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Understanding resistance to decrease risk of severe phoma stem canker on oilseed rape

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Contents

1. Abstract

The overall aim of this project was to decrease the risk of severe phoma stem canker on oilseed rape at increased temperature by improving understanding of operation of *Brassica napus* resistance against *Leptosphaeria maculans*. There were three related objectives/tasks:

1. To identify *L. maculans* races in UK regions with different climates to optimise the deployment of oilseed rape resistance genes
2. To investigate phenotypes of *R* gene-mediated and quantitative resistance (QR) against *L. maculans* in leaf and stem tissues in different environments
3. To develop an experimental system to investigate mechanisms of operation of *R* gene-mediated and QR against *L. maculans*

This work has identified a number of ways to decrease the risk of severe phoma stem canker epidemics on UK winter oilseed rape crops, both in the short-term and the long-term. These are discussed in the context of a series of recommendations, which could be implemented by different sections of the UK oilseed rape industry, including farmers, the AHDB, crop advisors and the breeding industry.

Short-term benefits to farmers

To guide regional deployment of oilseed rape cultivars with different resistance genes, there is a need to monitor regional populations of *L. maculans,* especially in relation to virulence at the *AvrLm1, AvrLm4* (populations currently differ between regions) and *Avrlm7* (to decrease risk of breakdown of *Rlm7* cultivar resistance) loci. It is recommended that AHDB deploys a set of spore samplers at some Recommended List (RL) winter oilseed rape trials sites to monitor races present in *L. maculans* populations in different regions. This should be done each growing season to detect frequencies of virulent isolates by using PCR of samples of airborne spores. Such information could then be made available to farmers to guide their choice of cultivars for the next growing season.

There is a need to develop a web-based scheme for guiding regional deployment of winter oilseed rape cultivars with different types of resistance. There is a need to continue monitoring ascospore release to provide better guidance for timing of fungicide applications. Results of this project suggest that deployment of a small number of spore samplers would be sufficient to provide such guidance. It is likely that a new generation of spore samplers will be developed that can provide 'real time' information about the timing of release of specific spores but, even if it is necessary to send weekly spore sampling tapes to a laboratory for quantitative PCR analysis, results could improve the accuracy of guidance for spray timing.

Long-term benefits to farmers

While some recommendations could be implemented quite quickly to decrease the risk of phoma stem canker, others are more long-term, since they need to be implemented by oilseed rape breeders.

R gene-mediated resistance should always be combined with QR to improve its effectiveness in different environments. To provide farmers with cultivars that have effective resistance against the stem canker pathogen at different locations and in different growing seasons, it is essential that *R* gene-mediated resistance is incorporated into backgrounds with good QR that is environmentally stable.

Relationships between temperature-resilience and effectiveness of resistance should be investigated. If factors that influence the effectiveness of resistance (differences between *R* genes; background QR) are associated with the temperature-resilience of that resistance, then breeders could screen potential cultivars/lines for temperature-resilient resistance as a means of selecting for effective resistance. This would save time and expense by comparison with field experiments at multiple locations over several seasons that are currently used to select for effective, environmentally stable resistance. Such screening could be used in initial selection stages, although the best material would then need to be tested in field experiments.

There is a need to understand interactions between the two pathogens (*L. maculans* and *L. biglobosa*) and cultivar *R* gene-mediated resistance to identify cultivars with resistance against both pathogens. Furthermore, although *L. maculans* is generally regarded as a more damaging pathogen than *L. biglobosa*, there is evidence that *L. biglobosa* was the dominant pathogen, responsible for severe stem base and upper stem lesions, in the 2011/2012 growing season.

There is a need to exploit new genomic information and genetic resources to improve our understanding of the operation of resistance against *Leptosphaeria* species and other extracellular pathogens. There are now unprecedented opportunities to exploit this novel genomic information through bioinformatics and biocomputational methods, together with the new host materials that have become available to improve our understanding of resistance against the phoma stem canker pathogens.

This new understanding can be exploited by breeders to develop new cultivars with more effective, durable, temperature-resilient resistance for the benefit of farmers and the whole agricultural industry in the UK.

2. Introduction

Use of host resistance is the most effective and environmentally friendly way to control crop plant diseases. To achieve effective control, understanding operation of host resistance is essential. In the context of global climate change, impacts of increased temperature on effectiveness of crop host resistance are of increasing relevance. Variation in temperature response between different resistance genes is poorly documented and the underlying mechanisms are not well understood. Phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) is a major disease problem on oilseed rape in the UK, causing losses worth £100M p.a., despite use of fungicides costing £20M p.a. (Fitt et al. 2006a; Stonard et al. 2010). These losses will increase if the most effective fungicides are no longer permitted - by EU legislation (EU directive 91/414). Furthermore, it is predicted that global warming will continue to increase the range and severity of UK phoma stem canker epidemics (Evans et al., 2008). Work in the CLIMDIS LINK project has shown that increased winter and spring temperature associated with climate change (Semenov 2009) is predicted to increase losses from phoma stem canker to 50% for susceptible cultivars in southern England (Butterworth et al. 2010), meaning that it may no longer be economic to grow oilseed rape there (Fig. 2.1). The modelling, based on more than 15 UK seasons of observed data, suggests that there are interactions between temperature and cultivar resistance in winter and spring but does not distinguish between the two types of resistance (*R* gene-mediated and quantitative). However, there is evidence that temperature affects operation of both types of resistance against the phoma stem canker pathogen in UK oilseed rape cultivars. Increased temperature both renders ineffective some resistance (*R*) genes (e.g. *Rlm6*) that operate in leaves in autumn and decreases effectiveness of quantitative resistance (QR) that operates as the pathogen spreads down leaf stalks in autumn/winter and colonises stem tissues in spring/summer (Huang et al, 2009). Therefore, there is a need to understand effects of temperature on effectiveness of resistance to improve strategies to decrease the risk of severe phoma stem canker epidemics on oilseed rape in the UK.

The two types of resistance against *L. maculans* identified in *B. napus* (Fig. 2.2) are major resistance (*R*) gene-mediated qualitative resistance that operates in cotyledons and leaves in autumn (Balesdent et al. 2001) and QR that operates in leaf stalk and stem tissues, during the period after initial leaf infection until harvest in the following summer (Pilet et al. 1998; Delourme et al. 2006; Huang et al. 2009). Qualitative resistance against *L. maculans* is associated with a gene-for-gene interaction in which the product of a pathogen effector (*Avr*) gene is recognised by the product of a host *R* gene so that the pathogen is unable to infect the host (i.e. resistant reaction). Therefore, *R* gene-mediated resistance against *L. maculans* is single-gene race specific resistance that is effective in protecting plants only if the corresponding avirulent allele (of the *Avr* gene) is predominant in the local *L. maculans* population (Balesdent et al. 2001; Rouxel et al. 2003). To optimise the use of *R* gene-mediated resistance and decrease the risk of breakdown of novel resistance, there is a need to monitor the races in *L. maculans* populations.

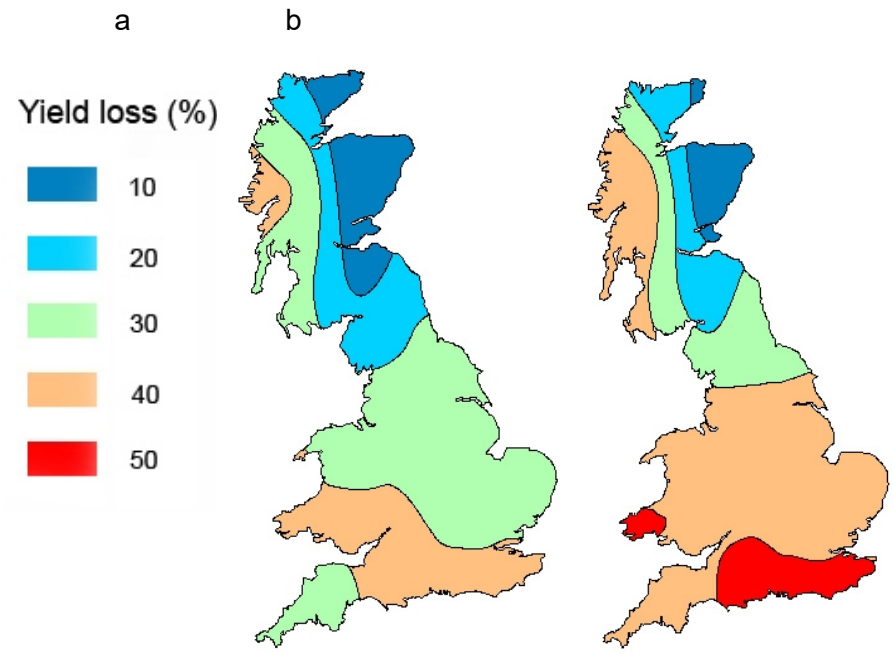


Fig. 2.1 Predicted losses from phoma stem canker for susceptible cultivars of winter oilseed rape for the 2020s (a) and 2050s (b) with increased temperature associated with climate change (Butterworth et al., Journal of the Royal Society Interface, 2010).

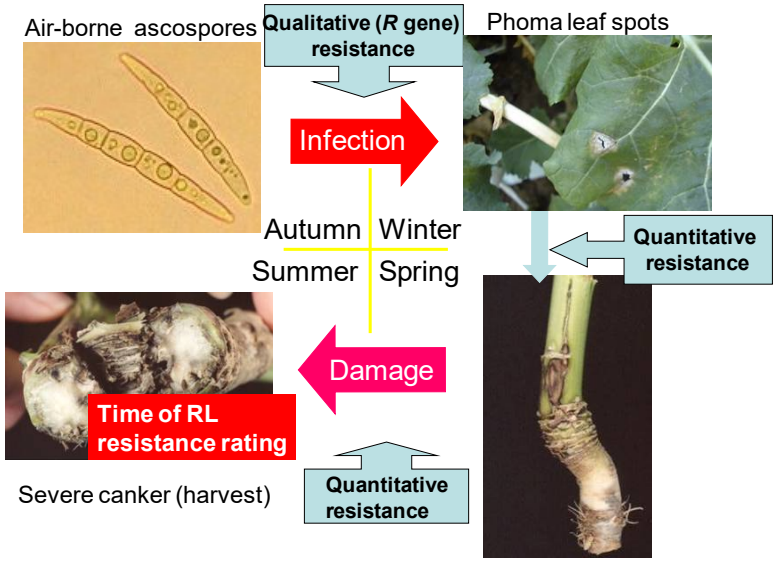


Fig. 2.2 Seasonal cycle of phoma stem canker epidemics in the UK in relation to components of oilseed rape (*Brassica napus*) resistance to *Leptosphaeria maculans*. Epidemics of this disease are initiated in autumn (September/October) by air-borne ascospores; the pathogen spreads along the leaf petioles to reach the stem, where stem base cankers or upper stem lesions develop by harvest. A gene (host *R* gene) for gene (pathogen *Avr* gene) specific host-pathogen interaction operates at the leaf infection stage but the basis for the background (quantitative) resistance that operates in the leaf petiole and stem is less clear. (Modified from Fitt et al., 2006).

