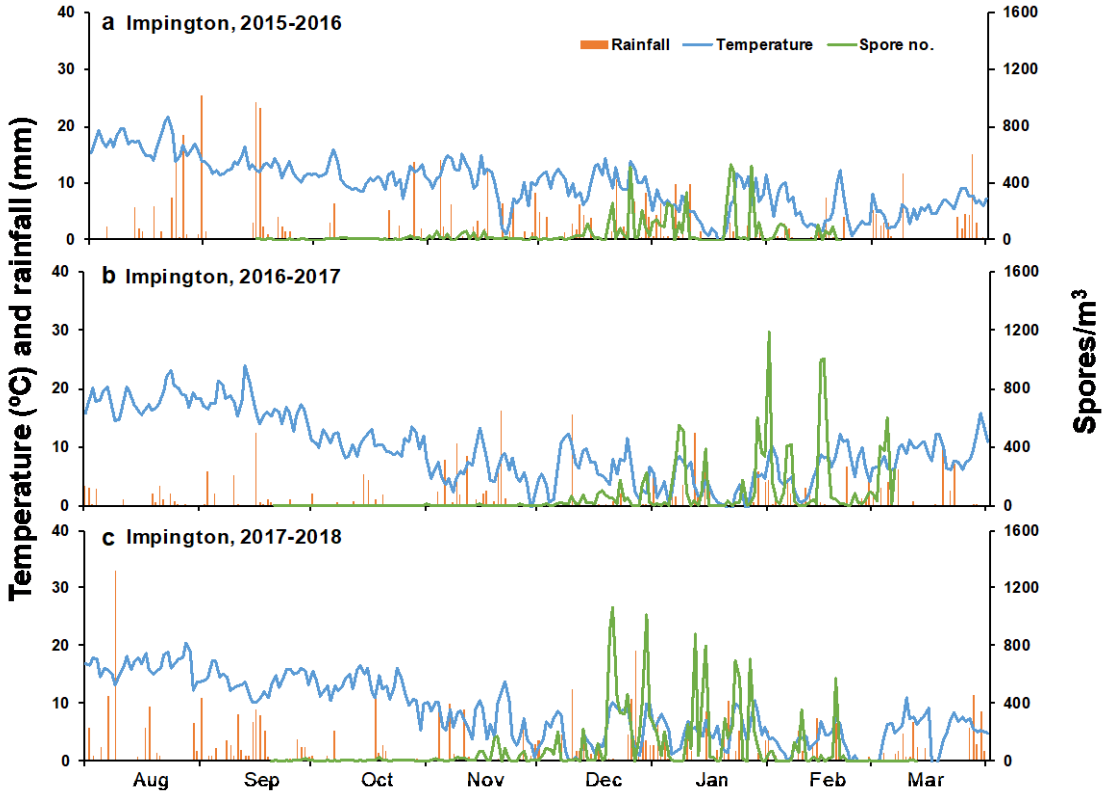

Fig. 4.9: Daily ascospore release in relation to daily temperature (°C) and rainfall (mm) in 2015-2016 (a), 2016-2017 (b) and 2017-2018 (c) at Impington. A Burkard spore sampler was set up at Impington, Cambridgeshire surrounded by crop debris from the previous growing season and ascospore release was monitored from September to March in each season. The daily temperature and rainfall were monitored at a weather station near the site.

At Impington, the daily temperature and rainfall in August-March varied between the three seasons (Fig. 4.9). Similar as at Bayfordbury, August and September were hot and dry in 2016 by comparison with 2015 and 2017. The mean temperature for August and September was greater in 2016 (17.61°C) than in 2015 (14.75°C) or 2017 (15.17°C). The number of days with rainfall >0.5 mm and the total rainfall in 2016 (17 days, 51.6 mm) were less than those in 2015 (22 days, 152.6 mm) and 2017 (32 days, 153.8 mm). The hot and dry weather in the summer 2016 led to later release of ascospores in 2016 than in 2015 and 2017 (Fig. 4.9). In 2015-2016, the daily temperature ranged from a maximum of 21.5°C in August to a minimum of -1.2°C in January (Fig. 4.9a). There was sporadic rainfall in August-September with eight days of heavy rainfall (>5 mm) interrupted by dry periods with no or very little rain. The weather was dry in October with only three days with rainfall >5 mm and no or very little rainfall in between these days. There was regular rainfall in November-February with several days of heavy rainfall (>5 mm). In 2016-2017 (Fig. 4.9b), the daily temperature ranged from a maximum of 23.8°C in September to a minimum of -1.5°C in January. There was very little rainfall in August-September with only three days with rainfall >5mm. The weather continued to be dry in October-November with only six days with rainfall >5 mm. In December-March, there was

little but regular rainfall. In 2017-2018 (Fig. 4.9c), the average daily temperature ranged from 20.5°C in August to -4.8°C in February. There was sporadic rainfall in August-September, with a heavy rainfall (33 mm) on 9 August followed by ten days of heavy rainfall (>5 mm) until September. There were fewer days with rain in October-November than in other months. There was regular rainfall during from December until March. The wet weather in 2017, especially in August-September, led to earlier release of ascospores than in the other two seasons (Fig. 4.9).

At Rothwell, the daily temperature and rainfall in August-March varied between the three seasons (Fig. 4.10). The mean temperature for August and September was greater in 2016 (16.42°C) than in 2015 (14.35°C) and 2017 (14.69°C). The number of days with rainfall >0.5 mm and the total rainfall in 2016 (20 days, 86.6 mm) were less than those in 2015 (19 days, 87.6 mm) and 2017 (28 days, 148.6 mm). In 2015/2016 (Fig. 4.10a), the average daily temperature ranged from 20.2°C in August to 1°C in January. There were several days with no rainfall in early August followed by constant daily rainfall (>5 mm) in mid-late August. Heavy rainfall (23 mm) was recorded on 18 August. It was very dry in September with only one day having rainfall >5 mm. It was dry in October with only five days that had rainfall >5 mm. There was regular rainfall from November until early March. Ascospores were released from late October and ascospore release continued until late February. In 2016/2017 (Fig. 4.10b), the daily temperature ranged from a maximum of 21.6°C in August to a minimum of -0.5°C in January. The weather was dry in early August followed by constant daily rainfall in late August with two days with rainfall >5 mm. In September, there was rainfall >5 mm on three days followed by small amounts of rainfall in the latter half of the month. There was regular rainfall from October until early March. Ascospore release started from late September until late February with very few or no spores released when temperature decreased <2°C. In 2017/2018 (Fig. 4.10c), daily temperature ranged from a maximum of 20.1°C in August to a minimum of -3.2°C in February. It was wet in August-September, with sporadic rainfall in August followed by small amounts of regular rainfall (0.2-5 mm) in September. The weather was mostly dry in October-November with a total of 69.4 mm rainfall. There was regular rainfall during December-March. Ascospore release was detected in mid-September on the first day of Burkard spore sampler operation indicating that spore release had already started. The spore release continued until late February. The spore sampler broke down twice; once in November for the whole month and then in late December for a week.

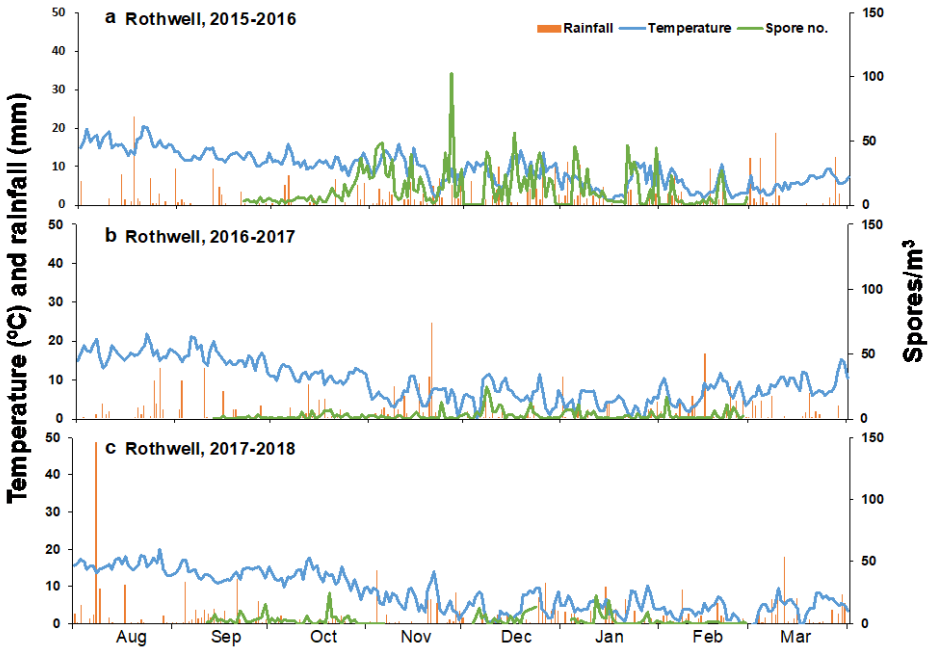

Fig. 4.10: Daily ascospore release in relation to daily temperature (°C) and rainfall (mm) in 2015-2016 (a), 2016-2017 (b) and 2017-2018 (c) at Rothwell. A Burkard spore sampler was set up at Rothwell, Lincolnshire surrounded by crop debris from the previous growing season and ascospore release was monitored from September to March in each season. The daily temperature and rainfall were monitored at a weather station near the site. The gaps in spore release in the 2017/2018 season (Fig. 4.10c) were due to breakdown of the spore sampler (broke down twice; once in November for the whole month and then in late December for a week).

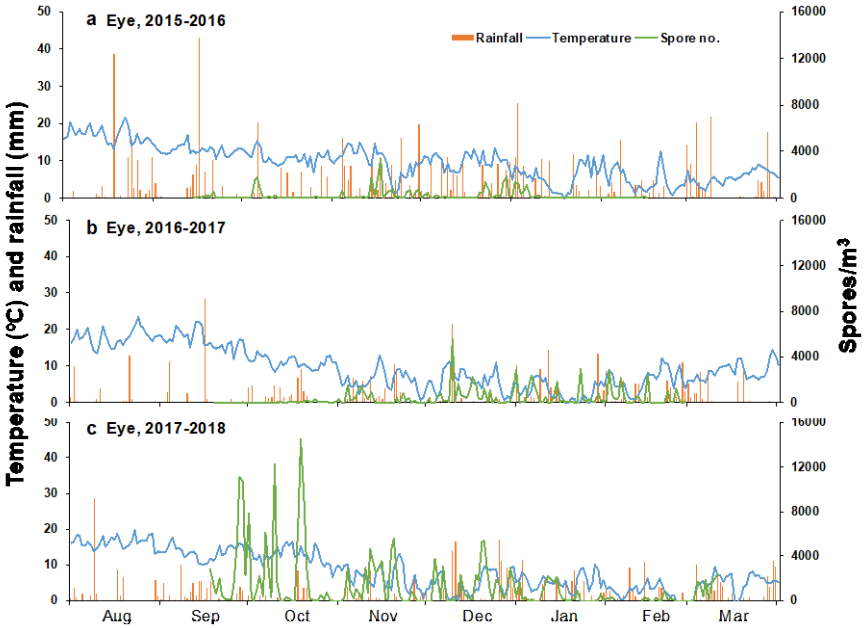

Fig. 4.11: Daily ascospore release in relation to daily temperature (°C) and rainfall (mm) in 2015-2016 (a), 2016-2017 (b) and 2017-2018 (c) at Eye. A Burkard spore sampler was set up at Eye, Suffolk surrounded by crop debris from the previous growing season and ascospore release was monitored from September to March in each season. The daily temperature and rainfall were monitored at a weather station near the site.

At Eye, the daily temperature and rainfall in August-March varied between the three seasons (Fig. 4.11). The mean temperature for August and September was greater in 2016 (17.5°C) than in 2015 (14.95°C) and 2017 (14.97°C). The number of days with rainfall >0.5 mm and the total rainfall in 2016 (15 days, 79.82 mm) were less than those in 2015 (26 days, 196.0 mm) and 2017 (26 days, 119.4 mm). In 2015/2016 (Fig. 4.11a), the daily temperature ranged from a maximum of 21.7°C on 22 August to a minimum -0.3°C on 19 January. Early August was generally dry, followed by heavy and regular rainfall in mid-late August. It was dry in early September followed by constant rainfall from mid-September onwards. There was regular rainfall for the rest of the season until February. A few ascospores were detected on the first day of Burkard spore sampler operation, indicating that spore release had already started in September. The ascospore release continued until January, following which there was very little spore release (Fig. 4.11a). In 2016/2017 (Fig. 4.11b), the daily temperature ranged from a maximum of 23.6°C in August to a minimum of -2°C in January. It was very dry in August-September with only four days with >5 mm rainfall followed by dry periods until early October. This was a period of constant rainfall in October interrupted by a dry period in November. From December to March, there were consecutive days with heavy rainfall. The ascospore release started in late October and continued until late February. In 2017/2018 (Fig. 4.11c), the daily temperature ranged from 19.6°C in August to -4.7°C in February. It was wet in August-September with heavy rainfall (28.8 mm) on 9 August followed by six days of rainfall >5mm and several days with 2-5 mm rainfall in September. There was regular rainfall from October to March. It was wet in December-January with a total rainfall of 177.8 mm. A large number of ascospores were detected on the very first day of Burkard sampler operation, indicating that spore release had started before the Burkard spore sampler was set up at this site. The spore release coincided with a very wet period of continuous rainfall in mid-September. Ascospores were continuously released from September until mid-March with very few spores detected on days when average daily temperature was <2°C (Fig. 4.11c).

4.2. Investigation of cultivar resistance against *L. biglobosa*

4.2.1. Cultivar resistance in field experiments

Differences in cultivar resistance against *L. maculans* and *L. biglobosa* were investigated using field experiments with six cultivars in three growing seasons and AHDB RL DOP experiments with 17 cultivars in two growing seasons.

4.2.1.1. Field experiments with six cultivars

There were significant differences between cultivars in the severity of phoma leaf spot and phoma stem canker in different seasons (P<0.05). In 2015/2016 at Boxworth, cv Quartz had the smallest numbers of *L. maculans* phoma leaf spots. However, there were no significant differences between Quartz and cvs Fencer, Harper and DK Cabernet (DKC) in numbers of *L. maculans* phoma leaf

spots. Cultivar Incentive had the greatest numbers of *L. maculans* phoma leaf spots. Cultivar Fencer had a significantly greater number of *L. biglobosa* phoma leaf spots than cvs DKC, PR46W21 and Incentive (Table 4.1). Cultivars Fencer, Harper, Quartz and DKC had significantly less severe phoma stem canker than PR46W21 and Incentive (Table 4.1). In 2016/2017, Fencer and Harper had a significantly smaller number of *L. maculans* phoma leaf spots and less severe phoma stem canker than cvs PR46W21, Incentive and DKC (Table 4.2). Cultivar Quartz had more *L. biglobosa* phoma leaf spots than other cultivars except for DKC. In 2017/2018, there were no significant differences between cultivars in numbers of *L. maculans* phoma leaf spots (Table 4.3). There were significant differences between cultivars in the number of *L. biglobosa* phoma leaf spots, with cv. Harper having the greatest numbers of *L. biglobosa* phoma leaf spots (Table 4.3). For phoma stem canker, cvs Fencer, Quartz, Harper and DKC had significantly less severe phoma stem canker than cvs Incentive and PR46W21 (Table 4.3).