Furthermore, we have shown that the effectiveness of such *R* gene-mediated qualitative resistance is often influenced by environmental factors such as temperature (Huang et al. 2006). We have demonstrated that, as temperature increases, the resistance to *L. maculans* mediated by the gene *Rlm6* no longer controls the disease at the leaf infection stage (Fig. 2.3; Huang et al, 2006). At least 12 *R* genes (*Rlm1* – *Rlm9*; *LepR1* – *LepR3*) for resistance against *L. maculans* have been identified. There is a need to investigate whether temperature affects the operation of all these *R* genes.

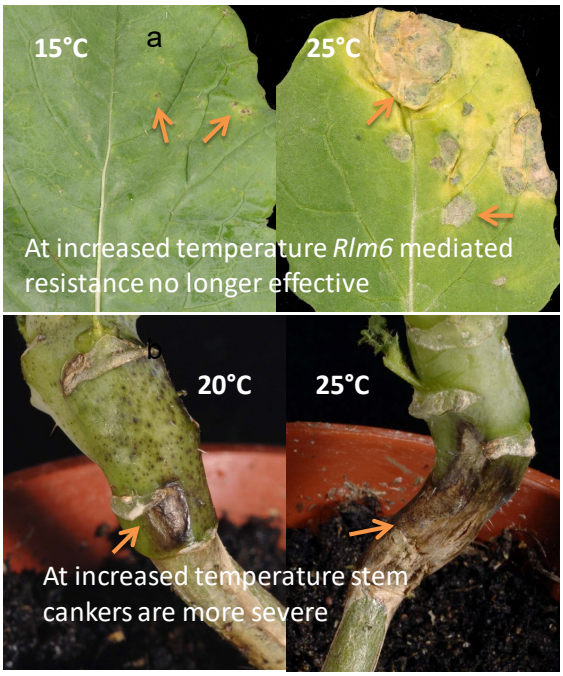


Fig. 2.3 Resistance to *Leptosphaeria maculans* is less effective at increased temperatures. This applies to both *R* genes' resistance operating in leaf laminas (a) and quantitative resistance operating in leaf stalks and stem tissues (b) (Huang et al., 2006 & 2009).

We have also obtained evidence that temperature influences the effectiveness of QR against *L. maculans* operating in leaf stalk and stem tissues. In controlled environment experiments, at lower temperatures (e.g. 15°C), more severe stem cankers developed on the varieties Eurol (without QR) than on Darmor (with QR) but there were no significant differences between Darmor and Eurol in stem canker severity when temperature increased to 25°C (Fig. 2.3; Huang et al, 2009). In France/Germany, where temperatures are greater than in the UK, phoma stem canker epidemics are generally more severe than in the UK (Huang et al. 2015). In Australia, when susceptible cultivars have been grown in Mediterranean conditions, phoma stem canker has caused 90% yield losses (Sprague et al. 2006). Therefore understanding the effects of temperature on effectiveness of qualitative and QR will provide valuable information for improving strategies to decrease risks of severe phoma stem canker epidemics on oilseed rape in the UK.

The overall aim of this project was to decrease the risk of severe phoma stem canker on oilseed rape at increased temperature by improving understanding of operation of *B. napus* resistance against *L. maculans*. There were three related objectives/tasks:

4. To identify *L. maculans* races in UK regions with different climates to optimise the deployment of oilseed rape resistance genes;
5. To investigate phenotypes of *R* gene-mediated and QR against *L. maculans* in leaf and stem tissues in different environments;
6. To develop an experimental system to investigate mechanisms of operation of *R* gene-mediated and QR against *L. maculans*.

3. Materials and methods

3.1. Differences in *L. maculans* races between different regional environments

3.1.1. Phoma leaf spot samples

To establish the current distribution of *L. maculans* races between UK regions (that differ in climate and cultivars; Stonard et al., 2010) for optimising deployment of oilseed rape cultivars with different *R* genes, field experiments were established, using a common protocol, by consortium members at 11 different sites (Fig. 3.1) using the very susceptible cultivar Drakkar in three growing seasons (2010/11, 2011/12, 2012/13). Since Drakkar has no *R* gene against *L. maculans*, it acts as a trap crop that is susceptible to all *L. maculans* races available in the local population. Leaves of Drakkar with phoma leaf spots were collected from different sites in autumn of each season. *L. maculans* isolates were obtained from the affected leaves using a method modified from that of West et al. (2002). In addition to 11 sites in the UK, there were four field experiment sites outside the UK. There was one site in Poland, one site in Germany and one site in France. Information from those sites enabled a comparison between *L. maculans* populations in the UK and those in other European countries.

Leaves were washed under running tap water and dried using tissue paper. A small piece of the leaf with a *L. maculans* type lesion (grey lesion with pycnidia) was cut out and put in a Petri dish lined with wet filter paper. The lid of the Petri dish was sprayed with distilled water to maintain high humidity and then incubated at 20°C with alternating 12h light and 12h darkness. After 3-5 days of incubation, pink cyrrhi were produced from pycnidia (Fig. 3.2). Under a dissection microscope, the cyrrhus from one mature pycnidium was collected using a sterile fine needle and transferred into a drop of sterilized water to make a spore suspension. The spore suspension was pipetted onto a plate containing PDA medium amended with 20 units/ml of penicillin and 40 units/ml of streptomycin, then incubated at 20°C in darkness. Isolates of *L. maculans* (each from a single pycnidium) were then sub-cultured onto V8 medium amended with 20 units/ml of penicillin and 40 units/ml of streptomycin, then incubated at 20°C with alternating 12h light and 12h darkness to induce pycnidial production. Conidial suspensions were made from each isolate. The conidial suspensions were used to identify avirulent/virulent alleles of *L. maculans Avr* genes using an established cotyledon test method (Balesdent et al. 2001) with a set of differential cultivars (Fig. 3.3). Seedlings were grown in trays

containing 40 wells with one seedling per well. Sticks were used to hold the plants to make sure that the cotyledons of the plants did not touch each other.

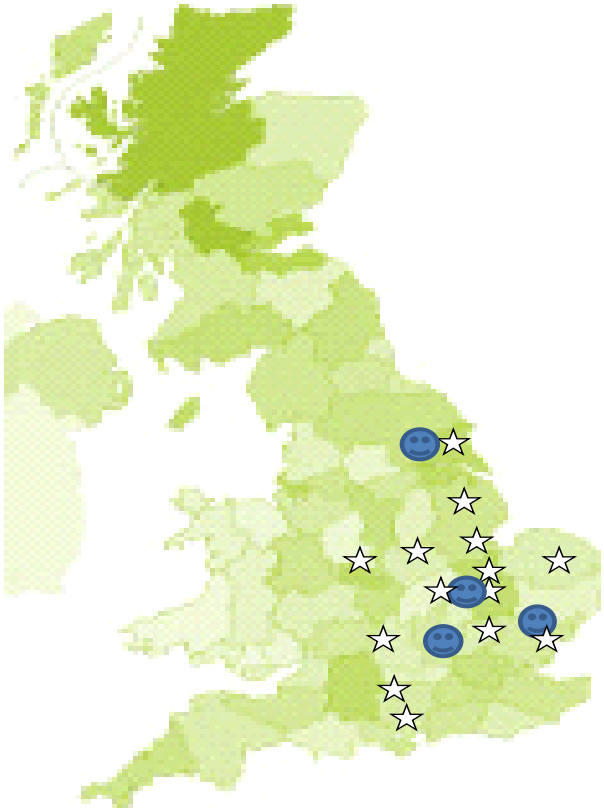


Fig. 3.1 Sites of 11 field experiments (stars) and 4 Burkard spore samplers (smiling faces) sampling air-borne ascospores of *L. maculans* populations over three cropping seasons (2010/2011, 2011/2012, 2012/2013).

Fig. 3.2 The deep cyrrhi from mature pycnidia produced on a phoma leaf spot lesion on an oilseed rape leaf (cultivar Drakkar) after 3 days of incubation at 20°C with 12h light and 12h darkness.

Plants were inoculated when they were 14 days old. True leaves were removed to make the cotyledons remain green for longer to allow for disease assessment. For each plant, a small hole was made at the edge of one of the cotyledon lobes (Fig. 3.3) with a Pasteur pipette to mark the

order of the isolates inoculated onto each plant. Very gently a sharp pin was used to wound the centre of each cotyledon lobe (Fig. 3.3) and an 11 µl drop of conidial suspension was directly placed onto the wound site (Fig. 3.4). After all the plants had been inoculated, plants were covered with a tray cover to maintain high humidity and incubated at 20°C with alternating 12h light and 12h darkness. At 12-20 days after inoculation, the disease was assessed using the 0-9 scale (Table 3.1) described by Koch et al. (1991).

Fig. 3.3 Plants were held up by sticks so that inoculated drops remained in place and cotyledons of different plants did not touch each other. A small hole was made on the edge of one of the cotyledon lobes with a Pasteur pipette.