Table 4.1: Numbers of *L. maculans* (Lm) and *L. biglobosa* (Lb) phoma leaf spots per plant and severity of phoma stem canker for untreated plots in 2015/2016 at Boxworth. Ten plants were randomly selected from each of the three replicate plots and assessed for numbers of Lm and Lb phoma leaf spots in autumn/winter. Twenty plants were randomly selected from each of the replicate plots for each cultivar and assessed for severity of phoma stem canker on a 0-7 scale before harvest.

Cultivar	Number of Lm phoma leaf spots per plant[1]	Number of Lb phoma leaf spots per plant[1]	Phoma stem canker severity[1]
DK-Cabernet	1.77 ^{ab}	0.20 ^{a}	1.82 ^{a}
Fencer	1.93 ^{ab}	1.20 ^{b}	0.92 ^{a}
Harper	1.87 ^{ab}	0.80 ^{ab}	1.08 ^{a}
Incentive	5.87 ^{c}	0.07 ^{a}	3.86 ^{c}
PR46W21	3.00 ^{b}	0.17 ^{a}	2.83 ^{b}
Quartz	1.57 ^{a}	0.37 ^{a}	1.68 ^{a}
Mean	2.670	0.467	2.031

[1]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

Table 4.2: Numbers of *L. maculans* (Lm) and *L. biglobosa* (Lb) phoma leaf spots per plant and severity of phoma stem canker for untreated plots in 2016/2017 at Terrington. Ten plants were randomly selected from each of the three replicate plots and assessed for numbers of Lm and Lb phoma leaf spots in autumn/winter. Twenty plants were randomly selected from each of the replicate plots for each cultivar and assessed for severity of phoma stem canker on a 0-7 scale before harvest.

Cultivar	Number of Lm phoma leaf spots per plant[1]	Number of Lb phoma leaf spots per plant[1]	Phoma stem canker severity[1]
DK-Cabernet	7.73 ^{c}	0.97 ^{ab}	2.03 ^{b}
Fencer	0.80 ^{a}	0.63 ^{a}	0.28 ^{a}
Harper	1.40 ^{a}	0.73 ^{a}	0.62 ^{a}
Incentive	6.20 ^{bc}	0.77 ^{a}	1.80 ^{b}
PR46W21	4.87 ^{b}	0.77 ^{a}	2.87 ^{c}
Quartz	2.73 ^{a}	1.20 ^{b}	0.80 ^{a}
Mean	3.960	0.844	1.400

[1]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

Table 4.3: Numbers of *L. maculans* (Lm) and *L. biglobosa* (Lb) phoma leaf spots per plant and severity of phoma stem canker for untreated plots in 2017/2018 at Terrington. Ten plants were randomly selected from each of the three replicate plots and assessed for numbers of Lm and Lb phoma leaf spots in autumn/winter. Twenty plants were randomly selected from each of the replicate plots for each cultivar and assessed for severity of phoma stem canker on a 0-7 scale before harvest.

Cultivar	Number of Lm phoma leaf spots per plant	Number of Lb phoma leaf spots per plant[1]	Phoma stem canker severity[1]
DK-Cabernet	3.13	1.43 ^{ab}	0.70 ^{a}
Fencer	0.47	0.73 ^{a}	0.10 ^{a}
Harper	0.73	1.90 ^{b}	0.85 ^{a}
Incentive	3.40	0.57 ^{a}	3.28 ^{b}
PR46W21	2.03	0.83 ^{a}	3.28 ^{b}
Quartz	1.50	1.30 ^{ab}	0.40 ^{a}
Mean	1.88	1.13	1.436

[1]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

4.2.1.2. AHDB RL DOP field experiments

There were significant differences between sites and cultivars in the numbers of *L. maculans* and *L. biglobosa* phoma leaf spots in the 2014/2015 cropping season ($P<0.05$). There were also interactions between site and cultivar in the numbers of *L. maculans* and *L. biglobosa* phoma leaf spots ($P<0.001$), with smaller numbers of *L. maculans* lesions and greater numbers of *L. biglobosa* lesions at Broughton than at Morley (Fig. 4.12). At Broughton, cv. RLDOP16 had significantly more *L. maculans* phoma leaf spots than all the other cultivars, while cultivar RLDOP8 had significantly more *L. biglobosa* phoma leaf spots than all other cultivars. At Morley, cv. RLDOP1 had significantly more *L. maculans* phoma leaf spots than all other cultivars, while cultivar RLDOP4 had significantly more *L. biglobosa* phoma leaf spots than all other cultivars (Fig. 4.12).

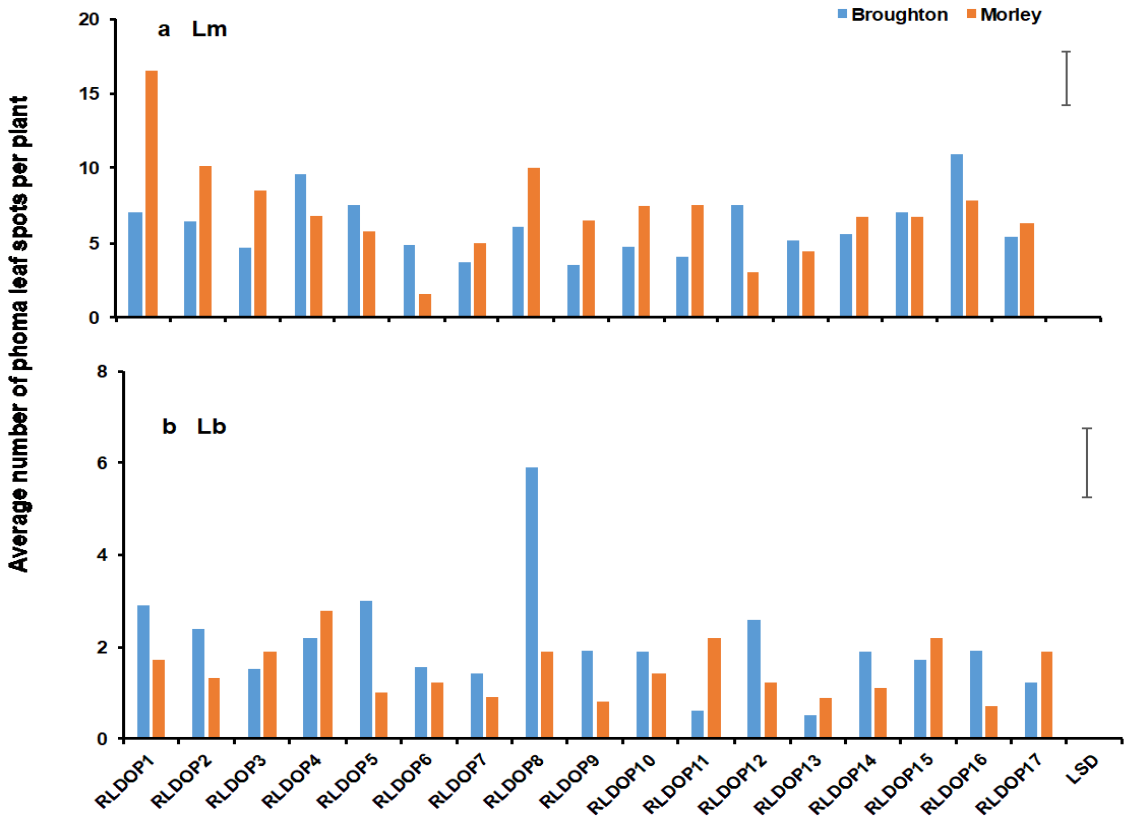

Fig. 4.12: Numbers of *L. maculans* (Lm) and *L. biglobosa* (Lb) phoma leaf spots per plant on 17 cultivars at Broughton in Hampshire and Morley in Norfolk in 2014/2015. Ten plants were randomly sampled from each of the 17 field plots and numbers of *L. maculans* and *L. biglobosa* phoma leaf spots on each plant were counted. The LSD bars represent the Least Significant Differences in the mean numbers of phoma leaf spots caused by Lm or Lb between different cultivars

In the 2015/2016, there were significant differences between sites and cultivars in the numbers of *L. maculans* and *L. biglobosa* phoma leaf spots ($P<0.01$; Fig. 4.13). There were significant interactions between site and cv. ($P<0.001$) with smaller numbers of *L. maculans* lesions and greater numbers

of *L. biglobosa* lesions at Broughton than at Morley (Fig. 4.13), similar as observed in the previous season. At Broughton, cv. RLDOP6 had the smallest number of *L. maculans* lesions than all the other cultivars, while cultivar RLDOP13 had the smallest number of *L. biglobosa* phoma leaf spots than all other cultivars. At Morley, cv. RLDOP21 had less *L. maculans* phoma leaf spots than all other cultivars, while cultivar RLDOP16 had significantly less *L. biglobosa* phoma leaf spots than all other cultivars (Fig. 4.13).

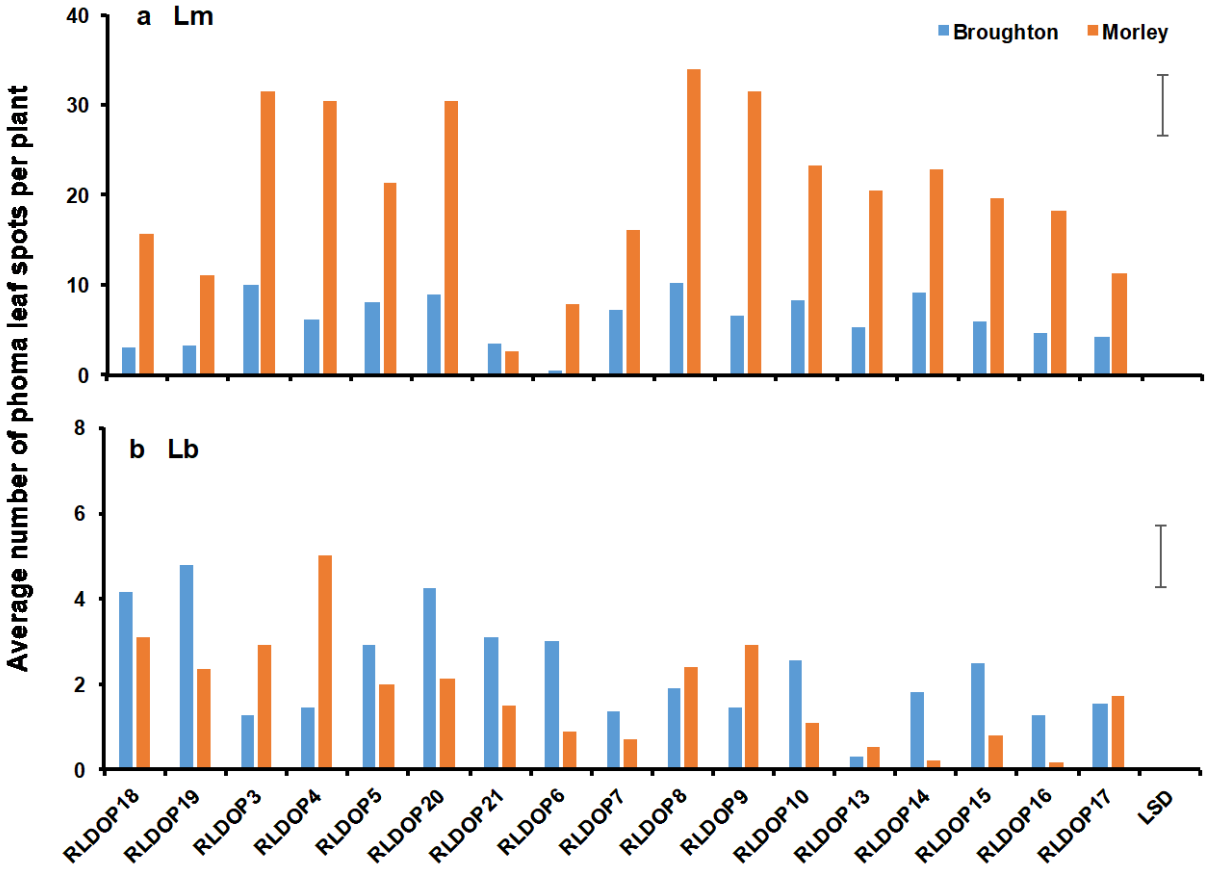

Fig. 4.13: Numbers of *L. maculans* (Lm) and *L. biglobosa* (Lb) phoma leaf spots per plant on 17 cultivars at Broughton in Hampshire and Morley in Norfolk in 2015/2016. Ten plants were randomly sampled from each of the 17 field plots and numbers of *L. maculans* and *L. biglobosa* phoma leaf spots on each plant were counted. The LSD bars represent the Least Significant Differences in the mean numbers of phoma leaf spots caused by Lm or Lb between different cultivars.

There were significant differences between sites and between cultivars in the severity of phoma stem canker (P<0.001) in both cropping seasons (Fig. 4.14). In 2014/2015, cvs had more severe phoma stem canker at Broughton than at Morley whereas in 2015/2016, cvs had more severe stem canker at Morley than at Broughton. In 2014/2015 at Broughton, cv RLDOP12 had the least severe stem canker than other cultivars, followed by RLDOP4 and RLDOP15 (Fig. 4.14a). In 2014/2015 at Morley, cv RLDOP6 had the least severe stem canker than other cultivars, followed by RLDOP14,

RLDOP4 and RLDOP5 (Fig. 4.14a). Cultivar RLDOP4 showed resistance at both Broughton and Morley, while RLDOP6 showed resistance at Morley but susceptibility at Broughton in 2014/2015. In 2015/2016, cultivars RLDOP6 and RLDOP21 showed resistance at both Broughton and Morley, while RLDOP19 showed resistance at Broughton but susceptibility at Morley (Fig. 4.14b).

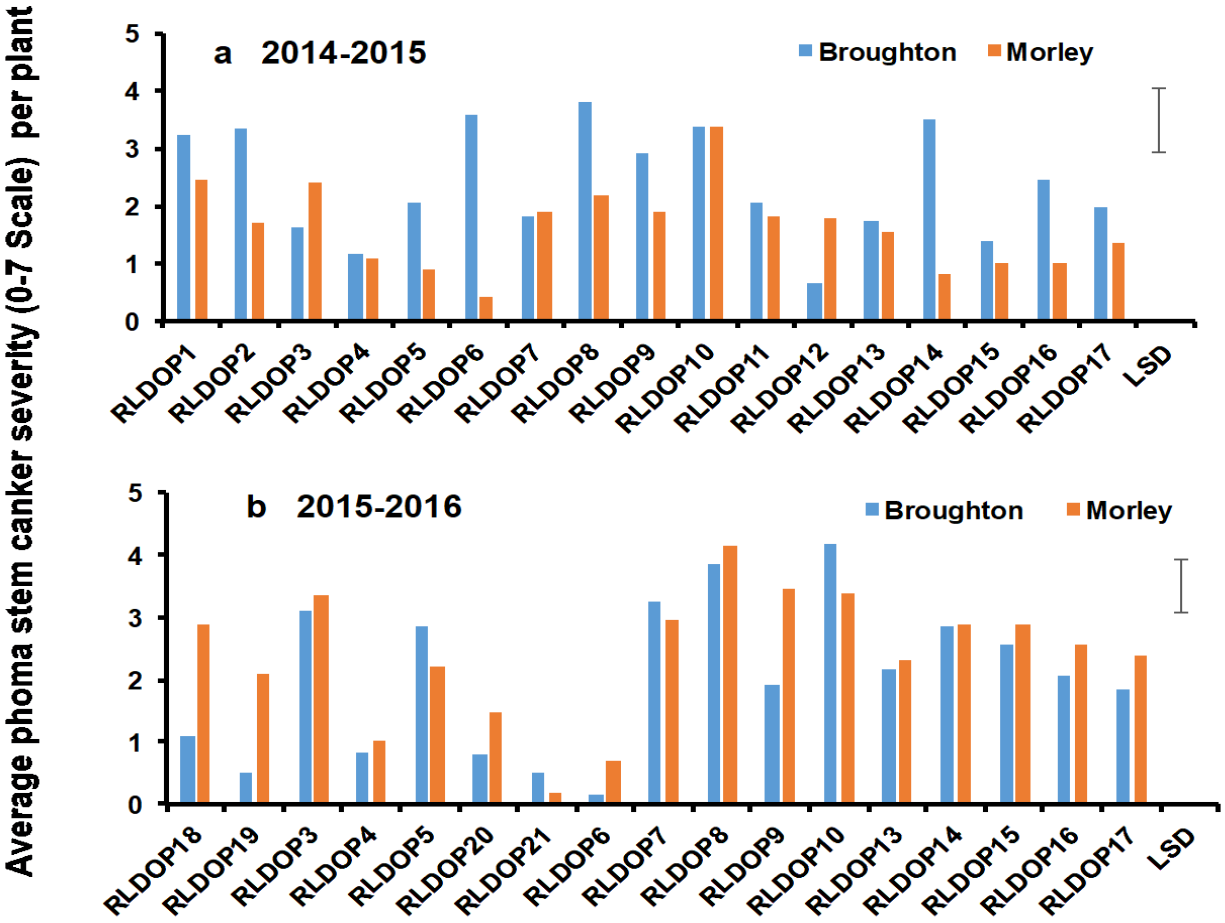

Fig. 4.14: Phoma stem canker severity on 17 cultivars at Broughton in Hampshire and Morley in Norfolk in the 2014/2015 and 2015/2016 cropping seasons. Twenty plants were randomly sampled from each plot in June/July before harvest and assessed for phoma stem canker severity on a 0-7 scale. The LSD bars represent the Least Significant Difference in mean severity of stem canker between different cultivars.

There were differences between cultivars and sites in the amounts of *L. maculans* and *L. biglobosa* DNA in stem base cankers in 2014/2015 and 2015/2016 cropping seasons (Figs. 4.15 & 4.16). In 2014/2015, there were significant differences between cultivars in the amount of *L. maculans* DNA (P<0.001) or *L. biglobosa* DNA (P<0.05) in stem cankers (Fig. 4.15). There were interactions between sites and cultivars in the amounts of *L. maculans* DNA (P<0.001) and *L. biglobosa* DNA (P<0.05) with more *L. maculans* DNA and less *L. biglobosa* DNA in stem cankers at Morley than at Broughton. Interestingly, at Broughton, a small amount of *L. maculans* DNA but large amount of *L. biglobosa* DNA was detected in stems of cultivar RLDOP12. Smaller amounts of both *L. maculans* DNA and *L. biglobosa* DNA in stems of RLDOP6 than in stems of all the other cultivars were detected

at both sites, suggesting that this cultivar is resistant to both *L. maculans* and *L. biglobosa*. However, the high stem canker severity score and low *L. maculans* DNA in RLDOP6 at Broughton suggest that RLDOP6 may have quantitative resistance to reduce the growth of *L. maculans* in the stem. Although small amounts of *L. maculans* and *L. biglobosa* DNA were also detected in stem base cankers of cultivars RLDOP5 and RLDOP15 in 2014/2015 at both sites (Fig. 4.15), large amounts of *L. maculans* DNA were detected in the next season 2015/2016 at both sites (Fig. 4.16) suggesting that cultivars RLDOP5 and RLDOP15 may have *R* gene mediated resistance which was partially broken-down in the season 2015/2016 due to pathogen population changes. In 2015/2016, there were significant differences between cultivars in the amount of *L. maculans* DNA ($P<0.01$) or *L. biglobosa* DNA ($P>0.05$) in stem cankers (Fig. 4.16). A small amount of *L. maculans* DNA but a large amount of *L. biglobosa* DNA was detected in stems of cultivar RLDOP21 at both sites, with similar results for cv RLDOP19 (Fig. 4.16), suggesting that these two cultivars were resistant to *L. maculans* but susceptible to *L. biglobosa*.

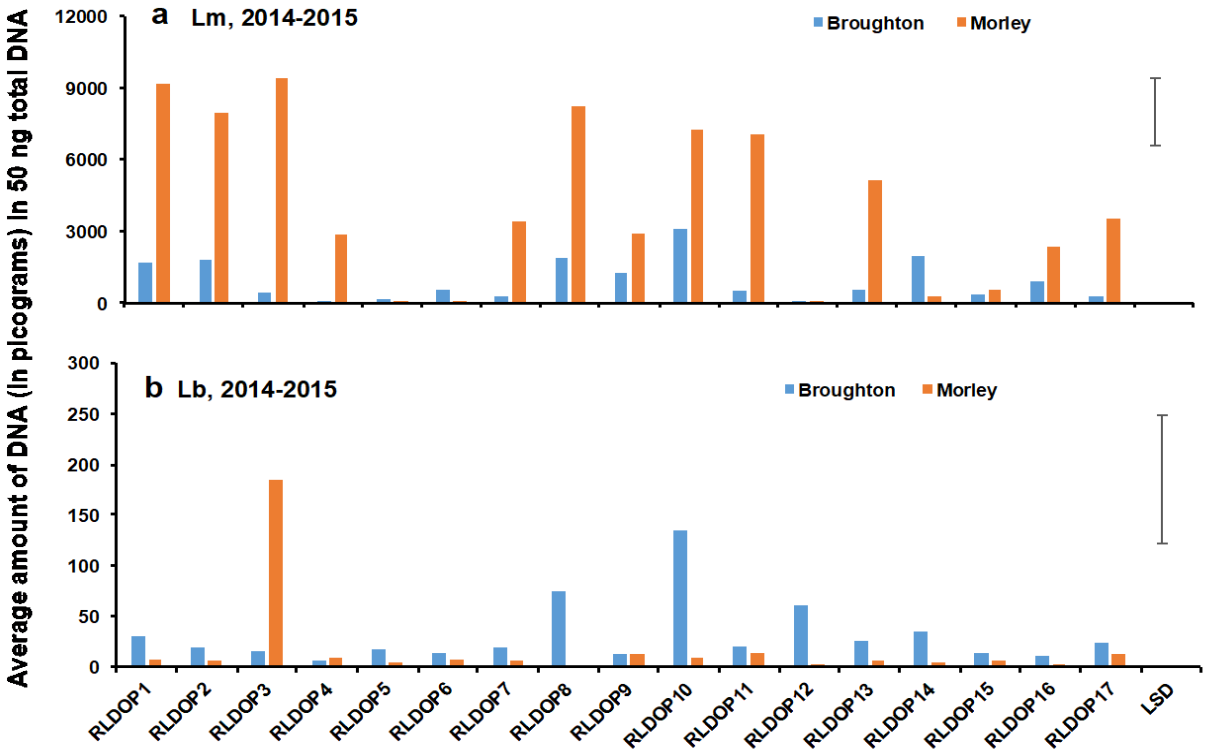

Figure 4.15: Amounts of *L. maculans* (Lm) and *L. biglobosa* (Lb) DNA in stem base cankers on different cultivars at Broughton in Hampshire and Morley in Norfolk in the 2014/2015 cropping season. Ten stems were randomly sampled from each of the 17 different cultivars in June/July before harvest. DNA was extracted from the stem samples and analysed using quantitative PCR (qPCR) to determine the amounts of Lm DNA or Lb DNA in stem base cankers of different cultivars. The LSD bars represent the Least Significant Difference in the mean amount of DNA between different cultivars.

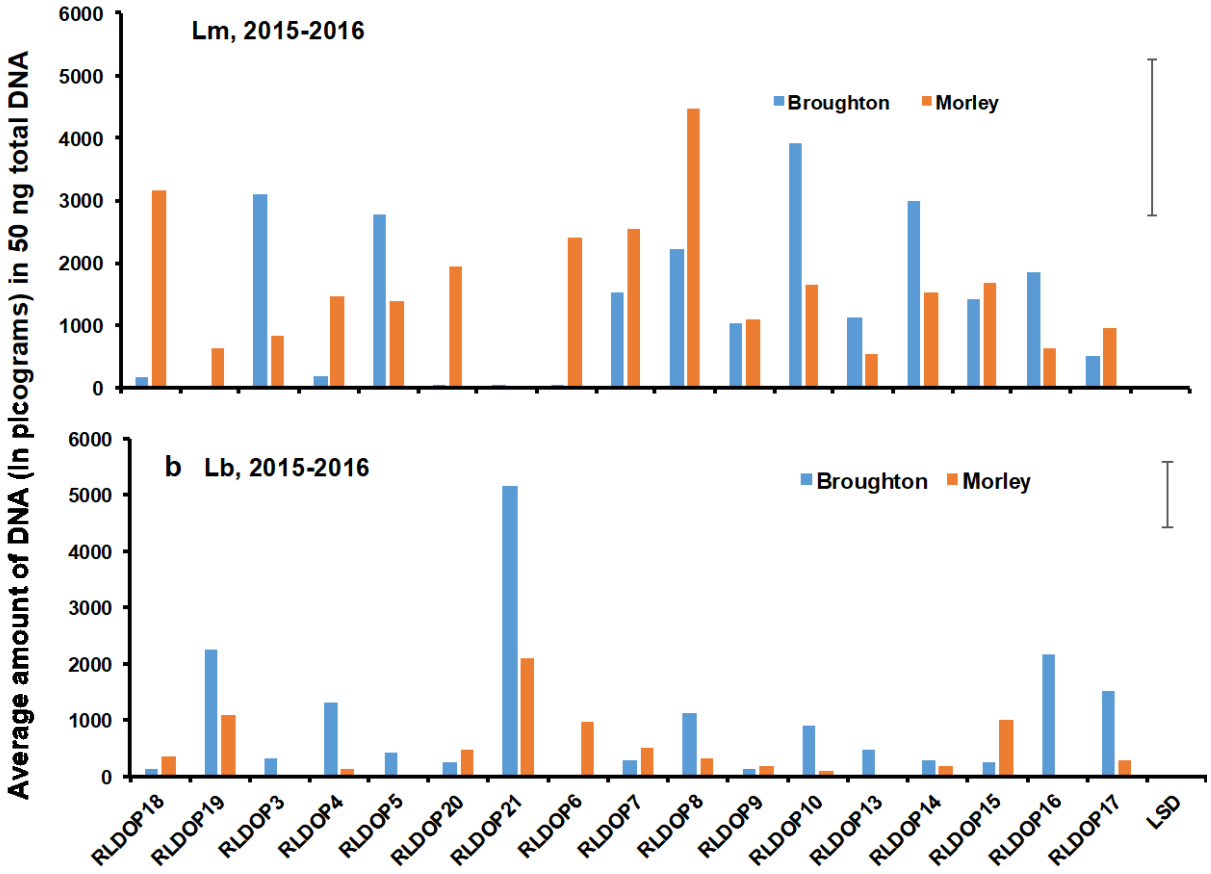

Figure 4.16: Amounts of *L. maculans* (Lm) and *L. biglobosa* (Lb) DNA in stem base cankers on different cultivars at Broughton in Hampshire and Morley in Norfolk in the 2015/2016 cropping season. Ten stems were randomly sampled from each of the 17 different cultivars in June/July before harvest. DNA was extracted from the stem samples and analysed using quantitative PCR (qPCR) to determine the amounts of Lm DNA or Lb DNA in stem base cankers of different cultivars. The LSD bars represent the Least Significant Difference in the mean amount of DNA between different cultivars.

4.2.2. Cultivar resistance in controlled environment experiments

There were differences between cultivars and isolates in the severity score and diameter of *L. maculans* or *L. biglobosa* phoma leaf spot lesions on cotyledons of different cultivars (Fig. 4.17 & 4.18). In Experiment 1, there were significant differences between cultivars in the severity score and diameter of *L. maculans* lesions (P<0.001). Cvs Drakkar and Topas had significantly greater severity scores and larger diameters of *L. maculans* lesions than cvs Fencer and Excel (Table 4.4). However, there were no significant differences between the two *L. maculans* isolates in the severity score or diameter of lesions (P>0.05) (Table 4.4). There were significant differences between *L. biglobosa* isolates and cultivars in the severity score and diameter of lesions (P<0.001) (Table 4.5). The severity score and diameter of *L. biglobosa* lesions were significantly greater on cv Drakkar than other cultivars. Cvs Excel and Topas had intermediate severity scores and diameters of *L. biglobosa*

lesions whereas cv Fencer had a significantly smaller severity score and diameter of *L. biglobosa* lesions compared to other cultivars (Table 4.5). Of the different *L. biglobosa* isolates, lesions caused by isolate BR16AS3 were less severe than those caused by other *L. biglobosa* isolates (Table 4.5), indicating differences in pathogenicity between different *L. biglobosa* isolates.

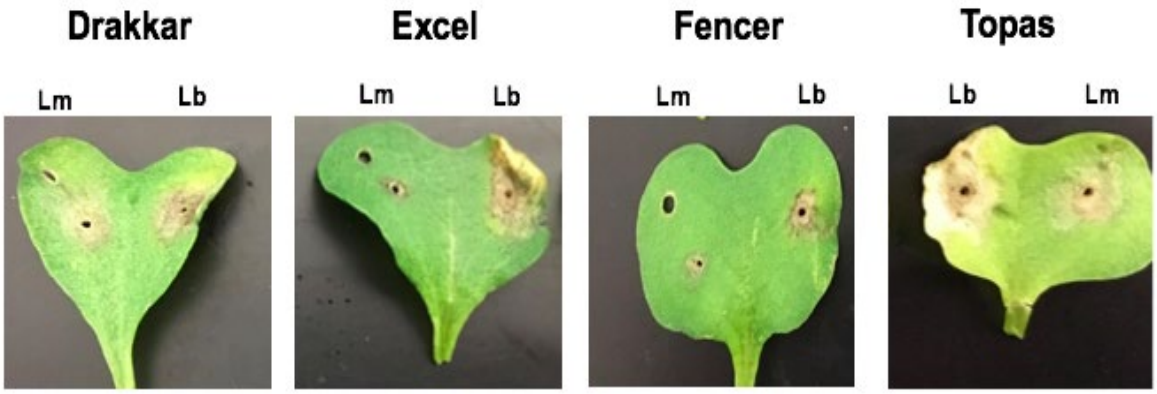

Figure 4.17: Symptoms of phoma leaf spots caused by *L. maculans* (Lm) or *L. biglobosa* (Lb) isolates on cotyledons of different cultivars at 16 days post inoculation in controlled environment experiment 1. Conidial suspensions (1x10^7 conidia/ml) of Lm isolate BR16AM14 or Lb isolate BR16AM9 were wound-inoculated onto cotyledons of four cultivars (Drakkar, Excel, Fencer and Topas).