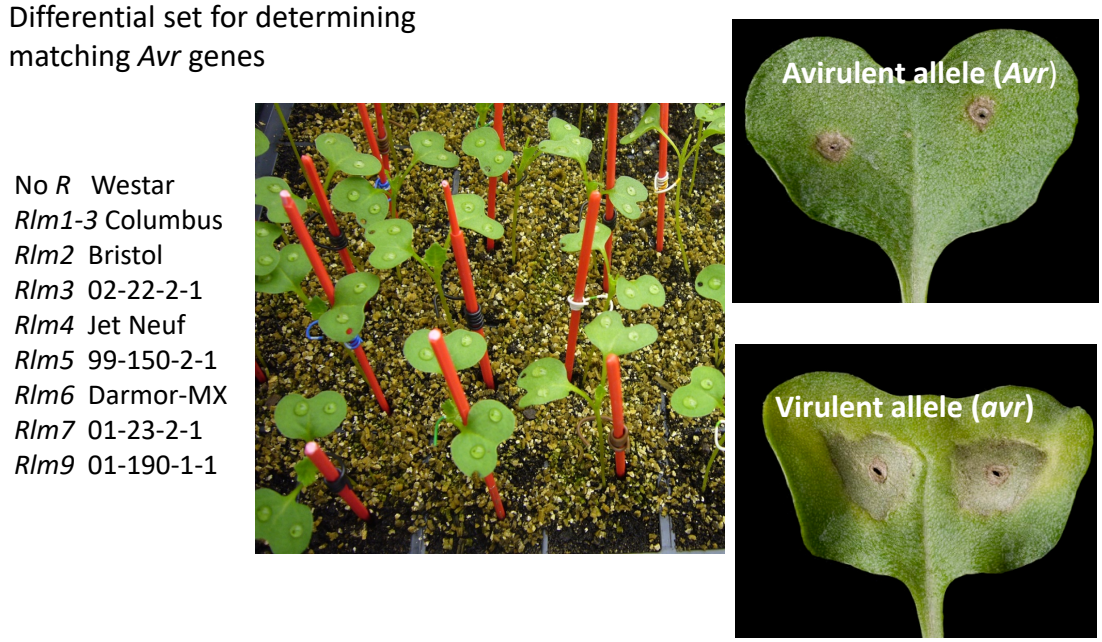


Fig. 3.4 Method used to determine races of *Leptosphaeria maculans* isolates collected from field experiments by identifying avirulent/virulent alleles of *Avr* genes through inoculation of cotyledons of a differential set of brassicas carrying different genes (listed on the left).

Table 3.1 Criteria for scoring *Leptosphaeria maculans* lesions on cotyledons on a 0-9 scale 12-20 days after inoculation (Koch et al. 1991).

Score	
0	No darkening around wounds, as in controls
1	limited blackening around wounds, lesion diameter 0.5-1.5 mm
3	dark necrotic lesions 1.5-3 mm
5	dark lesions, 3-6 mm diameter, brownish on lower surface
6	dark lesions 3-6 mm diameter, brownish on lower surface with a diffuse margin
7	as in 5, but less necrotic
8	grey-green lesions with no or few pycnidia
9	large grey-green lesions with profuse sporulation

3.1.2. Air-borne spore samples

Burkard 7-day continuously recording volumetric samplers (Burkard Manufacturing Company Ltd., Rickmansworth, UK) were operated at Rothamsted (2010/2011) or Bayfordbury (2011/2012, 2012/2013) and three other sites (Table 3.2) to sample air-borne *L. maculans* ascospores over the three growing seasons (2010/11, 2011/12, 2012/13). The Burkard samplers were operated from September to February when the major release of ascospores occurred. The Burkard samplers were surrounded radially by oilseed rape stems affected by phoma stem canker as described by Huang et al. (2005) (Fig. 3.5). At 7-day intervals, the 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. 3.5). One half was mounted onto a microscope slide for counting ascospores (Huang et al. 2005; Lacey and 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 future detection of frequencies of virulent/avirulent alleles of *AvrLm1* and *AvrLm6* in the air using qPCR (Van de Wouw et al., 2010).

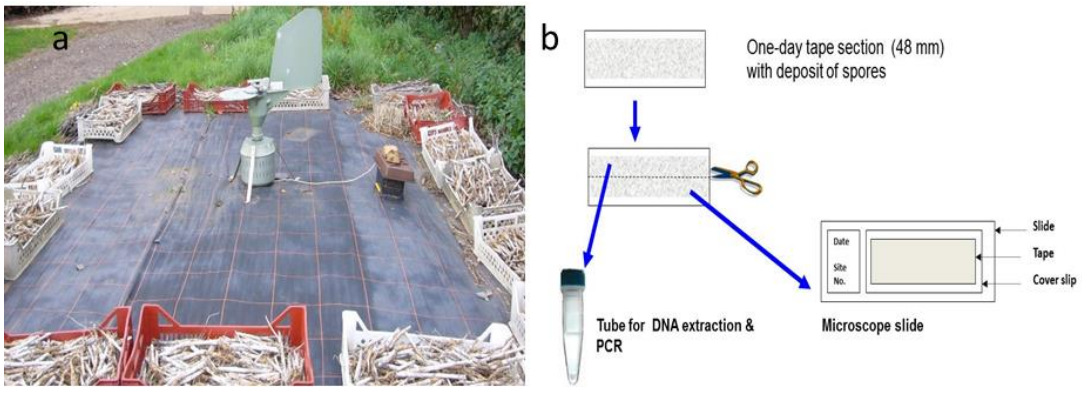


Fig. 3.5 The Burkard air sampler surrounded by oilseed rape stem debris used to sample ascospores of *L. maculans* released into the air (a) and the processing of air samples (b).

Table 3.2 Locations for collecting air-borne ascospores in the 2010/2011 growing season

Code	Organisation	Sponsor	Location
D	Limagrain	Jo Bowman	Rothwell, Market Rasen, Lincolnshire, LN7 6DT
H	Saaten Union	Richard Jennaway	Rosalie Field Station, Bradley Road, Cowlinge, Newmarket, Suffolk, CB8 9HU
I	Syngenta Seeds	Sean Burns	Syngenta Seeds Ltd, Hill Farm Raod, Whittlesford, Cambridge, CB22 4QT
K	Rothamsted	Yongju Huang	Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ

3.2. Interactions between *Rlm* gene and quantitative resistance in leaf and stem tissues in different environments

3.2.1. Effectiveness of *Rlm* genes in different backgrounds (with/without quantitative resistance) and quantitative resistance (with no known *R* genes) in field experiments in different regions

To examine effects of environment (e.g. temperature) and background QR on effectiveness of *R* genes, the consortium selected a set of 6 commercial cultivars (Table 3.3) carrying different *R* genes (*Rlm1*, *Rlm4* or *Rlm7*) in backgrounds with/without QR (e.g. one cultivar with *Rlm1* in a background with QR, one cultivar with *Rlm1* in a background without QR) for field experiments to investigate the effectiveness of the resistance in these cultivars under natural conditions. To examine the effects of environment on operation of QR, the consortium also selected two commercial cultivars (NK Grandia and Es-Astrid; Table 3.3) with good QR but no known *R* genes, for use in the field experiments to investigate the effectiveness of the resistance in these cultivars under natural conditions (different temperature/rainfall in different regions). The consortium selected a set of 11 sites representing different environments (Fig. 3.1). All the sites used the same set of cultivars, same source of untreated seed, same cultural practices and same disease assessment protocols.

In each growing season, the winter oilseed rape cultivars were sown in late August or early September. Plots were arranged in a randomised block design, with three replicates. The timing of phoma leaf spot assessment was adjusted from season to season according to the timing of the first major release of ascospores (i.e. the timing of the first appearance of phoma leaf spotting). The assessment was made on 10 plants per plot. Ten plants were sampled at random from each plot and individual data were collected for each plant, recording number of leaves, number of leaves with phoma leaf spots, severity of leaf spotting on a 0-3 scale (where 0, no leaf spotting; 1, <5 spots per plant; 2, 5-10 spots per plant; 3, >10 spots per plant). In addition to the severity assessment, for

experiments at Rothamsted, numbers of *L. maculans* type phoma leaf spots and *L. biglobosa* type phoma leaf spots were counted on each plant.

At the end of each season before harvest, severity of phoma stem canker was assessed by pulling up 30 stems per plot in late June or early July just before swathing or desiccation. Stems were pulled up at random throughout the plot. The stem base was cut to assess the stem base canker severity and the upper stem (>10 cm above the ground level) was cut to assess upper stem lesion severity. In addition, light leaf spot and sclerotinia stem rot were assessed. Stems were assessed by estimating % stem area with light leaf spot. Sclerotinia was assessed by visual observation of appearance of sclerotinia stem rot symptoms (recorded as yes or no). The severity of phoma stem canker was assessed by scoring the % necrotic tissue in cross-sections using a 0−6 scale (0, healthy stem with no affected tissue; 1, 1− 25% of cross-section affected; 2, 26 − 50% of cross-section affected; 3, 51− 75% of cross-section affected; 4, 76 − 99% of cross-section affected; 5, 100% of cross-section affected, plant still alive; 6, 100% affected, dead stem with a hollow or severely necrotic pith), modified from the 1-6 scale of Lô-Pelzer *et al*. (2009). The experiments were harvested and yields of individual plots were determined at 90% dry matter. In each season, information about weather and cultural practices was collected for each site when it was available (either from an on-site weather station or from a nearby weather station).

Table 3.3 Cultivars used in field experiments for studying operation of *R* gene-mediated resistance and quantitative resistance in natural conditions at 11 different sites (different temperatures etc.)

Cultivar	***R* gene**	**Quantitative resistance background**	**Current field resistance**	**AHDB RL rating (year)[1]**
NK Grandia	No	Yes	Resistant	6 (2010)
Es-Astrid	No	Yes	Resistant	7 (2010)
DK Cabernet	*Rlm1*	Yes	Resistant	6 (2010)
Adriana	*Rlm4*	Yes	Resistant	7 (2010)
Capitol	*Rlm1*	No	Susceptible	4 (2010)
Bilbao	*Rlm4*	No	Susceptible	4 (2010)
Excel	*Rlm7*	Yes	Resistant	9 (2010)
Roxel	*Rlm7*	No	Resistant	9 (2010)
Drakkar	No	No	Susceptible	_

[1] On a 1-9 scale, where 1 is susceptible, 9 is resistant.

3.2.2. Effectiveness of *Rlm* genes in backgrounds with/without quantitative resistance in leaf tissue in controlled environments at different temperatures

The effectiveness of different resistance genes *Rlm1*,*Rlm4, Rlm6, Rlm7, LepR1*, *LepR2 andLepR3*) in different cultivars/lines was tested in controlled environment (CE) experiments at different temperatures at the leaf infection stage using cotyledon inoculation methods (Huang et al. 2006). Cultivars were grown at different temperatures (20°C, 25°C) and cotyledons were inoculated with the corresponding isolates (i.e. the isolate producing a resistant phenotype on the cultivar carrying the corresponding *R* gene). To test the effectiveness of resistance genes *Rlm1* and *Rlm4* in backgrounds with/without quantitative resistance, different cultivars/lines with *Rlm1* and *Rlm4* were tested with different isolates in controlled environment (CE) experiments at different temperatures using cotyledon inoculation methods (Table 3.4). At 12-20 days after inoculation, the disease was assessed using the 0-9 scale (Table 3.1) as described by Koch et al. (1991). The phenotypic symptoms were also assessed; either small dark spots without pycnidia (resistant) or large grey lesions with pycnidia (susceptible).

Table 3.4 Cultivars/lines of oilseed rape and isolates of *Leptosphaeria maculans* used in the controlled environment experiments to assess effects of temperature on effectiveness of *R* gene or quantitative resistance (QR)

Cultivar	Isolate [1]	Inoculation [2]	CE condition [3]	Assessment
Adriana (*Rlm4* +QR)	V23.2.1 (Av**4**-5-6-7)	Cotyledon Petiole	20°C, 25°C	*R* gene & QR effects
Bilbao (*Rlm4*)	99-79 (Av2-**4**-7)	Cotyledon Petiole	20°C, 25°C	*R* gene & QR effects
Capitol (*Rlm1*) DK Cabernet (*Rlm1* +QR)	ME24 (Av**1**-6-7)	Cotyledon Petiole	20°C, 25°C	*R* gene & QR effects
	V23.11.9 (Av**1**-5-6-7)	Cotyledon Petiole	20°C, 25°C	*R* gene & QR effects
Columbus (*Rlm1+Rlm3*)	ME24 (Av**1**-6-7) V23.11.9 (Av**1**-5-6-7)	Cotyledon	20°C, 25°C	*R* gene effects
Jet Neuf (*Rlm4* +QR)	V23.2.1 (Av**4**-5-6-7) 99-79 (Av2-**4**-7)	Cotyledon	20°C, 25°C	*R* gene effects
A30 (no *R* genes, no QR) [4]	V23.11.9 (Av1-5-6-7) 99-79 (Av2-4-7)	Cotyledon Petiole	20°C, 25°C	Susceptible control
C119 (QR) [4]	V23.11.9 (Av1-5-6-7) 99-79 (Av2-4-7)	Petiole	20°C, 25°C	QR effects

[1] *L. maculans* isolate carrying different avirulent (Av) alleles of the *AvrLm* effector genes. The Av allele matching the corresponding host resistance (*R*) gene is in bold.

[2] Cotyledons or leaf petioles were inoculated with conidial suspensions.

[3] At all temperatures, the light was set with alternating 12 h light/12 h darkness.

[4]A30 and C119 are doubled haploid lines, not commercial cultivars.

3.2.3 Effectiveness of *Rlm* gene and quantitative resistance in stem tissues in controlled environments at different temperatures

To investigate whether temperature has similar effects on quantitative resistance (operating in leaf stalk and stem tissues) to those on qualitative *R* gene-mediated resistance (operating in leaf tissues), controlled environment experiments were done with two *R* genes (*Rlm1* or *Rlm4*) in different backgrounds. Two double haploid (DH) lines A30 (susceptible with no quantitative resistance) and C119 (with good quantitative resistance) were included in these experiments as controls (Table 3.4). Leaf petioles were inoculated with conidia of *L. maculans* isolates to allow the pathogen to grow along the leaf petiole to the stem and develop stem cankers (Huang et al. 2015). At each temperature, the time from inoculation to the appearance of stem canker and the severity of stem canker were assessed in a time course. Leaf petioles were inoculated with *L. maculans* isolates carrying the corresponding avirulent alleles of the *Avr* gene (Table 3.4). For petiole inoculation, the severity of stem canker was scored at a 0-6 scale (section 3.2.1). After stem canker assessment, individual stems were freeze dried for DNA extraction. The amount of *L. maculans* DNA in each stem was quantified using quantitative PCR (qPCR) (Huang et al. 2015).

3.3. Development of experimental systems to study mechanisms of operation of *R* gene-mediated resistance and quantitative resistance against *L. maculans*

3.3.1. Establishment of sets of material with different *R* genes in the same background or the same *R* gene in different backgrounds

One *L. maculans* isolate (Calm99-79) produced differential responses on the parents (resistant on Tapidor but susceptible on Ningyou7) (Fig. 3.6) of the *B. napus* DH mapping population BnaTNDH (Tapidor × Ningyou7) developed by Prof Jinling Meng at Huazhong Agricultural University (HAU). A set of differential isolates of *L. maculans* was tested on the parents using the cotyledon inoculation method (Balesdent et al. 2001). Preliminary results suggested that the *R* gene in Tapidor is *Rlm2*. To further confirm this, 202 DH lines from BnaTNDH were obtained from Prof Meng. The 202 DH lines were inoculated with the isolate Calm99-79. The *R* gene in Tapidor mapped on *B. napus* chromosome A10 close to another *R* gene (*LepR3*) which confirmed that the *R* gene in Tapidor is *Rlm2.* Furthermore, the *Rlm2* in Tapidor is not sensitive to temperature; the resistance in Tapidor was maintained when the temperature was increased from 15°C to 25°C. Results of preliminary experiments with Surpass400 (parent line for map-based cloning of *LepR3*) suggest that *LepR3* is sensitive to temperature; the same isolate (Ca99-79) produced a resistant phenotype on both Tapidor (*Rlm2*) and Surpass400 (*LepR3*) at 20°C, but a resistant phenotype on Tapidor (*Rlm2*) and a susceptible phenotype on Surpass400 (*LepR3*) at 25°C.

To investigate whether the observed differences between *LepR3* and *Rlm2* in response to temperature are due to effects attributable to genes at the resistance loci, it is necessary to establish near-isogenic lines with each of the genes in the same background. Our collaborators in Canada

have introduced *LepR3* (from Surpass400) and *Rlm2* (from Glacier) into a common susceptible Topas DH background by backcrossing (Fig. 3.7). To compare the operation of different *R* genes and minimise the background effects, it is necessary to have different *R* genes in the same background. Near-isogenic materials with *Rlm2* and *LepR3* in the same Topas background were produced by our Canadian collaborator during the life of the project.

Fig. 3.6 One *Leptosphaeria maculans* isolate (Ca99-79) produced a resistant reaction on cultivar Tapidor but susceptible reaction on cultivar Ningyou7, which are parents of the mapping population BnaTNDH. Photo was taken at 10 days after inoculation.

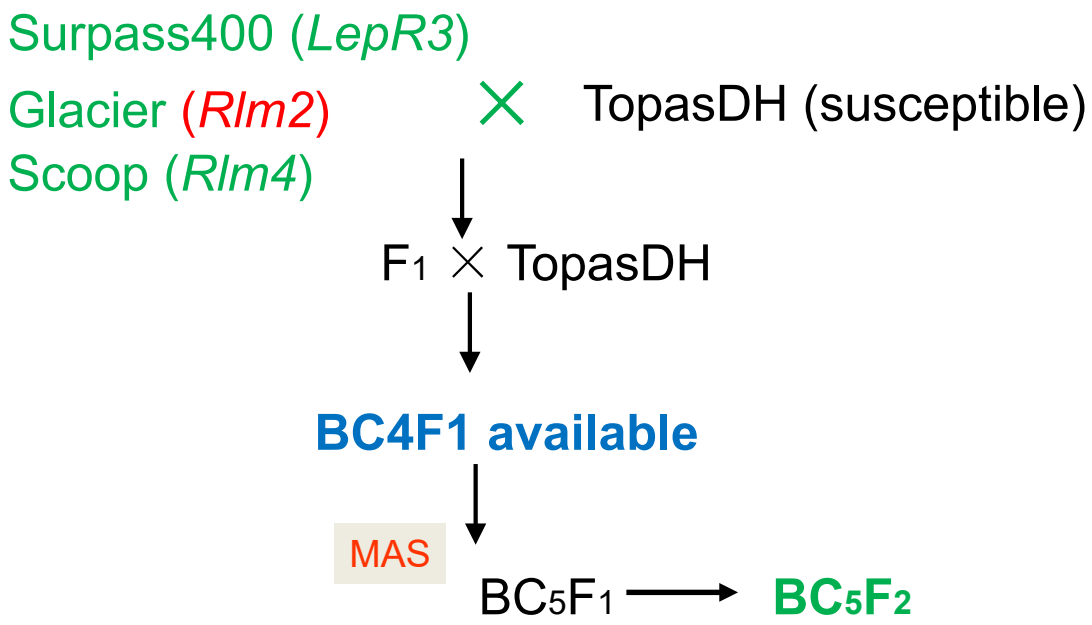


Fig. 3.7 Production of near-isogenic lines with three different *R* genes (*LepR3*, *Rlm2* and *Rlm4*). Through collaboration with Dr Derek Lydiate, Dr Nick Larkan and Dr Hossein Borhan (Saskatoon Research Centre, Agriculture and Agri-Food Canada), near-isogenic lines with these three *R* genes in a susceptible (Topas) background were obtained in October 2011 under a MTA.

3.3.2. Controlled environment experiments to investigate operation of *R* gene-mediated and quantitative resistance

To investigate whether the *R* gene-mediated resistance operating at the leaf infection stage is also operating at the stem colonisation stage (which is more relevant to 'field' resistance assessed before harvest) and whether effects of temperature on its operation in leaf tissues are similar to those in stem tissues, cultivars with *Rlm2* (Tapidor and Bristol) or *LepR3* (Suppass400) were inoculated with *L. maculans* isolates carrying the corresponding Avr genes (Table 3.5). To assess the effectiveness of *R* gene-mediated resistance in preventing leaf infection and the development of phoma leaf spots, cotyledons were inoculated at different temperatures. To assess the effectiveness of *R* gene-mediated resistance in preventing development of cankers in stem tissues, leaf petioles were inoculated with *L. maculans* to allow the pathogen to grow along the petiole to the stem and develop stem cankers at different temperatures. Two DH lines (without *R* genes) A30 (susceptible with no quantitative resistance) and C119 (with good quantitative resistance) (Huang et al. 2015) were included in these experiments as controls.