Table 4.4: Severity score and diameter of phoma leaf spot lesions caused by *L. maculans* (Lm) isolates on four cultivars in controlled environment experiment 1.

	Severity score of Lm lesions on each cultivar[1]				
Lm isolate	**Drakkar**	**Excel**	**Fencer**	**Topas**	**Mean[2]**
BR16AM14	8.50	1.22	1.20	8.50	4.335 [a]
BR16SH2	8.67	1.33	1.00	8.50	4.354 [a]
LSD	0.592 (55 d.f.)				0.288 (55 d.f.)
Mean	1.105 [a]	1.275 [a]	8.500 [b]	8.579 [b]	4.345
	Diameter of Lm lesions on each cultivar				
Lm isolate	**Drakkar**	**Excel**	**Fencer**	**Topas**	**Mean**
BR16AM14	1.46	0.12	0.12	1.35	0.715 [a]
BR16SH2	1.67	0.13	0.10	1.25	0.678 [a]
LSD	0.246 (55 d.f.)				0.120 (55 d.f.)
Mean	0.111 [a]	0.128 [a]	1.302 [b]	1.560 [c]	0.697

[1]Conidial suspensions (1x10^7 conidia/ml) of the two Lm isolates were wound-inoculated onto cotyledons of the four cultivars; the phoma leaf spot lesions on cotyledons were assessed on a 0-9 scale at 16 days post inoculation. [2]Values designated by different letters indicate that the differences are statistically significant (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

Table 4.5: Severity score and diameter of phoma leaf spot lesions caused by *L. biglobosa* (Lb) isolates on four cultivars in controlled environment experiment 1.

Lm isolates	Severity score of Lb lesions on each cultivar[1]				Mean
	Drakkar	Excel	Fencer	Topas	
BR16AM9	7.00 efghi	7.00 efghi	3.90 bc	7.50 fghi	6.169 bc
BR16AS3	7.55 ghi	4.00 bc	1.72 a	4.80 cd	4.446 a
BR16AS6	7.91 i	5.86 de	2.82 ab	7.00 efghi	5.725 bc
BR16CK4	7.09 efghi	6.57 efghi	3.09 b	6.46 efgh	5.660 b
BR16FN7	7.83 ghi	7.78 gi	3.10 b	7.80 ghi	6.408 c
BR16FN8	8.00 i	8.00 i	2.90 ab	7.60 fghi	6.414 c
BR16HP3	7.89 i	7.56 ghi	2.88 ab	7.50 efghi	6.248 bc
BR16HP6	7.78 gi	7.78 gi	3.13 b	7.00 efghi	6.257 bc
BR16SH10	6.44 efg	7.22 efghi	3.88 bc	7.25 efghi	6.019 bc
W10	7.46 ghi	6.00 def	3.00 b	7.00 efghi	5.689 b
LSD	1.478 (290 d.f.)				0.725 (290 d.f.)
Mean	7.479 c	6.703 b	3.034 a	6.952 b	5.904

Lb isolates	Diameter of Lb lesions on each cultivar				Mean
	Drakkar	Excel	Fencer	Topas	
BR16AM9	1.09 defghijkl	1.16 fghijklm	0.45 b	1.22 fhijklmn	0.939 cde
BR16AS3	1.14 efghijklm	0.49 b	0.18 a	0.60 bc	0.595 a
BR16AS6	1.91 hjklmn	0.85 cd	0.36 ab	0.98 defghijk	0.818 bcd
BR16CK4	0.97 defghij	0.91 cdef	0.38 ab	0.92 defg	0.773 b
BR16FN7	1.35 lmn	1.29 klmn	0.37 ab	1.20 fghiklmn	1.017 e
BR16FN8	1.42 mn	1.45 n	0.37 ab	1.06 defghijkl	1.050 e
BR16HP3	1.32 lmn	1.23 hklmn	0.36 ab	1.03 defghijkl	0.962 e
BR16HP6	1.18 fghijklm	1.28 klmn	0.41 ab	0.93 cdefgh	0.930 cde
BR16SH10	1.13 efghijklm	1.28 klmn	0.53 b	0.93 cdefgh	0.951 de
W10	1.14 efghijklm	0.87 cde	0.37 ab	0.94 defghi	0.809 bc
LSD	0.288 (290 d.f.)				0.141 (290 d.f.)
Mean	1.182 c	1.059 b	0.376 a	0.974 b	4.865

[1]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

In Experiment 2, there were significant differences between *L. maculans* isolates and cultivars in the severity score and lesion diameters on different cultivars (P<0.001; Table 4.6; Fig. 4.14).

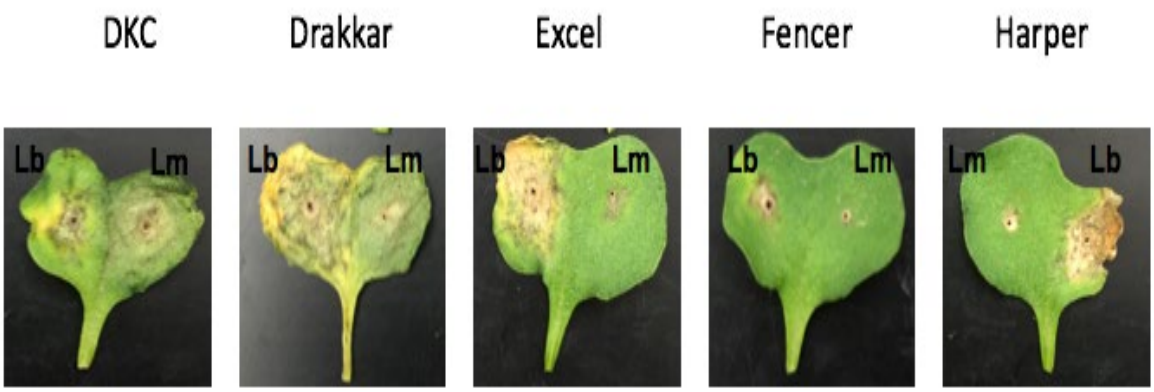

Fig. 4.18: Symptoms of phoma leaf spots caused by *L. maculans* (Lm) or *L. biglobosa* (Lb) isolates on cotyledons of different cultivars at 16 days post inoculation in controlled environment experiment 2. Conidial suspensions (1x10^7 conidia/ml) of Lm isolate NF16AM2 or Lb isolate NF16AM7 were wound-inoculated onto cotyledons of five cultivars (DKC - DK Cabernet, Drakkar, Excel, Fencer and Harper).

Table 4.6: Severity score and diameter of phoma leaf spot lesions caused by *L. maculans* (Lm) isolates on five cultivars in controlled environment experiment 2.

Lm isolates	Severity score of Lm lesions on each cultivar[1]					Mean[2]
	DKC	Drakkar	Excel	Fencer	Harper	
NF16AM2	4.69	6.75	1.13	0.82	1.13	2.947 ^{bc}
NF16FN5	1.60	2.50	0.50	0.71	0.71	1.207 ^{a}
NF16HP1	4.63	7.50	1.30	1.39	1.73	3.306 ^{c}
NF16HP5	3.63	6.20	0.90	0.23	1.09	2.397 ^{b}
LSD	1.738 (191 d.f)					0.767 (191 d.f)
Mean	3.782 ^{b}	6.001 ^{c}	0.992 ^{a}	0.793 ^{a}	1.209 ^{a}	2.464
Lm isolates	**Diameter of Lm lesions on each cultivar**					**Mean**
	DKC	**Drakkar**	**Excel**	**Fencer**	**Harper**	
NF16AM2	0.69 ^{c}	0.95 ^{cd}	0.11 ^{a}	0.09 ^{a}	0.11 ^{a}	0.396 ^{bc}
NF16FN5	0.15 ^{a}	0.29 ^{ab}	0.05 ^{a}	0.07 ^{a}	0.07 ^{a}	0.123 ^{a}
NF16HP1	0.69 ^{c}	1.17 ^{d}	0.13 ^{a}	0.15 ^{a}	0.23 ^{ab}	0.476 ^{c}
NF16HP5	0.43 ^{c}	1.04 ^{d}	0.09 ^{a}	0.02 ^{a}	0.14 ^{a}	0.329 ^{b}
LSD	0.303 (191 d.f)					0.134 (191 d.f)
Mean	0.508 ^{b}	0.912 ^{c}	0.099 ^{a}	0.086 ^{a}	0.144 ^{a}	

[1]Conidial suspensions (1x10^7 conidia/ml) of the four Lm isolates were wound-inoculated onto cotyledons of the five cultivars; the phoma leaf spot lesions on cotyledons were assessed on a 0-9 scale at 16 days post inoculation. [2]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

Table 4.7: Severity score and diameter of phoma leaf spot lesions caused by *L. biglobosa* (Lb) isolates on four cultivars in controlled environment experiment 2.

Lb isolates	Severity score of Lb lesions on each cultivar[1]					Mean
	DKC	Drakkar	Excel	Fencer	Harper	
NF16AM7	3.44 bcdef	6.00 ghi	4.00 cdef	1.27 a	6.75 hi	4.143 a
NF16DKC4	4.33 efg	1.86 ab	2.71 abcde	2.14 abc	4.86 fgh	3.284 a
NF16DKC7	3.88 cdef	3.17 bcdef	2.25 abc	2.33 abcd	3.57 bcdef	3.086 a
NF16FN2	4.20 defg	2.67 abcde	2.80 abcde	2.29 abc	7.00 i	3.810 a
LSD	1.891 (141 d.f.)					0.839 (141 d.f.)
Mean	3.928 c	3.604 bc	3.026 b	1.955 a	5.686 d	
Lb isolates	**Diameter of Lb lesions on each cultivar**					**Mean**
	DKC	**Drakkar**	**Excel**	**Fencer**	**Harper**	
NF16AM7	0.63 cdefg	0.99 fh	0.76 defgh	0.18 a	0.81 efgh	0.660 a
NF16DKC4	0.61 cdef	0.23 ab	0.43 abcde	0.31 abc	0.79 efgh	0.487 a
NF16DKC7	0.58 bcde	0.42 abcde	0.33 abc	0.32 abc	0.47 abcde	0.431 a
NF16FN2	0.63 cdef	0.33 abc	0.40 abcd	0.34 abc	1.14 h	0.571 a
LSD	0.382 (141 d.f.)					0.169 (141 d.f.)
Mean	0.614 b	0.522 b	0.499 b	0.282 a	0.814 c	

[1]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

Lesions caused by isolate NF16HP1 were more severe than isolates NF16HP5 and NF16FN5 (Table 4.6). The lesion diameter and severity score were significantly greater on Drakkar than on all other cultivars. DK Cabernet had intermediate severe lesions whereas cvs Excel, Fencer and Harper had least severe lesions. There were significant differences between cultivars in the severity score and diameter of lesions formed by *L. biglobosa* isolates (P<0.001). There were no significant differences between different *L. biglobosa* isolates (P>0.05; Table 4.7). Cultivar Harper had a significantly greater severity score and diameter of lesions than other cultivars. Cvs DK Cabernet, Drakkar and Excel had an intermediate severity score whereas cv. Fencer had a smaller severity score. Isolate NF16AM7 caused severe lesions on cvs Harper, Excel and Drakkar, intermediately severe lesions on DK Cabernet and least severe lesions on cv. Fencer (Figure 4.18). Lesions caused by isolate NF16DKC4 had significantly greater severity scores and diameters on cvs Harper and DK Cabernet than on other three cultivars. For three cultivars with *Rlm7* resistance, the isolate NF16FN2 caused more severe lesions on cv Harper than on cvs Excel and Fencer (Table 4.7).

4.3. Effects of fungicides on control of *L. biglobosa* compared to *L. maculans*

4.3.1. Effects of fungicides on control of phoma stem canker on different cultivars in field experiments

The six cultivars with different 'field' phoma stem canker resistance ratings (Table 3.2) were used for fungicide field experiments in three growing seasons (2015/2016, 2016/2017 and 2017/2018). These cultivars were treated with two fungicides (Proline and Refinzar) at two spray timings (early spray and late spray). Effects of the two fungicides on control of phoma leaf spot and phoma stem canker were assessed.

4.3.1.1. Effects of fungicides on severity of phoma leaf spots caused by *L. biglobosa* and *L. maculans* on different cultivars

There were differences in severity of phoma leaf spot on different cultivars between the three cropping seasons. In 2015/2016, there were no differences between untreated and fungicide-treated plots in numbers of *L. maculans* or *L. biglobosa* phoma leaf spots per plant for all cultivars (Table 4.8). These data were not totally reliable as the crops were badly damaged by cabbage stem flea beetle; plots were very patchy. There were no interactions between cultivar and fungicide treatment in the average number of *L. maculans* or *L. biglobosa* phoma leaf spots. Cultivars

Table 4.8: Numbers of *L. biglobosa* (Lb) and *L. maculans* (Lm) phoma leaf spots per plant for untreated and fungicide-treated plots in 2015/2016 at Boxworth.

Cultivar	Number of Lm phoma leaf spots per plant[1]			Mean[2]
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	1.77	3.33	4.60	3.233 [c]
Fencer	1.93	1.33	1.00	1.422 [a]
Harper	1.87	1.70	1.00	1.522 [a]
Incentive	5.87	6.07	4.87	5.600 [d]
PR46W21	3.00	3.00	3.13	3.044 [bc]
Quartz	1.57	3.10	1.43	2.033 [ab]
LSD	0.816 (34 d.f.)			1.154 (34 d.f.)
Mean	2.670 [a]	3.090 [a]	2.670 [a]	
Cultivar	Number of Lb phoma leaf spots per plant			Mean
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	0.20	0.20	0.17	0.189 [a]
Fencer	1.20	0.57	0.30	0.689 [b]
Harper	0.80	0.77	0.70	0.756 [b]
Incentive	0.07	0.63	0.43	0.378 [a]
PR46W21	0.17	0.23	0.40	0.267 [a]
Quartz	0.37	0.27	0.20	0.278 [a]
LSD	0.212 (34 d.f.)			0.300 (34 d.f.)
Mean	0.467 [a]	0.444 [a]	0.367 [a]	

[1]Ten plants were randomly sampled from each of the 54 plots and assessed for numbers of Lm and Lb phoma leaf spots in autumn/winter. [2]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

Fencer and Harper had less *L. maculans* phoma leaf spots than cvs DK-Cabernet, Incentive and PR46W21. However, cvs Fencer and Harper had more *L. biglobosa* phoma leaf spots than all the other cultivars.