3.4. Exploitation and technology transfer

3.4.1. Exploitation and dissemination of new knowledge

Through involvement of AHDB, Co-operative Farms, NFU, DSV, Elsoms, Limagrain, Saaten Union, LS Plant Breeding, Monsanto, Pioneer, Grainseed, Syngenta and Agricultural Trusts (many trust committee members are farmers) in the consortium, results were **exploited directly** in the arable sector of the **agricultural industry** during the course of the project. Project meetings were held every 6 months to discuss results and deliver them to industry partners. New developments were conveyed to the **industry** through AHDB, Co-op Farms, NFU and breeders' open days, workshops and conferences. Recommendations for growers were made through AHDB topic sheets, at road-shows and events like Cereals'. Recommendations/suggestions for breeders were made through project meetings, phone calls and email communications.

3.4.1. Publications and conferences

New information about pathogen populations, air-borne pathogen spores and host resistance in field and controlled environment experiments was disseminated through publications and conference presentations. After publication of research papers, the press and other media were used during the course of the project to highlight the benefits and novelty of the research. Findings were communicated as refereed papers in international journals and as presentations/publications in scientific conference proceedings and seminars. Results of the project contributed directly to the

Table 3.5 Isolates of *Leptosphaeria maculans* used in controlled environment experiments to assess effects of *Rlm2* and *LepR3* in preventing leaf infection and preventing subsequent development of stem canker at different temperatures

Cultivar	Isolate [1]	Inoculation [2]	CE condition [3]	Assessment
Tapidor (*Rlm2*, QR)	87-41 (Av1-**2**-5-6-7)	Cotyledon	20°C, 25°C	Leaf spotting
	99-79 (Av**2**-4-6-7)			
	87-41 (Av1-**2**-5-6-7)	Petiole inoculation	20°C, 25°C	Canker development
	99-79 (Av**2**-4-6-7)			
Bristol (*Rlm2*, no QR)	87-41 (Av1-**2**-5-6-7)	Cotyledon	20°C, 25°C	Leaf spotting
	99-79 (Av**2**-4-6-7)			
Supass400 (*LepR3*, no QR)	87-41 (Av**1**-**2**-5-6-7)	Cotyledon	20°C, 25°C	Leaf spotting
	99-79 (Av**2**-4-6-7)			
	87-41 (Av**1**-**2**-5-6-7)	Petiole inoculation	20°C, 25°C	Canker development
	99-79 (Av**2**-4-6-7)			
C119 (no *R* genes, QR) [4]	ME24 (Av1-6-7)	Cotyledon	20°C, 25°C	Leaf spotting
	99-79 (Av2-4-6-7)			
	ME24 (Av1-6-7)	Petiole inoculation	20°C, 25°C	Canker development
	99-79 (Av2-4-6-7)			
A30 (no *R* genes, no QR) [4]	ME24 (Av1-6-7)	Cotyledon	20°C, 25°C	Leaf spotting
	99-79 (Av2-4-6-7)			
	ME24 (Av1-6-7)	Petiole inoculation	20°C, 25°C	Canker development
	99-79 (Av2-4-6-7)			

[1] *L. maculans* isolate carrying different avirulent (Av) alleles of the *AvrLm* effector genes. The Av allele matching the corresponding host resistance (*R*) gene is in bold.

[2] Cotyledons or leaf petioles were inoculated with conidial suspension (Huang et al., 2014).

[3] At all temperatures, the light was set with alternating 12 h light/12 h darkness.

[4] A30 and C119, doubled haploid lines without *R* genes, not commercial cultivars were used as controls for leaf infection (cotyledon inoculation) or canker development (petiole inoculation).

Current international debates on adaptation to climate change and food security and opportunities were taken to publicise the benefits of breeding crop cultivars with resistance that is effective at increased temperature at international conferences that addressed climate change/food security issues (e.g. International Congress of Plant Pathology, Beijing, August 2013).

4. Results

4.1. Differences in *L. maculans* races between different regional environments

4.1.1. *L. maculans* races in field populations in different regions

4.1.1.1. *L. maculans* races in field populations in the 2010/2011 growing season

In the 2010/2011 growing season, field experiments with cultivar Drakkar were set up at 15 sites (Table 4.1) to sample *L. maculans* populations in different regions. Leaves of Drakkar with phoma leaf spots were collected in autumn/winter from 12 sites (Table 4.2). In total, 347 single pycnidial isolates were obtained from those leaf samples (Table 4.1).

Table 4.1. Locations and sponsors of the field experiments in the 2010/2011 growing season

No.	Code	Sponsor	Location
1	A-Banbury	John Sweatman	Top Dawkins Barn, Wardington Road, Wardington, Banbury, Oxon, OX17 1FE, UK
2	B-Spalding	Mark Nightingale	Elsoms Seeds Ltd, Pinchbeck Road, Spalding, Lincolnshire, PE11 1QG, UK
3	C-TAG Morley	Neil Groom	TAG Morley, Wymondham, Norfolk, NR18 9DB, UK
4	D-Rothwell	Jo Bowman	Rothwell, Market Rasen, Lincolnshire, LN7 6DT, UK
5	E-Bainton	Craig Padley	Bainton, Nr Driffield, North Yorkshire, YO25 9EH, UK
6	F1- Harper Adams	Matthew Clarke	F1, Harper Adams, Shropshire, TF10 8NB, UK
7	F2- Stockbridge	Matthew Clarke	Stockbridge, Hampshire, SO21 3NE, UK
8	G1-Schenklengsfeld	Tony Betambeau	Schenklengsfeld, 36277, Germany
9	G2- Zlotoryja	Tony Betambeau	59-500 Zlotoryja, Southern Poland
10	H-Cowlinge	Richard Jennaway	Rosalie Field Station, Bradley Road, Cowlinge, Newmarket, Suffolk, CB8 9HU, UK
11	I1-Cranwell	Nigel Padbury	Cranwell, Sleaford, Grantham, Lincolnshire, NG32 3HJ, UK
12	I2-Verpillieres	Michaela Wille	Verpillières, F-80700, France
13	I3-Bad-Salzuflen	Andrea Libske	Zum Knipkenbach20, 32107 Bad Salzuflen, Germany
14	J-Oadby Lodge Farm	Robin Nurse	Stoughton Estate, Oadby Lodge, Farm, Gartree Road, Leicester, LE2 2FG, UK
15	K-Harpenden	Yongju Huang	Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK

To determine the races of these isolates, a set of differential cultivars/lines with known *R* genes was used for cotyledon inoculation tests. A total of 58 isolates from different sites were characterised by cotyledon inoculation. The results showed that the avirulent allele of *AvrLm7* was predominant at all the sites sampled, with a mean frequency of 100% (Fig. 4.1). This indicates that the corresponding resistance gene *Rlm7* is effective. Other predominant avirulent alleles were *AvrLm5* and *AvrLm6.* The frequencies of *AvrLm5* (92%) and *AvrLm6* (95%) avirulent alleles were greater than those of *AvrLm4* (47%) and *AvrLm1* (11%). The populations of *L. maculans* were 100% virulent at *AvrLm2, AvrLm3* and *AvrLm9* loci, suggesting that the corresponding resistance genes *Rlm2*, *Rlm3* and *Rlm9* are not effective against *L. maculans*.

There were differences between sites in frequency of *AvrLm1* and *AvrLm4* (Fig. 4.2) or *AvrLm5* and *AvrLm6* (Fig. 4.3). For example, frequencies of *AvrLm4* in Norfolk and Shropshire were 100% and 90%, respectively, which were greater than those at other locations (mean frequency 30% at Rothwell, Stockbridge and Cowlinge). Virulent alleles of *AvrLm6* were detected only in isolates from Germany and France (Fig. 4.3), all UK isolates were avirulent against the corresponding resistance gene *Rlm6*.

Table 4.2. Numbers of *L. maculans* isolates obtained from field experiments in the 2010/2011 growing season (for locations, see Table 4.1).

No.	**Code**	**Drakkar sampled**	**Isolates from Drakkar**			
1	A-Banbury	No phoma	*			
2	B-Spalding	15/02/2011	16			
3	C-TAG Morley	18/11/2010	30			
4	D-Rothwell	01/02/2011	30			
5	E-Bainton	25/10/2010	30	plus 15 from Roxet & Excel		
6	F1- Harper Adams	01/02/2011	27			
7	F2- Stockbridge	01/12/2010	30			
8	G1-Germany	27/4/2011	7			
9	G2-Poland	30/11/2010	8			
10	H-Cowlinge	17/11/2010	30			
11	I1-Cranwell	No phoma	*			
12	I2-Verpillieres	16/11/2010	28			
13	I3-Bad-Salzuflen	16/11/2010	30			
14	J-Castle Farm	No phoma	*			
15	K-Harpenden	07/12/2010	30	plus 36 from the 8 cultivars		

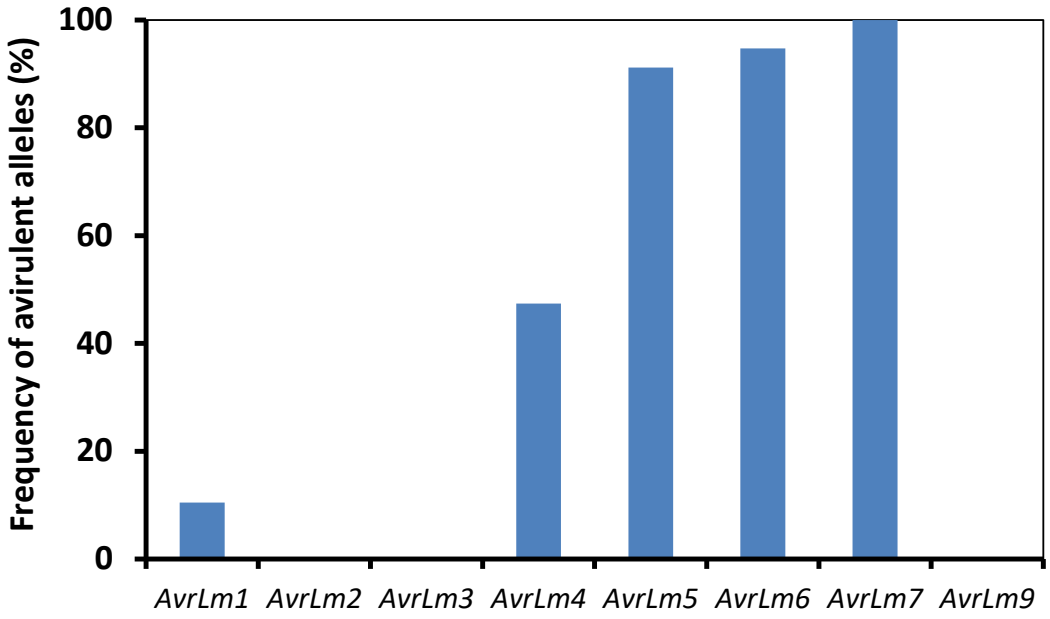


Fig. 4.1 Frequency of avirulent alleles of *L. maculans Avr* genes in the field populations sampled from Drakkar from ten different sites in the 2010/2011 growing season.