In 2016/2017, both fungicides significantly reduced the number of *L. maculans* or *L. biglobosa* phoma leaf spots on all treated cultivars compared to the untreated plots (Table 4.9). There were no significant interactions between cultivar and fungicide treatment in the number of *L. biglobosa* phoma leaf spots, but there were significant interactions between cultivar and fungicide treatment for the number of *L. maculans* phoma leaf spots. Both fungicides were equally effective in reducing the number of *L. maculans* phoma leaf spots on cvs Incentive, PR46W21 and DK-Cabernet with no significant effects on cvs Fencer and Harper.

In 2017/2018, both fungicides significantly reduced the average number of *L. maculans* or *L. biglobosa* phoma leaf spots on all treated cultivars compared to the untreated (Table 4.10). There were no significant interactions between cultivar and fungicide treatment in the number of *L. maculans* phoma leaf spots but there were significant interactions between cultivar and fungicide treatment in the number of *L. biglobosa* phoma leaf spots. Both fungicides significantly reduced the number of *L. biglobosa* phoma leaf spots on cvs DK-Cabernet, Harper and Quartz with no significant effects on cvs Fencer, Incentive and PR46W21.

Table 4.9: Numbers of *L. biglobosa* (Lb) and *L. maculans* (Lm) phoma leaf spots per plant for untreated and fungicide-treated plots in 2016/2017 at Terrington.

Cultivar	Number of Lm phoma leaf spots per plant[1]			Mean[2]
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	7.73 [e]	0.57 [a]	0.57 [a]	2.960 [c]
Fencer	0.80 [a]	0.37 [a]	0.27 [a]	0.480 [a]
Harper	1.40 [a]	0.37 [a]	0.43 [a]	0.730 [ab]
Incentive	6.20 [d]	0.87 [a]	0.50 [a]	2.520 [c]
PR46W21	4.87 [c]	1.37 [a]	0.83 [a]	2.360 [c]
Quartz	2.73 [b]	0.63 [a]	0.53 [a]	1.300 [b]
LSD	0.544 (34 d.f.)			0.769 (34 d.f.)
Mean	3.960 [b]	0.690 [a]	0.520 [a]	
Cultivar	Number of Lb phoma leaf spots per plant			Mean
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	0.97	0.70	0.33	0.667 [a]
Fencer	0.63	0.17	0.37	0.389 [a]
Harper	0.73	0.47	0.80	0.667 [a]
Incentive	0.77	0.47	0.40	0.544 [a]
PR46W21	0.77	0.30	0.43	0.500 [a]
Quartz	1.20	0.27	0.40	0.622 [a]
LSD	0.155 (34 d.f.)			0.219 (34 d.f.)
Mean	0.844 [b]	0.394 [a]	0.456 [a]	

[1]Ten plants were randomly sampled from each of the 54 plots and assessed for numbers of Lm and Lb phoma leaf spots in autumn/winter. [2]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

Table 4.10: Numbers of *L. biglobosa* (Lb) and *L. maculans* (Lm) phoma leaf spots per plant for untreated and fungicide-treated plots in 2017/2018 at Terrington.

Cultivar	Number of Lm phoma leaf spots per plant[1]			Mean[2]
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	3.13	0.73	0.93	1.600 [a]
Fencer	0.47	0.20	0.20	0.290 [a]
Harper	0.73	0.50	0.10	0.444 [a]
Incentive	3.40	0.87	0.30	1.520 [a]
PR46W21	2.03	0.40	0.30	0.910 [a]
Quartz	1.50	0.47	0.90	0.960 [a]
LSD	0.928 (34 d.f.)			1.313 (34 d.f.)
Mean	1.880 [b]	0.530 [a]	0.460 [a]	
Cultivar	Number of Lb phoma leaf spots per plant			Mean
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	1.43 [ef]	0.83 [abcde]	0.67 [abcd]	0.978 [bc]
Fencer	0.73 [abcd]	0.73 [abcd]	0.77 [abcd]	0.744 [ab]
Harper	1.90 [f]	1.17 [cde]	0.43 [ab]	1.167 [c]
Incentive	0.57 [abc]	0.83 [abcde]	0.20 [a]	0.533 [a]
PR46W21	0.83 [abcde]	0.93 [bcde]	0.80 [abcde]	0.856 [abc]
Quartz	1.30 [def]	0.27 [a]	0.50 [ab]	0.689 [ab]
LSD	0.265 (34 d.f.)			0.375 (34 d.f.)
Mean	1.128 [b]	0.794 [a]	0.561 [a]	

[1]Ten plants were randomly sampled from each of the 54 plots and assessed for numbers of Lm and Lb phoma leaf spots in autumn/winter. [2]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

4.3.1.2. Effects of fungicides on severity of phoma stem canker

There were differences between cultivars in the severity of phoma stem canker in different seasons. In the 2015/2016 growing season, both fungicides reduced the severity of phoma stem canker on cultivars as compared to untreated plots, especially for cvs DK-Cabernet, Incentive and PR46W21 (Table 4.11a). In 2016/2017, similarly both fungicides significantly reduced the severity of phoma stem canker on cultivars compared to the untreated plots, especially for DK-Cabernet, Incentive and PR46W21 (Table 4.11b). There were significant interactions between cultivars and fungicides for the severity of phoma stem canker (P<0.05). Fungicide Refinzar was more effective than Proline in reducing the severity of phoma stem canker on cv. Incentive compared to untreated plots. For cvs Fencer and Harper, there were no significant effects of either fungicide in reducing phoma stem canker severity (Table 4.11b).

In 2017/2018, both fungicides significantly reduced the average severity of phoma stem canker on cultivars compared to the untreated plots (Table 4.11c). There were significant interactions between cultivar and fungicide in the severity of phoma stem canker (P<0.05). Both fungicides significantly and equally reduced the severity of phoma stem canker on cvs Incentive and PR46W21 (P<0.05), with no significant effects on DK-Cabernet, Fencer, Harper and Quartz.

Table 4.11: Severity of phoma stem canker for untreated and fungicide-treated plots for six cultivars in three growing seasons (2015/16, 2016/2017, 2017/2018)

Cultivar	**Table 4.11a: 2015/2016, Boxworth[1]**			Mean[2]
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	1.82	0.74	0.61	1.054 [b]
Fencer	0.92	0.43	0.27	0.539 [a]
Harper	1.08	0.52	0.33	0.644 [ab]
Incentive	3.86	1.90	1.80	2.518 [d]
PR46W21	2.83	1.16	1.98	1.993 [c]
Quartz	1.68	0.51	0.97	1.053 [b]
LSD	0.310 (34 d.f.)			0.438 (34 d.f.)
Mean	2.031[b]	0.876 [a]	0.993 [a]	1.300
Cultivar	**Table 4.11b: 2016/2017, Terrington**			Mean
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	2.03 [f]	0.87 [de]	0.72 [cd]	1.206 [b]
Fencer	0.28 [abc]	0.13 [a]	0.15 [a]	0.189 [a]
Harper	0.62 [bcd]	0.18 [ab]	0.33 [abc]	0.378 [a]
Incentive	1.80 [f]	1.27 [e]	0.43 [abcd]	1.167 [b]
PR46W21	2.87 [g]	0.83 [de]	0.85 [de]	1.517 [c]
Quartz	0.80 [d]	0.25 [ab]	0.20 [ab]	0.417 [a]
LSD	0.187 (34 d.f.)			0.265 (34 d.f.)
Mean	1.400 [b]	0.589 [a]	0.447 [a]	0.812
Cultivar	**Table 4.11c: 2017/2018, Terrington**			Mean
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	0.70 [abcd]	0.63 [abc]	0.70 [abcd]	0.678 [b]
Fencer	0.10 [ab]	0.27 [ab]	0.05 [a]	0.139 [a]
Harper	0.85 [bcd]	0.47 [abc]	0.13 [ab]	0.483 [ab]
Incentive	3.28 [f]	1.65 [e]	1.07 [cde]	2.000 [c]
PR46W21	3.28 [f]	1.62 [e]	1.40 [de]	2.100 [c]
Quartz	0.40 [abc]	0.47 [abc]	0.18 [ab]	0.350 [ab]
LSD	0.313 (34 d.f.)			0.442 (34 d.f.)
Mean	1.436 [b]	0.850 [a]	0.589 [a]	0.958

[1]Twenty plants were randomly sampled from each of the 54 plots and assessed for severity of phoma stem canker on a 0-7 scale in June/July before harvest. [2]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

4.3.1.3. Effects of fungicide on the amounts of *L. maculans* and *L. biglobosa* DNA in stem base cankers on different cultivars

There were differences between cultivars in the effects of fungicides on the amounts of *L. maculans* and *L. biglobosa* DNA in stem base cankers in different seasons. In 2015/2016, there were

significant differences between cultivars in the amounts of *L. maculans* or *L. biglobosa* DNA (Table 4.12). There were also significant interactions between cultivar and fungicide for the amounts of *L. maculans* or *L. biglobosa* DNA in stem base cankers *(P*<0.05*)*. Refinzar reduced the amount of *L. maculans* DNA in stem cankers only on cv. Quartz. Fungicide Proline had no effects in reducing amount of *L. maculans* DNA on any cultivars. Both fungicides were equally effective in reducing the amount of *L. biglobosa* DNA in stem base cankers on cvs Fencer, Harper and Quartz. For cv. Incentive, Proline reduced the amount of *L. biglobosa* DNA in stem cankers while Refinzar had no significant effect in reducing the amount of *L. biglobosa* DNA in stem cankers.

Table 4.12: Amounts of *L. maculans* (Lm) or *L. biglobosa* (Lb) DNA in stem base cankers on different cultivars from untreated and fungicide-treated plots in 2015/2016 at Boxworth, Cambridge

Cultivar	Amount of Lm DNA in stem cankers[1]			Mean[2]
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	3.00 ^{bcdef}	2.64 ^{bcde}	2.76 ^{bcde}	2.806 ^{ab}
Fencer	2.55 ^{bcd}	2.50 ^{bcd}	2.03 ^{ab}	2.340 ^{a}
Harper	2.28 ^{abc}	2.35 ^{abcd}	2.16 ^{ab}	2.253 ^{a}
Incentive	3.79 ^{f}	3.16 ^{cdef}	3.78 ^{f}	3.606 ^{c}
PR46W21	3.21 ^{cdef}	3.16 ^{bcdef}	3.64 ^{f}	3.354 ^{bc}
Quartz	3.52 ^{ef}	3.44 ^{def}	1.30 ^{a}	2.674 ^{a}
LSD	0.408 (55 d.f.)			0.581 (55 d.f.)
Mean	3.130 ^{b}	2.905 ^{ab}	2.691 ^{a}	
Cultivar	Amount of Lb DNA in stem cankers			Mean
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	1.32 ^{abcd}	1.13 ^{abc}	1.84 ^{bcdef}	0.903 ^{bc}
Fencer	2.24 ^{efg}	0.94 ^{a}	0.96 ^{a}	0.728 ^{ab}
Harper	2.59 ^{fg}	1.162 ^{abcd}	1.22 ^{abcd}	0.631 ^{a}
Incentive	1.87 ^{bcdef}	1.07 ^{a}	1.75 ^{abcdef}	0.983 ^{c}
PR46W21	1.30 ^{abcd}	1.08 ^{ab}	1.52 ^{abcde}	0.982 ^{c}
Quartz	2.95 ^{g}	1.16 ^{abcd}	1.98 ^{bdef}	0.591 ^{a}
LSD	0.339 (55 d.f.)			0.483 (55 d.f.)
Mean	2.005 ^{c}	1.093 ^{a}	1.589 ^{b}	

[1]Ten stems were randomly sampled from each plot in the first replicate for DNA extraction and qPCR to determine the amounts of Lm and Lb DNA in 50 ng total DNA in stem base cankers. For statistical analysis, the amount of DNA was natural logarithm (ln) transformed. [2]Values designated by different letters indicate significant differences (*P*<0.05) in the Least Significant Difference (LSD) multiple comparison test.

In 2016/2017, there were significant differences between cultivars in the amount of *L. maculans* DNA but no differences in amount of *L. biglobosa* DNA (Table 4.13). Both fungicides were ineffective in reducing the amount of *L. maculans* DNA in stem base cankers on all cultivars compared to untreated plots. However, both fungicides were equally effective in reducing the amount of *L. biglobosa* DNA on all cultivars compared to untreated plots (Table 4.13).

Table 4.13: Amounts of *L. maculans* (Lm) or *L. biglobosa* (Lb) DNA in stem base cankers on different cultivars from untreated and fungicide-treated plots in 2016/2017 at Terrington, Norfolk.

Cultivar	Amount of Lm DNA in stem cankers[1]			Mean[2]
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	2.47	2.50	2.33	2.435 [b]
Fencer	1.93	1.44	2.04	1.804 [a]
Harper	2.54	1.96	2.40	2.308 [b]
Incentive	3.35	3.03	2.71	3.048 [c]
PR46W21	3.68	3.30	2.92	3.325 [c]
Quartz	2.25	2.80	2.46	2.495 [b]
LSD	0.249 (83 d.f.)			0.352 (83 d.f.)
Mean	2.716 [a]	2.517 [a]	2.481 [a]	
Cultivar	Amount of Lb DNA in stem cankers			Mean
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	1.91	1.74	1.52	1.734 [a]
Fencer	2.60	1.78	1.34	1.946 [a]
Harper	2.33	1.47	1.51	1.795 [a]
Incentive	2.40	1.80	1.76	2.008 [a]
PR46W21	2.35	2.02	1.69	2.038 [a]
Quartz	2.78	1.72	1.79	2.139 [a]
LSD	0.290 (83 d.f.)			0.410 (83 d.f.)
Mean	2.390 [b]	1.760 [a]	1.602 [a]	

[1]Ten stems were randomly sampled from each plot in the first replicate for DNA extraction and qPCR to determine the amounts of Lm and Lb DNA in 50 ng total DNA in stem base cankers. For statistical analysis, the amount of DNA was natural logarithm (ln) transformed. [2]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

In 2017-2018, there were significant differences between cultivars in the amounts of *L. maculans* or *L. biglobosa* DNA (Table 4.14). There were significant interactions between cultivar and fungicide on the effects of these fungicides on the amount of *L. maculans* or *L. biglobosa* DNA in stem base cankers *(P*<0.05*)*. Both fungicides were equally effective in reducing the amount of *L. maculans* DNA on cvs DK-Cabernet and Fencer. For cv Quartz, Refinzar significantly reduced the amount of *L. maculans* DNA in stem cankers compared to untreated plots while Proline did not. Both fungicides were equally effective in reducing the amount of *L. biglobosa* DNA in stem cankers of cvs DK-Cabernet and Quartz compared to untreated plots. For cvs PR46W21 and Harper, Proline significantly reduced the amount of *L. biglobosa* DNA but Refinzar did not. Surprisingly, for the susceptible cultivar Incentive, there was more *L. biglobosa* DNA in stems of untreated plants than treated plants for both fungicides (Table 4.14).

Table 4.14: Amounts of *L. maculans* (Lm) or *L. biglobosa* (Lb) DNA in stem base cankers on different cultivars from untreated and fungicide-treated plots in 2017/2018 at Terrington, Norfolk.

Cultivar	Amount of Lm DNA in stem cankers[1]			Mean[2]
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	3.00 efg	1.87 b	1.90 b	2.329 b
Fencer	2.02 bc	2.87 def	0.71 a	1.953 a
Harper	2.59 cdef	2.32 bcd	2.55 bcde	2.486 b
Incentive	3.38 g	3.04 efg	2.96 efg	3.154 c
PR46W21	3.08 efg	3.18 efg	3.25 fg	3.160 c
Quartz	2.93 efg	2.80 def	1.09 a	2.396 b
LSD	0.229 (57 d.f.)			0.335 (57 d.f.)
Mean	2.921 b	2.734 b	2.243 a	
Cultivar	Amount of Lb DNA in stem cankers			Mean
	Untreated	Proline treated	Refinzar treated	
DK-Cabernet	2.25 l	1.12 abcd	1.10 abc	1.569 ab
Fencer	1.55 defgh	2.22 l	0.95 a	1.616 b
Harper	2.35 l	1.36 abcdef	2.20 gkl	1.981 c
Incentive	0.99 ab	1.61 efghij	1.55 defghi	1.346 a
PR46W21	1.93 ghjkl	1.24 abcde	1.71 fghijk	1.641 b
Quartz	2.18 l	1.53 cdef	1.55 acdefg	1.795 bc
SED	0.192 (57 d.f.)			0.281 (57 d.f.)
Mean	1.811 b	1.479 a	1.486 a	

[1]Ten stems were randomly sampled from each plot in the first replicate for DNA extraction and qPCR to determine the amounts of Lm and Lb DNA in 50 ng total DNA in stem base cankers. For statistical analysis, the amount of DNA was natural logarithm (ln) transformed. [2]Values designated by different letters indicate significant differences (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

4.3.2. Effects of fungicides on control of the growth of *L. maculans* and *L. biglobosa in vitro*

The individual components of fungicides Proline (prothioconazole-desthio) and Refinzar (penthiopyrad + picoxystrobin) were tested with different *L. biglobosa* and *L. maculans* isolates for efficacy measured by EC_{50} value (effective concentration at which 50% of growth is inhibited). A total of 18 *L. biglobosa* and 21 *L. maculans* isolates were tested with prothioconazole-desthio. A total of 11 *L. biglobosa* and 12 *L. maculans* isolates were tested with penthiopyrad and picoxystrobin. At 12 dpi, the diameters of the radial mycelial growth of *L. biglobosa* and *L. maculans* were measured and EC_{50} values were calculated for the three fungicides to determine the concentrations of fungicides that gave half maximal response in percentage of diameter reduction for these isolates.

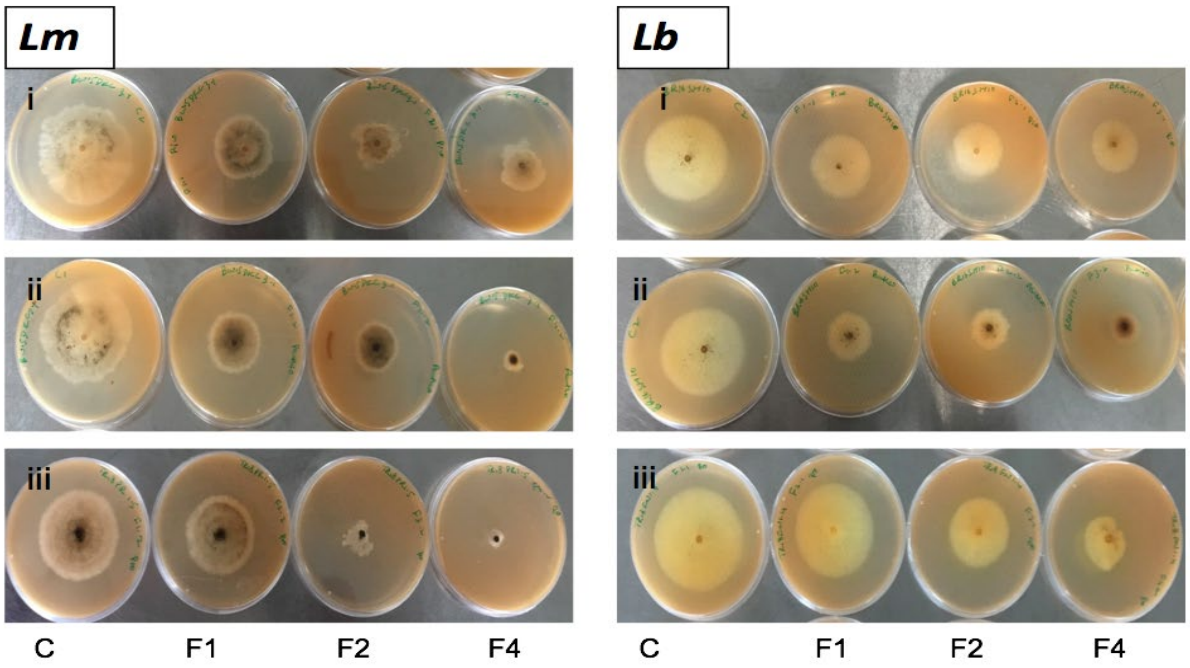

Fig. 4.19: Radial mycelial growth of *Leptosphaeria maculans* (Lm) or *L. biglobosa* (Lb) isolates on V8 agar media amended with different fungicides. Concentrations F1 (0.05 μg/ml), F2 (0.1 μg/ml) and F4 (0.5 μg/ml) of fungicides picoxystrobin (i), penthiopyrad (ii) and prothioconazole-desthio (iii). C represents control with no fungicide. Growth of Lm (BW15DKC3.1) on (i) and (ii) and TR18PR1.5 on (iii)) or Lb (BR16SH10 on (i) and (ii) and TR18FN1.4 on (iii)) isolates on V8 agar plates at 12 days post inoculation.

There were differences between isolates in radial mycelial growth on V8 plates amended with different fungicides; generally the growth of isolates decreased with the increased fungicide concentration (Fig. 4.19). There were significant differences in EC_{50} values of prothioconazole-desthio for *L. biglobosa* or *L. maculans* isolates (P<0.05). For isolates collected from untreated plants, prothioconazole-desthio (Proline) had greater EC_{50} values for *L. biglobosa* isolates than *L. maculans* isolates (Fig. 4.20). For *L. maculans* isolates collected from plants sprayed with Proline (prothioconazole-desthio) or Refinzar (penthiopyrad + picoxystrobin), *L. maculans* isolates collected from plants sprayed with Proline had greater EC50 than isolates collected from plants sprayed with Refinzar (Fig. 4.20). *L. maculans* isolates collected from plants sprayed with Proline also had greater EC50 than isolates collected from untreated plants.

For EC_{50} values of penthiopyrad, there were significant differences between *L. biglobosa* and *L. maculans* isolates (P<0.01). For isolates collected from untreated plants, penthiopyrad had greater EC_{50} values for *L. maculans* isolates than *L. biglobosa* isolates (Fig. 4.21). For *L. maculans* isolates collected from plants sprayed with Proline or Refinzar, *L. maculans* isolates collected from plants sprayed with Proline had greater EC50 than isolates collected from plants sprayed with Refinzar (Fig. 4.21). *L. maculans* isolates collected from plants sprayed with Proline also had greater EC50 than isolates collected from untreated plants or from treated with Refinzar.

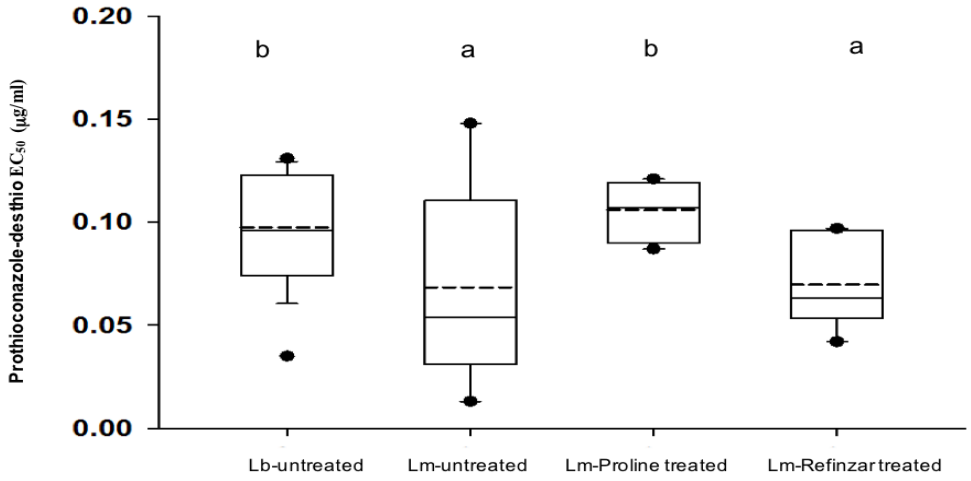

Fig. 4.20: Sensitivities of *Leptosphaeria biglobosa* (Lb) and *L. maculans* (Lm) isolates to prothioconazole-desthio. A total of 39 *Leptosphaeria* isolates (18 Lb and 21 Lm) were tested on V8 media amended with different concentrations (0, 0.05, 0.1, 0.25, 0.5 µg/ml) of prothioconazole-desthio. EC_{50} values for each isolate were calculated as 'effective concentration at which 50% of growth is inhibited'. Of the 21 Lm isolates, eight isolates were collected from untreated plants (Lm-untreated), seven isolates were collected from plants sprayed with Proline (Lm-Proline treated) and six isolates were from plants sprayed with Refinzar (Lm-Refinzar treated). The straight line in the box plot denotes the median value, the dotted line denotes mean value, the box edges show the upper quartiles (75%) and lower quartiles (25%), the upper and the lower dashes outside the box show the 95% to 5% values whereas the dots outside the box indicate the outliers. Values designated by different letters indicate that the differences are statistically significant (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

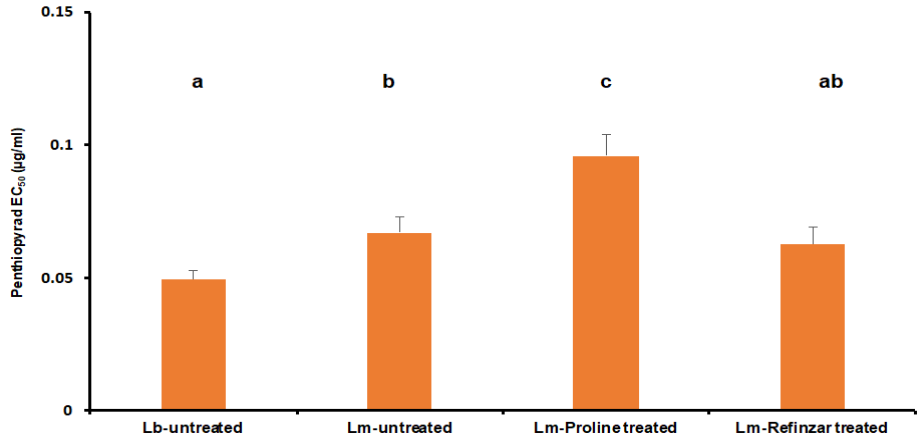

Fig. 4.21: Sensitivities of *Leptosphaeria biglobosa* (Lb) and *L. maculans* (Lm) isolates to penthiopyrad. A total of 20 isolates (11 Lb and 9 Lm) were tested on V8 media amended with diifferent concentrations (0, 0.05, 0.1, 0.25, 0.5 µg/ml) of penthiopyrad. Of the 9 Lm isolates, four were from untreated plants (Lm-untreated), two from plants sprayed with Proline (Lm-Proline treated) and three from plants sprayed with Refinzar (Lm-Refinzar treated). Error bars represent standard errors of means. Bars indicated by different letters indicate significant difference (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

For the EC_{50} values of picoxystrobin, there were no significant differences between *L. biglobosa* and *L. maculans* isolates (Fig. 4.22). For *L. maculans* isolates, the EC_{50} values were greater for isolates collected from untreated plants than those isolates collected from plants treated with Proline or Refinzar; however, the differences were not statistically significant.

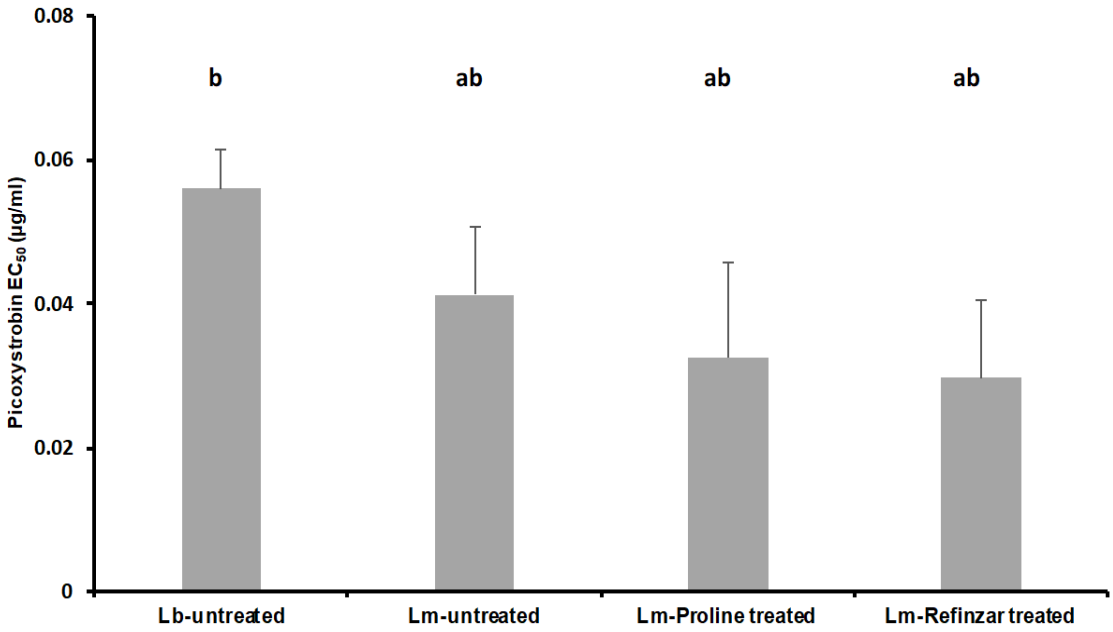

Fig. 4.22: Sensitivities of *Leptosphaeria biglobosa* and *L. maculans* isolates to picoxystrobin. A total of 20 isolates (11 Lb and 9 Lm) were tested on V8 media amended with idifferent concentrations (0, 0.05, 0.1, 0.25, 0.5 µg/ml) of picoxystrobin. Of the 9 Lm isolates, four were from untreated plants (Lm-untreated), two from plants sprayed with Proline (Lm-Proline treated) and three from plants sprayed with Refinzar (Lm-Refinzar treated). Error bars represent standard errors of means. Bars indicated by different letters indicate significant difference (P<0.05) in the Least Significant Difference (LSD) multiple comparison test.