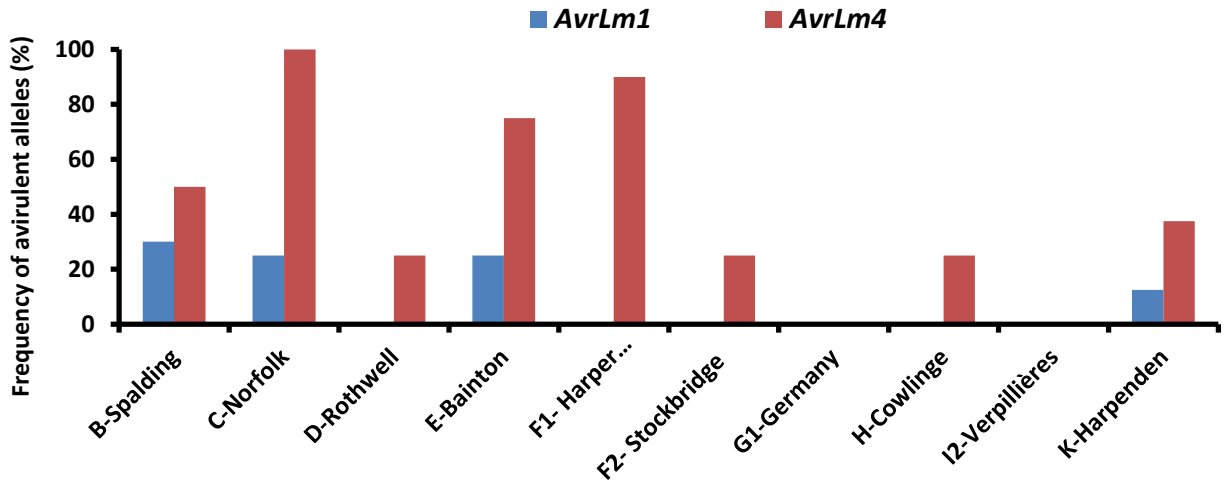


Fig. 4.2 Frequency of avirulent alleles of *AvrLm1* and *AvrLm4 Avr* genes in the field populations sampled by Drakkar at different locations in the 2010/2011 growing season.

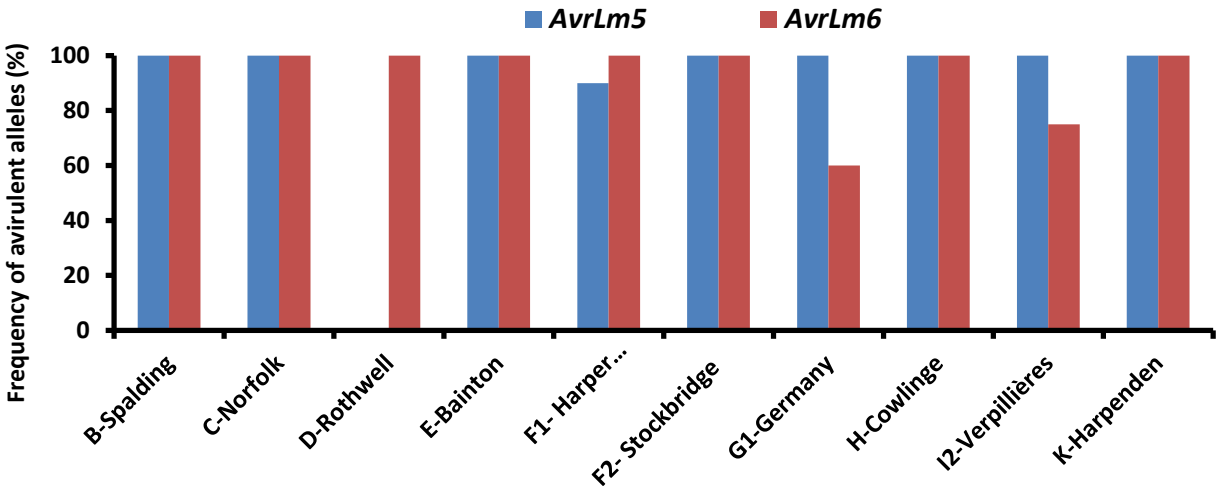


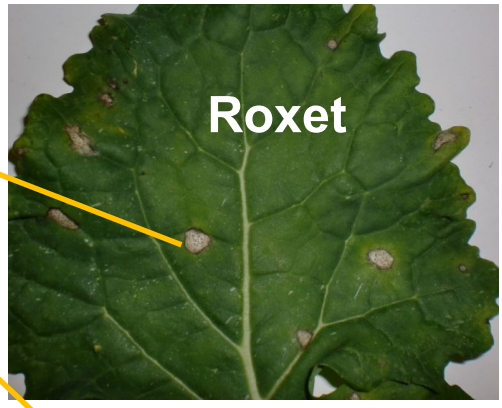
Fig. 4.3 Frequency of avirulent alleles of *AvrLm5* and *AvrLm6 Avr* genes in the field populations sampled from Drakkar at different locations in the 2010/2011 growing season.

Table 4.3. Numbers of *Leptosphaeria maculans* (Lm) or *L. biglobosa* (Lb) isolates obtained from field experiments in the 2010/2011 growing season.

Cultivar	**Lesion type**	**Site**	**Isolation**	**Lm**	**Lb**	**Lm%**	**Lb%**
Roxet	Big grey lesions	E-Bainton	6	6	0		
		H-Cowlinge	7	6	1		
		K-Harpenden	8	8	0		
						95.2	4.8
Excel	Big grey lesions	E-Bainton	6	4	2		
		H-Cowlinge	7	5	2		
		K-Harpenden	4	4	0		
						76.5	23.5
Roxet	Small dark lesions	E-Bainton	8	2	3		
		K-Harpenden	5	2	3		
						30.8	46.2
Excel	Small dark lesions	E-Bainton	8	1	4		
		K-Harpenden	5	0	5		
						7.7	69.2

Surprisingly, in the field experiments in the 2010/2011 growing season, phoma leaf spots were observed on cultivars carrying the effective resistance gene *Rlm7* (Excel and Roxet), but the phenotypes of phoma leaf spots were different from those on the susceptible cultivar Drakkar: the lesions on Excel and Roxet were smaller than those on Drakkar and these lesions had dark margins (no dark margins around lesions on Drakkar, Fig. 4.4). For the field experiment at Rothamsted, in addition to obtaining isolates from the trap cultivar Drakkar, single pycnidial isolates of *L. maculans* were also obtained from cultivars with quantitative resistance (with no known *R* gene) (Es-Astrid and NK Grandia). To investigate whether those lesions on the *Rlm7* cultivars were caused by *L. maculans* or *L. biglobosa*, isolates were obtained from Roxet and Excel. To compare them with isolates from cultivars with *Rlm1*, isolates from Capitol (*Rlm1*) and DK Cabernet (*Rlm1* + QR) were also obtained. The frequencies of *Avr* alleles in those isolates were investigated by cotyledon inoculation. This was done to investigate whether host background (quantitative) resistance affects selection for *Avr* alleles and whether there were any *L. maculans* isolates that were virulent against the resistance gene *Rlm7*. It was found that there were some host selection effects; for example the frequency of the avirulent allele *AvrLm7* was 100% in populations obtained from Capitol but only 80% in populations obtained from DK-Cabernet (Fig. 4.5). There were also differences between isolates obtained from Roxet and Excel (Fig. 4.6). For further analysis, the isolates were cultured to harvest mycelium for DNA extraction and molecular characterisation. Most of the isolates from small dark lesions on Excel or Roxet were *L. biglobosa* (Table 4.3)

- ***L. maculans* or *L. biglobosa*?**
- **Virulent *L. maculans* (*AvrLm7* or *avrLm7*)*?***
- **Early detection of *avrLm7* to avoid breakdown of *Rlm7?***



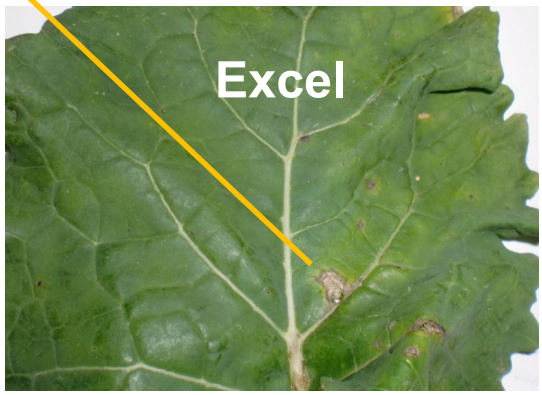


Fig. 4.4 Symptoms of phoma leaf spot on Drakkar (no *R* genes) and Roxet (*Rlm7*) and Excel (*Rlm7*) in a field experiment at Rothamsted in the 2010/2011 growing season.