4.4. Exploitation and technology transfer

4.4.1. Exploitation and dissemination of new knowledge

Through involvement of AHDB and industry partners of two Innovate UK projects, results of this project were exploited directly in the arable sector of the agricultural industry during the course of the project. This project is related to two Innovate UK projects that had a wide range of partners including oilseed rape breeders (Limagrain, LS Plant Breeding, Monsanto, KWS and Grainseed), an agrochemical company (DuPont, now is Corteva Agriscience) and an agricultural service provider (Hutchinsons). New developments were conveyed to the industry through AHDB, breeders' open days, Hutchinsons's agronomists and farmer conferences. Knowledge on identification of symptoms of phoma leaf spots caused by *L. biglobosa* and *L. maculans* and knowledge on cultivar differences in resistance against the two phoma stem canker causal pathogens were passed on to growers through AHDB topic sheets, farm open days and events like Cereals'. Results were also presented

to AHDB through annual reports and annual project monitoring meetings. In each growing season, the timing and abundance of *L. biglobosa* and *L. maculans* ascospores released at four different regions were passed onto growers to guide timing of fungicide applications through Hutchinsons. Results of cultivar resistance against by *L. biglobosa* and *L. maculans* from field experiments and controlled environment experiments were passed onto growers through AHDB and Hutchinsons agronomists for guidance on choice of cultivars. For details of knowledge transfer activities see Appendix 1.

4.4.2. Publications and conferences

New information about proportions of the two phoma stem canker causal pathogens (*L. biglobosa* and *L. maculans*) in different regions, differences in cultivar resistance against the two pathogens and effectiveness of different fungicides on control of these two pathogens in field experiments and *in vitro* was disseminated through publications and conference presentations. New findings from this project and relevant projects were communicated as research papers in international journals and as presentations or publications in scientific conference proceedings and seminars. During the course of this project, seven research papers related this project have been published in peer reviewed international journals. Two presses related to two publications were released to highlight the benefits and novelty of the research. Results of this project and related projects were presented at six international conferences and six national conferences. In addition, new results were also presented at the University of Hertfordshire annual research conference in the School of Life and Medical Sciences and departmental research seminars. For details of conference presentations and research papers see Appendix 1.

5. Discussion

5.1. Regional differences in proportions of *L. biglobosa* in pathogen populations

The patterns of *L. biglobosa* and *L. maculans* ascospore release differed between locations and between cropping seasons. In comparison within sites over the three seasons for all the four sites, ascospores were released earlier in 2015/2016 and 2017/2018, when weather conditions in August-September were wet, than in 2016/2017 when weather conditions in August-September were very dry. The results agree with previous studies that the timing of ascospore release depends on weather conditions, especially temperature and rainfall in August-September. It is reported that rainfall in summer is an important factor for pseudothecial maturation and ascospore release (Toscano-Underwood et al., 2003; Huang et al., 2005). In comparison between sites over the three seasons, ascospore release was considerably later at Impington than at other three sites in all seasons. Analysis of weather data in August-September (a key period of pseudothecial maturation and subsequent timing of ascospore release) at Impington indicated that there were little differences in temperature between Impington and other three sites. The temperature was -0.2 – 0.4, 0.1 – 1.2 and

0.2 – 0.4°C greater than other three sites in 2015, 2016 and 2017, respectively. For the total rainfall, Impington had 28.2 – 48.8 mm less rainfall than the other three sites in 2016, but had more rainfall in 2017 (0.5 – 34.4 mm) than the other three sites and in 2015 (23.9 – 65.0 mm) than other two sites (except for Eye). These data suggest that the temperature and rainfall in August-September may not be the main cause of differences in the timing of ascospore release between Impington and the other three sites. There is a need to investigate other factors, such as the use of stems of cultivars with different resistances or with/without fungicide treatments. Following observation of first major ascospore release, the spore release continued until February and March on days after rainfall. The results agree with previous work that the numbers of ascospores released in the air increased rapidly following regular rainfall whereas continuous dry weather conditions without rainfall led to delays in release of ascospores even if mature pseudothecia were present (Huang et al., 2005).

The results of the qPCR analysis indicated that there were differences between sites and seasons in numbers of *L. biglobosa* and *L. maculans* ascospores in the air. In general, *L. biglobosa* and *L. maculans* ascospores were released at a similar time at Bayfordbury, Impington and Eye, whereas at Rothwell *L. maculans* ascospores were released earlier than *L. biglobosa* ascospores. Previous studies suggested that *L. biglobosa* and *L. maculans* pseudothecia mature at a similar rate at temperatures of 15-20°C whereas *L. biglobosa* pseudothecia mature at a slower rate than Lm when temperature decreases to <10°C (Toscano-Underwood et al., 2003). In the UK, where temperature generally decreases to <10°C in winter, it was observed that *L. maculans* ascospores were mainly released in autumn/winter while *L. biglobosa* ascospores were released later in the spring as observed at Rothwell (Huang et al., 2011).

Weather data collected at all sites in all the three seasons indicated that temperature had little or no effect on ascospore release when temperatures was >5°C. However, there was little or no ascospore release at temperatures <2°C, indicating that as temperature decreases to <2°C, pseudothecial maturation and ascospore release is reduced. Previous studies done in Poland indicated that the numbers of ascospores detected in the air decreased in winters where temperature fluctuated close to 0°C. Several weather-based prediction models have been formulated to predict the onset of *Leptosphaeria* spp. ascospore release (Huang et al., 2007; Salam et al., 2003). However, previous models did not differentiate the timing of *L. biglobosa* and *L. maculans* ascospore release. There is a need to develop new models that can effectively predict the timing of *L. biglobosa* and *L. maculans* ascospore release, which can be used to guide the choice of cultivars and fungicides.

5.2. Cultivar resistance against *L. biglobosa* and *L. maculans*

These results from field experiments showed that cultivars with *R* gene-mediated resistance (e.g. *Rlm7*) against *L. maculans* had more *L. biglobosa* phoma leaf spots and more *L. biglobosa* DNA in stem base cankers than cultivars without any resistance genes. These results agree with previous

studies that cultivars with *R* gene-mediated resistance generally had more *L. biglobosa* DNA than *L. maculans* DNA in stem base cankers (Huang et al., 2014). These results indicated that cultivars with effective *R* genes against *L. maculans* may be more susceptible to colonisation by *L. biglobosa*. Consistent use of cultivars with *R* gene-mediated resistance against *L. maculans* may lead to increased population of *L. biglobosa* in local pathogen populations. Previous studies showed that *L. biglobosa* is less sensitive to some triazole fungicides than *L. maculans* (Huang et al., 2011; Eckert et al., 2010); increased proportions of *L. biglobosa* in local pathogen populations may lead to severe *L. biglobosa* phoma stem canker epidemics. Effective control of phoma stem canker epidemics needs to involve control of both *L. biglobosa* and *L. maculans* by cultivar resistance and fungicides. Results of qPCR analysis on 17 cultivars from AHDB RL DOP field trials showed that it is possible to select cultivars with resistance against both *L. biglobosa* and *L. maculans*.

Results from controlled environment experiments suggested that cultivars with smaller values of AHDB RL resistance ratings (i.e. susceptible) and no *R* genes developed more severe phoma leaf spot lesions on cotyledons when inoculated with either *L. biglobosa* or *L. maculans* isolates, indicating that these cultivars were susceptible to both *Leptosphaeria* spp. All cultivars with *R* gene-mediated resistance against *L. maculans* produced a typical resistance phenotype (small lesions with darkening around the inoculation sites) when inoculated with *L. maculans* isolates. However, most cultivars with *R* gene-mediated resistance except cv Fencer formed very large necrotrophic phoma leaf spot lesions when inoculated with *L. biglobosa* isolates indicating that these cultivars were susceptible to *L. biglobosa*. These results agree with the results from field experiments that cultivars with *R* gene-mediated resistance against *L. maculans* were more susceptible to *L. biglobosa.* However, cv Fencer with resistance gene *Rlm7* against *L. maculans* also showed an intermediate resistance to *L. biglobosa* isolates forming less severe *L. biglobosa* lesions than Excel (also with resistance gene *Rlm7*). There is a need to further investigate whether the differences between Fencer and Excel in resistance against *L. biglobosa* is due to the background quantitative resistance or a major gene resistance against *L. biglobosa*. Previous research suggested that combination of major resistance gene mediated qualitative resistance and quantitative resistance provide better control of *L. maculans* (Brun et al., 2010; Huang et al., 2018). Results of this study suggest that combination of major gene resistance and quantitative resistance (e.g. Fencer) may also provide better control of *L. biglobosa.*

5.3. Effects of fungicides on control of *L. biglobosa* compared to *L. maculans*

Results from field experiments suggested that fungicides Proline (prothioconazole-desthio) and Refinzar (penthiopyrad + picoxystrobin) were effective in reducing the severity of *L. maculans* phoma leaf spotting on susceptible winter oilseed rape cultivars (i.e. cultivars with smaller AHDB RL resistance ratings; for example cultivars Incentive and PR46W21). Both fungicides had no or small effects in reducing severity of *L. maculans* phoma leaf spot on resistant cultivars, especially cultivars with effective *R* gene (e.g. *Rlm7*) resistance against *L. maculans*. Both fungicides had similar effects

either in reducing severity of *L. biglobosa* phoma leaf spotting on all cultivars or in reducing severity of phoma stem canker with greater effects on susceptible cultivars than on resistant cultivars. Previous studies showed that fungicides Proline and Refinzar had similar effects in reducing the severity of *L. biglobosa* and *L. maculans* phoma leaf spots and severity of phoma stem canker on an oilseed rape cultivar Catana (Sewell et al., 2016). These results indicated that Proline and Refinzar are still effective fungicides for control of phoma stem canker in the UK.

Further investigation of fungicide-sensitivity of *L. biglobosa* and *L. maculans* isolates to individual components of Proline (prothioconazole-desthio) and Refinzar (penthiopyrad + picoxystrobin) showed that *L. biglobosa* isolates were less sensitive to prothioconazole-desthio than *L. maculans* isolates. The results agree with previous studies that *L. biglobosa* isolates were less sensitive to some triazole fungicides than *L. maculans* isolates (Eckert et al., 2010; Huang et al., 2011). However, *L. biglobosa* isolates were more sensitive to penthiopyrad than *L. maculans* isolates, while there were no differences in sensitivity to picoxystrobin between *L. biglobosa* and *L. maculans* isolates.

The results from *in vitro* tests for *L. maculans* isolates collected from fungicide treated plants suggested there is a risk of development of insensitivity to azole (e.g. prothioconazole-desthio) and non azole (e.g. penthiopyrad) fungicides if use of Proline continues. The EC_{50} values of fungicides prothioconazole-desthio or penthiopyrad were greater for *L. maculans* isolates collected from Proline-treated plants than for *L. maculans* isolates collected from untreated plants. However, there were no significant differences between *L. maculans* isolates collected from Refinzar treated plants and *L. maculans* isolates collected from untreated plants in EC_{50} values of all the three fungicides tested (prothioconazole-desthio, penthiopyrad and picoxystrobin). These results suggest that there is a very low risk of development of insensitivity to azole (e.g. prothioconazole-desthio) and non-azole (e.g. penthiopyrad, picoxystrobin) fungicides if use of Refinzar continues. However, there were only a limited number of *L. maculans* isolates collected from fungicide treated plants in this study. There is a need to collect more *L. maculans* and *L. biglobosa* isolates from fungicide treated plants at different regions in the UK and test their sensitivities to different fungicides to monitor any potential fungicide insensitivity/resistance development in *Leptosphaeria* populations.

5.4. Benefits from AHDB undergraduate student bursary projects

This project has benefited from the AHDB Cereals & Oilseeds undergraduate bursary scheme, which has not only added value to the main project but also inspired two undergraduate students to pursue PhD degrees after they have obtained their first degrees. During the course of this project, Dr Huang applied for two 10-week student bursary projects to be taken in the summer of 2017 and 2018, respectively. Both applications were successful. James Fortune worked on the AHDB student bursary project 'Identification of phoma stem canker pathogens (*Leptosphaeria maculans* and *L. biglobosa*) on different winter oilseed rape cultivars using quantitative PCR' in summer 2017. With this student bursary project, we were able to determine whether the stem cankers on stems of 17

AHDB RL cultivars, from disease observation plots at Broughton in Hampshire and Morley in Norfolk sampled in July 2016, were caused by *L. biglobosa* or *L. maculans* using quantitative PCR (by quantifying the amount of *L. biglobosa* and/or *L. maculans* DNA in the stems). The results are presented in Fig. 4.16. In addition, James helped us with assessing and processing stem samples from our Agri-tech project (for more information, see AHDB student bursary report 2017). With the research experience and new knowledge about the importance of plant pathology for food security, James has decided to do a PhD in plant pathology.

Similarly, Laura Sapelli worked on the AHDB student bursary project 'Identification of ascospores of phoma stem canker causal pathogens (*Leptosphaeria maculans* and *L. biglobosa*) on stems of winter oilseed rape cultivars with different resistances' in summer 2018. Results of the main project showed that greater amounts of *L. biglobosa* DNA than *L. maculans* DNA were detected in stem cankers on cultivars with the resistance gene *Rlm7*. However, it is not clear whether the *L. biglobosa* or *L. maculans* infected stems can produce *L. biglobosa* or *L. maculans* ascospores to infect the crops in the next season. With this AHDB bursary project, we were able to compare the development of pseudothecia (sexual fruiting body containing ascospores) and determine whether those pseudothecia contained *L. biglobosa* or *L. maculans* ascospores. Stems of six winter oilseed rape cultivars, five of them carrying the resistance gene *Rlm7*, were used for this bursary project. Results showed that these *L. biglobosa* or *L. maculans* infected stems can produce *L. biglobosa* or *L. maculans* ascospores. However, there were differences between cultivars or between stem bases and upper stems in proportions of *L. biglobosa* and *L. maculans* ascospores (for more information, see the AHDB student bursary report 2018). In addition to the lab-based research, Laura was provided with the opportunity to attend the AHDB Duxford Monitor Farm launch event. During the farm tour, she obtained first-hand knowledge that weeds and diseases are major threats to our food production, which has inspired her to do PhD in plant pathology.

In summary, the AHDB Cereals & Oilseeds undergraduate bursary research project scheme is a very good scheme, which not only adds value to the main project but also provides undergraduate students with opportunities to gain research experience, which may lead to training of the next generation of young agricultural researchers to benefit the UK agricultural industry. In addition, this undergraduate bursary scheme also helps early career researchers to gain supervisory experience. For example, two PhD students (Harika Gajula and Asna Javaid) in our group have gained supervisory experience by working with the two bursary students James and Laura. We will continue to apply for AHDB Cereals & Oilseeds undergraduate bursary research projects in future if we have any AHDB funded projects.