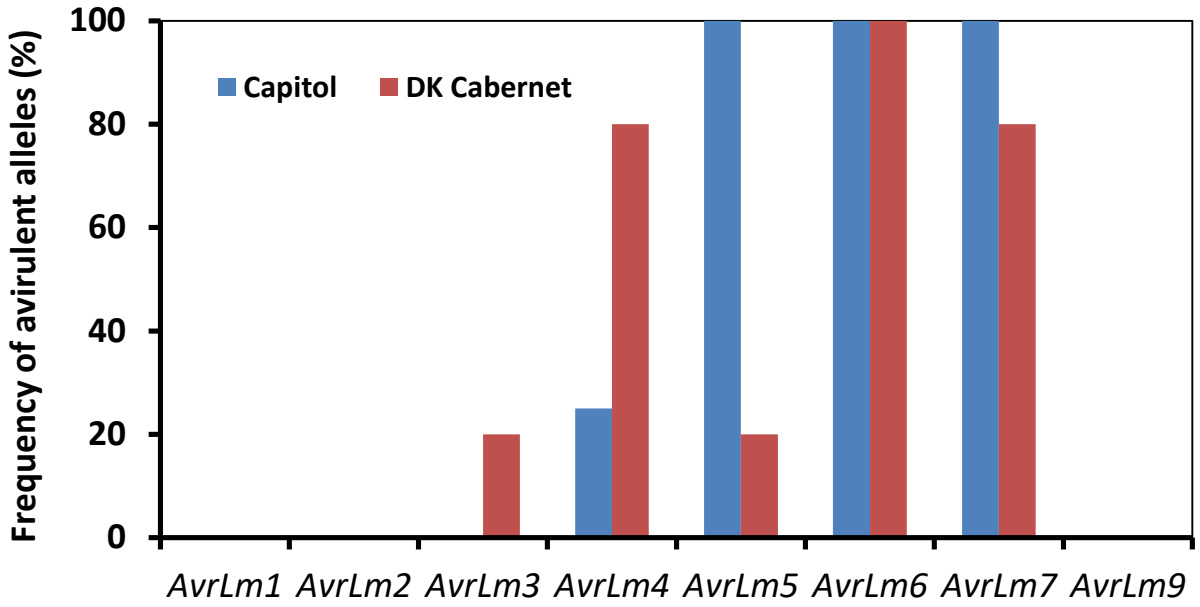


Fig. 4.5 Frequency of avirulent alleles *Avr* in *L. maculans* isolates obtained from cultivars Capitol and DK Cabernet from the Rothamsted field experiment in the 2010/2011 growing season.

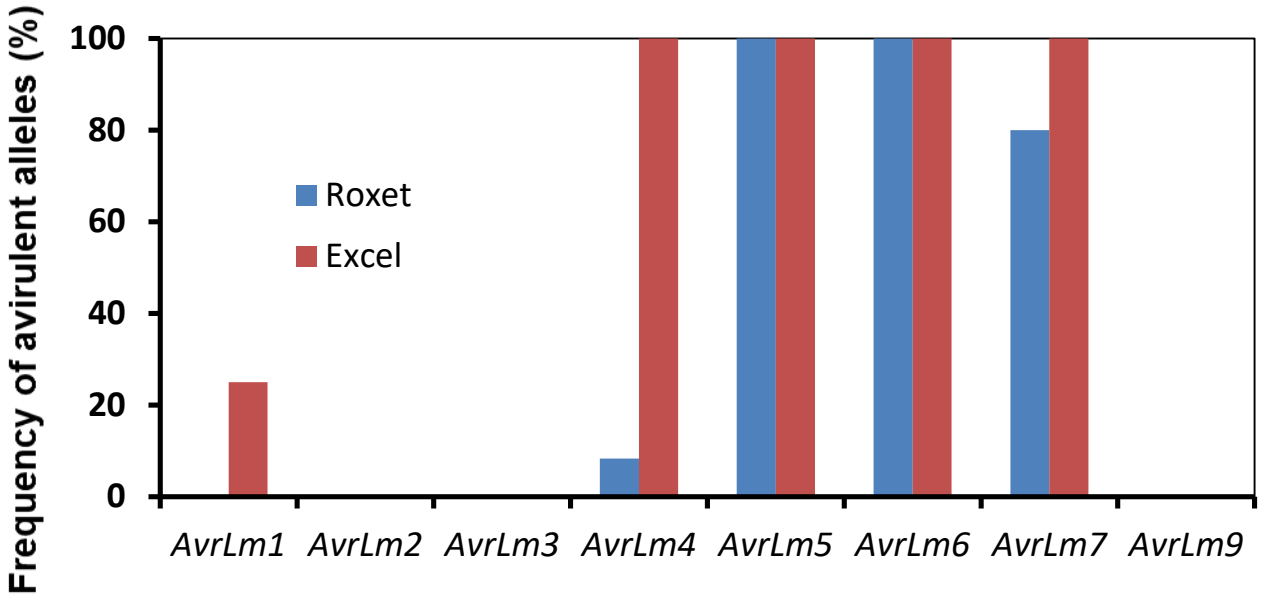


Fig. 4.6 Frequency of avirulent alleles *Avr* in *L. maculans* isolates obtained from cultivars Roxet and Excel from the Rothamsted field experiment in the 2010/2011 growing season.

4.1.1.2. *L. maculans* races in field populations in the 2011/2012 growing season

In the 2011/2012 growing season, field experiments with cultivar Drakkar were set up at the same 15 locations (Table 4.1) but in different fields to collect *L. maculans* populations from different regions. Leaves of Drakkar with phoma leaf spots were collected in autumn/winter from 12 sites (Table 4.4). In total, 98 single pycnidial isolates were obtained from Drakkar leaf samples. To monitor the occurrence of races that are virulent against *Rlm7*, leaves of Roxet and Excel with phoma leaf spots were also sampled for a PhD project.

To determine the races of those isolates, a set of differential cultivars/lines with known *R* genes was used for cotyledon inoculation tests. A total of 88 isolates from different sites were characterised by cotyledon inoculation. The results showed that the avirulent allele of *AvrLm7* was still predominant at all the sites sampled but the virulent allele of *AvrLm7* was detected (frequency 3%) (Fig. 4.7). This indicates that the corresponding resistance gene *Rlm7* is effective but there is a need to monitor the pathogen population so that this *R* gene can be deployed effectively. Other avirulent alleles that were predominant were *AvrLm5* and *AvrLm6.* The populations of *L. maculans* were 100% virulent at *AvrLm2* and *AvrLm9* loci, as in the 2010/2011 season, suggesting that the corresponding resistance genes *Rlm2* and *Rlm9* are not effective against *L. maculans*. However, the isolates that had virulent alleles of *AvrLm7* also had avirulent alleles of *AvrLm3*.

Table 4.4. Numbers of *Leptosphaeria maculans* isolates obtained from field experiments at different locations in the 2011/2012 growing season.

Code	Drakkar sampled	Isolates from Drakkar	Leaf samples of *Rlm7*
A-Banbury	5/3/2012	24	No
B-Spalding	21/02/2012	13	Yes
C-Norfolk	01/02/2012	8	Yes
D-Rothwell	25/01/2012	12	Yes
E-Bainton	20/01/2012	9	Yes
F1- Harper Adams	22/02/2012	12	Yes
F2- Stockbridge	21/03/2012	8	-
G1-Germany	No phoma	-	-
G2-Poland	No phoma	-	-
H-Cowlinge	16/12/2011	10	Yes
I1-Cranwell	No sample	-	-
I2-Verpillieres	31/01/2012	12	Yes
I3-Bad-Salzuflen	03/12/2011	12	Yes
J-Oadby Lodge Farm	06/03/2012	20	Yes
K-Harpenden	25/01/2012	10	Yes

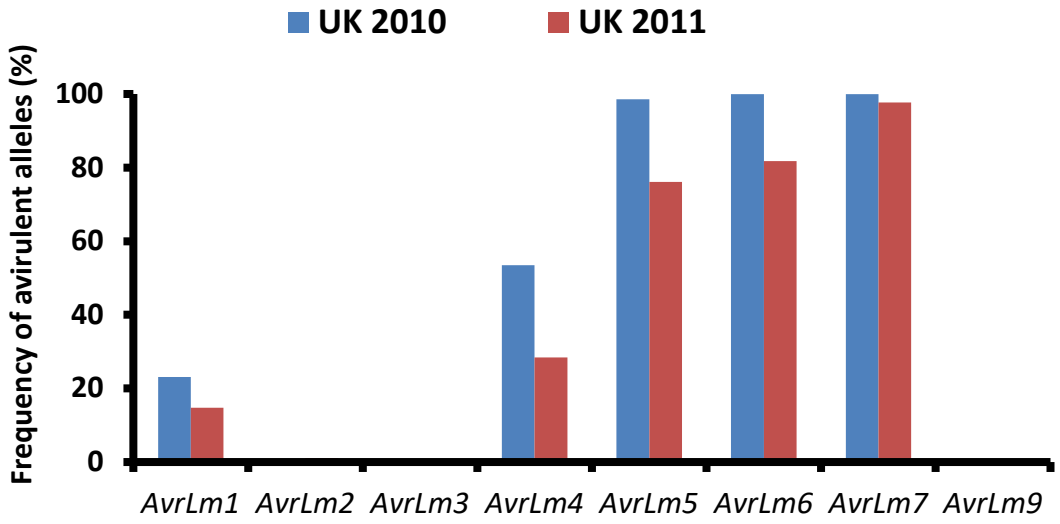


Fig. 4.7 Frequency of avirulent alleles of *Leptosphaeria maculans Avr* genes in the field populations obtained from Drakkar from ten different UK sites in the 2010/2011 (UK 2010) and 2011/2012 (UK 2011) growing seasons.

There were also differences between sites in frequencies of avirulent *AvrLm1, AvrLm4, AvrLm5* and *AvrLm6* alleles (Fig. 4.8). For example, frequencies of *AvrLm4* in Norfolk and Shropshire were 100% and 90%, respectively, which were greater than those at other locations (mean frequency 30% at Rothwell, Stockbridge and Cowlinge). Virulent alleles of *AvrLm6* were detected at most UK sites (Fig. 4.8), whereas they were not detected in the UK in 2010/2011 (Fig. 4.3).

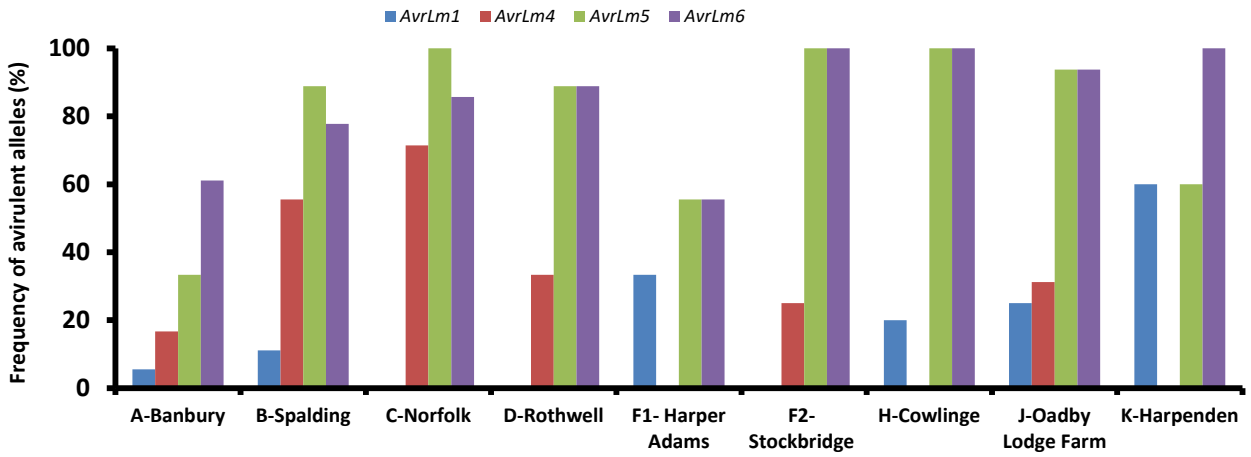


Fig. 4.8 Frequencies of avirulent alleles of *AvrLm1*, *AvrLm4*, *AvrLm5* and *AvrLm6 Avr* genes in *Leptosphaeria maculans* populations obtained from Drakkar from different locations in the 2011/2012 growing season.