6. References

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Williams, R. H. & Fitt, B. D. L. 1999. Differentiating A and B groups of *Leptosphaeria maculans*, causal agent of stem canker (blackleg) of oilseed rape. Plant Pathology*,* 48**,** 161-175.

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7. Appendices

Appendix 1: List of publications and knowledge transfer activities (2015–19)

Papers in refereed International Journals

Huang YJ, Paillard S, Kumar V, King GJ, Fitt BDL, Delourme R (2019). Oilseed rape (*Brassica napus*) resistance to growth of *Leptosphaeria maculans* in leaves of young plants contributes to quantitative resistance in stems of adult plants. PLoS ONE 14(9): e0222540. https://doi.org/10.1371/ journal.pone.0222540.

Huang YJ, Mitrousia GK, Sidique SNM, Qi AM, Fitt BDL (2018). Combining *R* gene and quantitative resistance increases effectiveness of cultivar resistance against *Leptosphaeria maculans* in *Brassica napus* in different environments. PLoS ONE 13(5): e0197752. https://doi.org/10.1371/ journal.pone.0197752.

Mitrousia GK, Huang YJ, Qi AM, Fitt BDL (2018). Effectiveness of *Rlm7* resistance against *Leptosphaeria maculans* (phoma stem canker) in UK winter oilseed rape cultivars. *Plant Pathology 56, 1339-1353.* DOI:10.1111/ppa.12845.

Cai X, Huang YJ, Jiang DH, Fitt BDL, Li GQ, Yang L (2018). Evaluation of oilseed rape seed yield losses caused by *Leptosphaeria biglobosa* in central China. *European Journal of Plant Pathology* 150: 179–190, DOI: 10.1007/s10658-017-1266-x.

Sewell TR, Hawkins NJ, Moloney S, Stotz HU, Huang YJ, Kelly SL, Kelly DE, Fraaije B, Fitt BDL (2017). Azole sensitivity in *Leptosphaeria* pathogens of oilseed rape: the role of lanosterol 14α-demethylase. *Scientific Reports* 7, Article number: 15849. DOI:10.1038/s41598-017-15545-9.

Luo Z, Wang M, Long Y, Huang YJ, Shi L, Zhang C, Liu X, Fitt BDL, Xiang J, Mason AS, Snowdon RJ, Liu PF, Meng J, Zou J (2017). Incorporating pleiotropic quantitative trait loci in dissection of complex traits: seed yield in rapeseed as an example. *Theoretical and Applied Genetics* 130, 1569-1585. DOI: 10.1007/s00122-017-2911-7.

Sewell TR, Moloney S, Ashworth M, Ritchie F, Mashanova A, Huang YJ, Stotz HU, Fitt BDL (2016) Effects of a penthiopyrad and picoxystrobin fungicide mixture on phoma stem canker *Leptosphaeria* spp.) on UK winter oilseed rape. *European Journal of Plant Pathology* 145: 675–685, DOI 10.1007/s10658-016-0916-8.

Edited short conference papers

Mitrousia GK, Huang YJ, Qi AM, Sidique SNM, Fitt BDL (2018). Prediction of ascospore release of *Leptosphaeria* spp. to improve timing of fungicide applications. *IOBC-WPRS Bulletin* in press.

Huang YJ, West JS, Mitrousia GK, Wood T, Fitt BDL (2017). Identification of pest and disease resistance in the UK OREGIN Brassica biodiversity collection. *Crop Production in Southern Britain. Aspects of Applied Biology* 134, 207-210.

Javaid A, Gajula H, Fitt B D L, Huang YJ (2017). Investigating the risk of severe phoma stem canker caused by *Leptosphaeria biglobosa* on winter oilseed rape in the UK. *Crop Production in Southern Britain. Aspects of Applied Biology* 134, 53-58.

Javaid A, Fitt B D L, Huang YJ (2016). Decreasing the risk of severe phoma stem canker caused by *Leptosphaeria biglobosa* on winter oilseed rape. *IOBC-WPRS Bulletin* 116, 97-101.

Abstracts of conferences

Huang YJ, Gajula LH, Karandeni-Dewage CS, Fitt BDL (2019). Game of hide and seek - interactions between different effector genes in the fungal pathogen *Leptosphaeria maculans.* BSPP presidential meeting, Bristol, UK, 2-3 September 2019. Oral presentation.

Javaid A, Gajula H, Fitt BDL, Huang YJ (2019). Effects of cultivar resistance and fungicides on coexisting fungal pathogens *Leptosphaeria mculans* and *L. biglobosa.* British Society for Plant Pathology (BSPP) presidential meeting, Bristol, UK, 2-3 September 2019. Oral presentation.

Qi A, Huang YJ, Malcolm-Brown M, Sidique SN, Fitt BDL (2019). Predicting timing of release of ascospores of *Leptosphaeria spp*. to improve control of phoma stem canker on oilseed rape in the UK. The *15th International Rapeseed Congress*, 16-19 June 2019, Berlin. Oral presentation.

Huang YJ, Javaid A, Gajula LH, Karandeni-Dewage CS, Mitrousia GK, Fitt, B.D.L. (2019). Host resistance affects coexistence of two related fungal pathogens *Leptosphaeria maculans* and *L. biglobosa. The 15th International Rapeseed Congress*, 16-19 June 2019, Berlin. Oral presentation.

Gajula LH, Fitt BDL, Huang YJ (2019). Changes in race structure of *Leptosphaeria maculans* populations on oilseed rape in the UK. *The 15th International Rapeseed Congress*, 16-19 June 2019, Berlin. Oral presentation.

Javaid A, Gajula LH, Fitt BDL, Huang YJ (2019). Regional differences in proportions of *Leptosphaeria maculans* and *L. biglobosa* in Eastern England. *15th International Rapeseed Congress*, 16-19 June 2019, Berlin. Poster presentation.

Huang YJ, Javaid A, Gajula LH, Karandeni-Dewage CS, Mitrousia GK, Fitt BDL (2018). Ignored fungal pathogen sibling - *Leptosphaeria biglobosa*. *Brassica Workshop*, 1-4 July 2018, St Malo, France. Oral presentation.

Lange R, Rempel C, Punja Z, Wu PS, Huang YJ, Qi A, Fitt BDL (2018). Oilseed rape crop debris and potential spread of *Leptosphaeria maculans* (phoma stem canker) into China. *Brassica 2018* - 21st Crucifer Genetics Conference*,* 1-4 July 2018, St Malo, France. Poster presentation.

Javaid A, Gajula LH, Fitt BDL, Huang YJ (2018). Investigating regional differences in proportions of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* in southern England. *International Congress of Plant Pathology (ICPP)*, 29 July - 3 August 2018, Boston, USA. Poster presentation.

Gajula LH, Mitrousia GK, Fitt BDL, Huang YJ (2018). Molecular mechanisms of mutation to virulence in *Leptosphaeria maculans* populations in the UK. *International Congress of Plant Pathology (ICPP)*, 29 July - 3 August 2018, Boston, USA. Poster presentation.

Javaid A, Fitt BDL, Huang YJ (2018). Effects of fungicides on *Leptosphaeria maculans* and *L. biglobosa. BSPP Presidential Meeting,* 10-11 December 2018, Warwick, UK. Poster presentation.

Javaid A, Gajula LH, Fitt BDL, Huang YJ (2017). Investigating the risk of severe phoma stem canker caused by *Leptosphaeria biglobosa* on winter oilseed rape in the UK. *Association of Applied Biologists (AAB) conference - Crop Production in Southern Britain.* 15-16 Feb 2017, Peterborough, UK. Oral presentation.

Huang YJ, West JS, Mitrousia GK, Wood T, Fitt BDL (2017). Identification of pest and disease resistance in the UK OREGIN Brassica biodiversity collection. *Crop Production in Southern Britain.* 15-16 Feb 2017, Peterborough, UK. Oral presentation.

Mitrousia GK, Winter M, Jedryczka M, Gajula LH, Kaczmarek J, Sidique SN, Huang YJ, Fitt BDL (2017). Coexistence of *Leptosphaeria maculans* and *L. biglobosa* on oilseed rape crops. The 12th *European Foundation for Plant Pathology* conference, 29 May – 2 June 2017, Dunkirk, France.

Poster presentation

Huang YJ, Javaid A, Gajula LH, Karandeni-Dewage CS, Mitrousia GK, Fitt BDL (2017). Ignored until recently – the story of the fungal pathogen *Leptosphaeria biglobosa.* Oral presentation. BSPP-BMS (British Mycological Society) conference, 11-13 Sept 2017, Nottingham, UK. Oral presentation.

Javaid A, Fitt B D L, Huang YJ (2017). Phoma stem canker: Are we missing something important? *Agri-Tech East's REAP (Realising our Economic and Agricultural Potential) conference 2017, Cambridge, UK.* 7th November 2017. Oral presentation.

Gajula LH, Fitt BDL, Huang YJ (2016). Identification of new virulent races of *Leptosphaeria maculans* populations on oilseed rape in the UK. *IOBC-WPRS conference,* 7-9 September 2016, Tartu, Estonia. Oral presentation.

Javaid A, Gajula H, Fitt B D L, Huang YJ (2016). Decreasing the risk of severe phoma stem canker caused by *Leptosphaeria biglobosa* on winter oilseed rape. *IOBC-WPRS conference,* 7-9 September 2016, Tartu, Estonia. Oral presentation.

Mitrousia GK, Huang YJ, Sidique SN, Gajula LH, Fitt BDL (2016). Coexistence of *Leptosphaeria* spp. on oilseed rape crops in the UK. *Brassica 2016* - 20th Crucifer Genetics Conference*,* 3-7 October 2016, Melbourne, Australia. Oral presentation.

Mitrousia GK, Huang YJ, Noel K, Stots H, Larkan N, Borhan H, Fitt BDL (2016). Effects of increased temperature on B. napus resistance against *Leptosphaeria maculans*. *Brassica 2016* - 20th Crucifer Genetics Conference*,* 3-7 October 2016, Melbourne, Australia. Poster presentation.

Huang YJ, Cai X, Karandeni-Dewage CS, Gajula LH, Javaid A, Li GQ, Fitt BDL (2016). Understanding phoma stem canker epidemics caused by *Leptosphaeria biglobosa* in the UK and China. *Brassica 2016* - 20th Crucifer Genetics Conference*,* 3-7 October 2016, Melbourne, Australia. Oral presentation.

Huang YJ, Fitt BDL (2016). Sustainable disease management in oilseed rape in the UK. *Association of Applied Biologists (AAB) conference – Advances in IPM.* 16-17 November 2016, Ramada Resort Grantham, Marston, Lincolnshire, UK. Oral presentation

Events/ KT Activities

NIAB START Farm open day at Stanaway Farm, Otley, Suffolk on 22 May 2019, information on this project was presented as leaflets. (Yongju Huang)

Cereals 2019 at Boothby Graffoe, Lincolnshire, 12 -13 June 2019, information on this project was presented at the Hutchinsons stand. (David Ellerton)

Cereals 2018, Chrishall Grange, Duxford, Cambridge, 13-14 June 2018, information on this project was presented at University of Hertfordshire stand. (Yongju Huang and Bruce Fitt)

Attended the AHDB Duxford Monitor Farm launch event on 19 June 2018 at Rectory Farm in Great Chishill, Hertfordshire. (Yongju Huang)

Agri-Tech East meeting at University of Hertfordshire on 5 November 2018, information on this project was presented. (Yongju Huang and Bruce Fitt)

The Agri-Tech East's REAP (Realising our Economic and Agricultural Potential) meeting 2017, Cambridge, UK, 7th November 2017. Information on this project was presented. (Asna Javaid)

Hutchinsons Winter Farmer Technical conference, Kingsgate Centre, Peterborough, 15 November 2017, Information on this project was presented. (David Ellerton, Bruce Fitt, Yongju Huang)
AFCP (AgriFood Charities Partnership) Forum at University of Hertfordshire, 5 April 2017. Information of this project was presented. (Asna Javaid, Yongju Huang)

Hertfordshire County Show, 27-28 May 2017, Dunstable Road, Redbourn, Information of this project was presented at University of Hertfordshire stand. (Yongju Huang)
Cereals 2016, 15th – 16th June 2016, Chrishall Grange, Nr Duxford, Cambridgeshire. Information on this project was presented at University of Hertfordshire stand. (Yongju Huang and Bruce Fitt)

Hutchinsons Winter Farmer Technical conference, East of England Showground Peterborough. 19 Nov 2015. Information on this project was presented. (David Ellerton, Bruce Fitt, Yongju Huang)

Scientific conference presentations

Oral presentations at the British Society for Plant Pathology (BSPP) presidential meeting, Bristol, UK, 2-3 September 2019. (Yongju Huang and Asna Javaid)

Oral presentation at the 15th International Rapeseed Congress (IRC), 16-19 June 2019, Berlin. (Yongju Huang)

Oral presentation at Brassica 2018 - 21st Crucifer Genetics Conference, 1-4 July 2018, St Malo, France. (Yongju Huang)

Poster presentations at the International Congress of Plant Pathology (ICPP), 29 July - 3 August 2018, Boston, USA. (Yongju Huang, Asna Javaid and Bruce Fitt)

Poster presentations at the British Society for Plant Pathology (BSPP) presidential meeting, 10-11 November 2018, Warwick, UK. Yongju Huang, Asna Javaid and Bruce Fitt)

Oral presentations at the Association of Applied Biologists (AAB) conference - Crop Production in Southern Britain, 15-16 Feb 2017, Peterborough, UK. (Yongju Huang and Asna Javaid)

Poster presentation at the 12th European Foundation for Plant Pathology conference, 29 May – 2 June 2017, Dunkirk, France. (Yongju Huang)

Oral presentation at the BSPP-BMS (British Mycological Society) conference, 11-13 Sept 2017, Nottingham, UK. (Yongju Huang)

Oral presentation at the Agri-Tech East's REAP (Realising our Economic and Agricultural Potential) conference 2017, Cambridge, UK. The 7th of November 2017. (Asna Javaid)

Oral presentation at the *IOBC-WPRS conference,* 7-9 September 2016, Tartu, Estonia. (Asna Javaid)

Oral presentation at the *Brassica 2016* - 20th Crucifer Genetics Conference, 3-7 October 2016, Melbourne, Australia. (Yongju Huang)

Oral presentation at the Association of Applied Biologists (AAB) conference – Advances in IPM, 16-17 November 2016, Ramada Resort Grantham, Marston, Lincolnshire, UK. (Yongju Huang)

Press articles (release)

'Speeding the breeding of phoma-resistant OSR', Farmers Guardian, 16 Sept 2019. (Results related to this project)

'Twin pathogens prompt review of OSR phoma risk & control', Arable Farming, 1 Oct 2018. (Results from this project)

'Raising awareness of phoma B threat', 1 Oct 2018. (Results from this project)

‘Twin pathogens prompt review of OSR phoma risk & control’, Farmers Guardian, 9 November 2018. (Results from this project)

‘Crop resistance works better together’, University of Hertfordshire news release, 31 May 2018. (Results related to this project)

‘New research warns of a resistance gene at risk in oilseed rape’, University of Hertfordshire news release, 1 May 2018. (Results related to this project).