4.1.1.3. *L. maculans* races in field populations in the 2012/2013 growing season

In the 2012/2013 growing season, field experiments with cultivar Drakkar were set up at the same 15 locations (Table 4.1) but in different fields to collect *L. maculans* populations from different regions. Due to heavy rain, the field experiment at site J (Oadby Lodge Farm) was flooded and the experiment was abandoned. Since isolates virulent against the resistance gene *Rlm7* were detected in the 2011/2012 growing season, in 2012/2013 *L. maculans* isolates were also obtained from cultivars carrying *Rlm7* (Table 4.5). In total, 101 isolates were obtained from Drakkar, 92 isolates were obtained from Excel and 109 isolates were obtained from Roxet.

A total of 28 *L. maculans* isolates from Drakkar were characterised by cotyledon inoculation. The results showed that the avirulent allele of *AvrLm7* was still predominant at all the sites sampled but the virulent allele of *AvrLm7* was detected at a very low frequency (Fig. 4.9). This indicated that the corresponding resistance gene *Rlm7* was still effective but that there is a need to monitor the *L. maculans* populations so that this *R* gene can be deployed effectively. Compared with the previous two growing seasons, the frequencies of avirulent alleles of *AvrLm5* and *AvrLm6* were less in 2012/2013. The populations of *L. maculans* were 100% virulent at *AvrLm2* and *AvrLm9* loci, as in the previous two seasons suggesting that the corresponding resistance genes *Rlm2* and *Rlm9* are not effective against *L. maculans* in the UK field conditions.

Table 4.5. Numbers of *Leptosphaeria maculans* isolates obtained from oilseed rape field experiments at different locations in the 2012/2013 growing season.

Code	Isolates from Drakkar	Isolates from Excel	Isolates from Roxet
A-Banbury	8	4	8
B-Spalding	14	5	0
C-Norfolk	8	8	10
D-Rothwell	10	11	12
E-Bainton	0	11	14
F1- Harper Adams			
F2- Stockbridge			
G1-Germany			
G2-Poland	9	0	6
H-Cowlinge	8	16	20
I1-Cranwell	4	9	0
I2-Verpillieres	10	0	4
I3-Bad-Salzuflen	2	1	1
J-Oadby Lodge Farm			
K-Harpenden	20	24	21

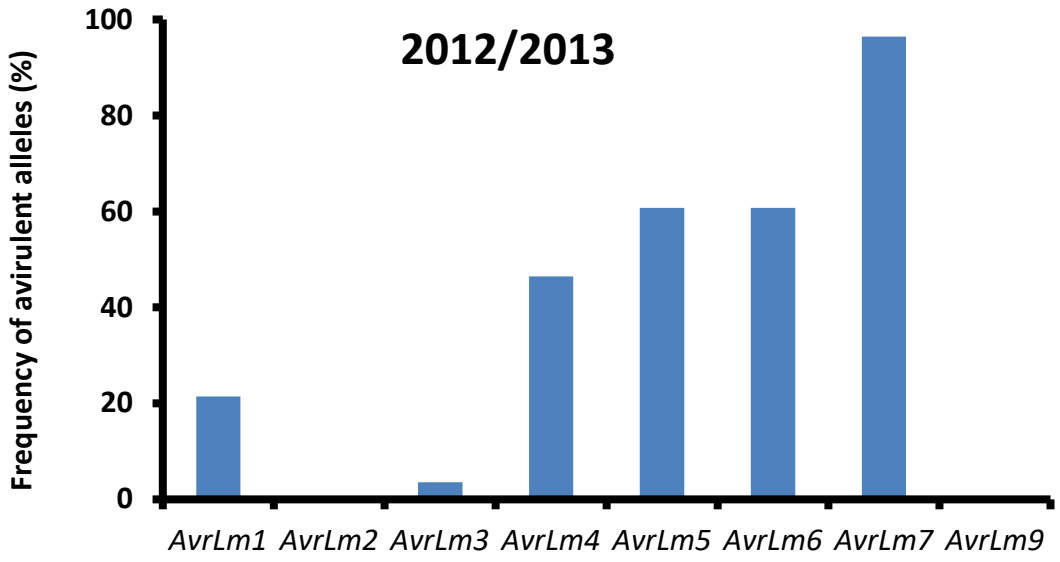


Fig. 4.9 Frequency of avirulent alleles of *Leptosphaeria maculans Avr* genes in the field populations obtained from Drakkar from ten different sites in the 2012/2013 growing season.

In addition to testing isolates obtained from Drakkar, 33 *L. maculans* isolates obtained from cultivars carrying the *Rlm7* gene were tested. Surprisingly, results showed that the majority of isolates obtained from cultivars carrying *Rlm7* were still avirulent against *Rlm7* (Fig. 4.10).

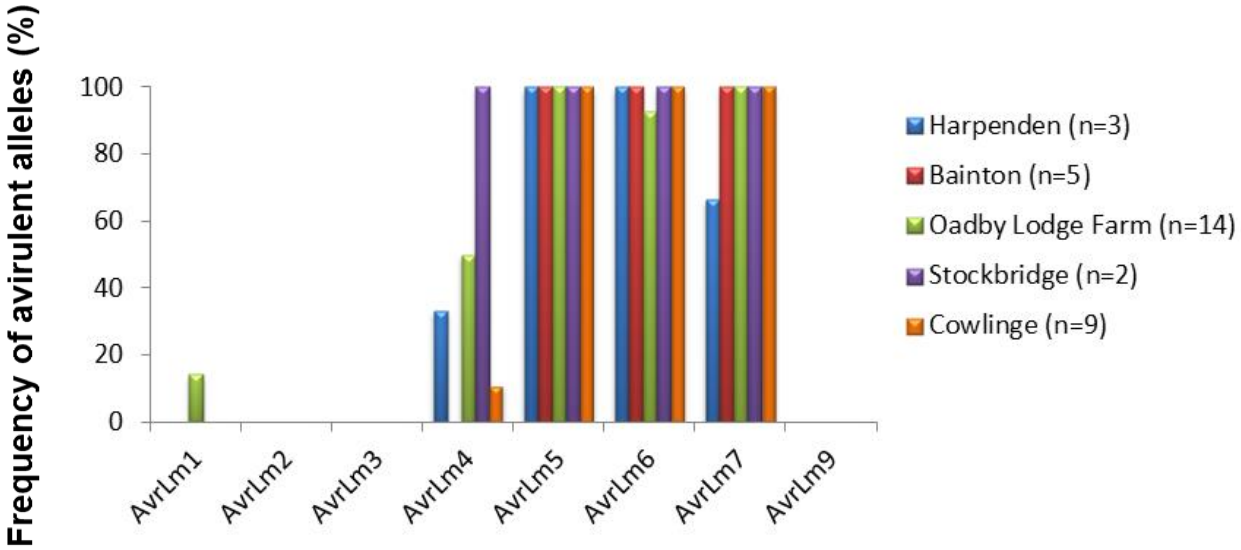


Fig. 4.10 Frequency of avirulent alleles of *Leptosphaeria maculans Avr* genes in the field populations obtained from cultivars carrying *Rlm7* from different locations in the 2012/2013 growing season.

4.1.2. Patterns of ascospore release in different regions

Burkard 7-day continuously recording volumetric samplers were operated at four locations over the three growing seasons. The patterns of ascospore release differed between locations in 2010/2011 (Fig. 4.11), 2010/2012 (Fig. 4.12) and 2012/2013 (Fig. 4.13). Ascospore release patterns also differed between seasons; for example at Rothamsted/Bayfordbury in Hertfordshire the date when the maximum ascospore release occurred was more than 2 months later in the 2011/2012 season than that in the 2010/2011 season (Fig. 4.14). Interestingly, although the patterns of ascospore release differed between locations, the days when the maximum ascospore release occurred were similar at the four locations in each season. There was only one to five days difference between locations (Table 4.6).

Timing of ascospore release is related to rainfall in August and September (Evans et al. 2008). For example, at Bayfordbury in 2011/2012 season, very little rainfall occurred in August and September in 2011 and this delayed the maturation of pseudothecia. Major ascospore release was not observed until January 2012 after heavy rainfall in December 2011 (Fig. 4.15).

4.2. Interactions between *R* gene and quantitative resistance in leaf and stem tissues in different environments

4.2.1. Effectiveness of *R* genes in different backgrounds (with/without quantitative resistance) and quantitative resistance (with no known *R* genes) in field experiments in different regions

4.2.1.1. Severity of phoma leaf spots

In the 2010/2011 growing season, field experiments with 8 cultivars carrying different *R* genes in backgrounds with/without QR were set up at 15 sites (Table 4.1) to investigate the effectiveness of resistance in each cultivar under field conditions. The severity of phoma leaf spots was assessed on a 0-3 scale at 7 sites in the autumn. There were differences in severity of phoma leaf spot between locations (Fig. 4.16). Phoma leaf spot was more severe in the field experiment in France than in field experiments in the UK. Among the field experiments in the UK, phoma leaf spot was more severe at Rothwell than other sites. There were also differences in severity of phoma leaf spot between cultivars. Cultivar Drakkar always had more phoma leaf spots than other cultivars at all the locations. The two cultivars with *Rlm7* (Roxet and Excel) generally had less phoma leaf spots than other cultivars. For the cultivars with *Rlm1* or *Rlm4* in backgrounds with/without QR, cultivars DK Cabernet (*Rlm1* + QR) and Adriana (*Rlm4* + QR) generally had less phoma leaf spots than cultivars Capitol (*Rlm1*) and Bilbao (*Rlm4*) (Fig. 4.16).

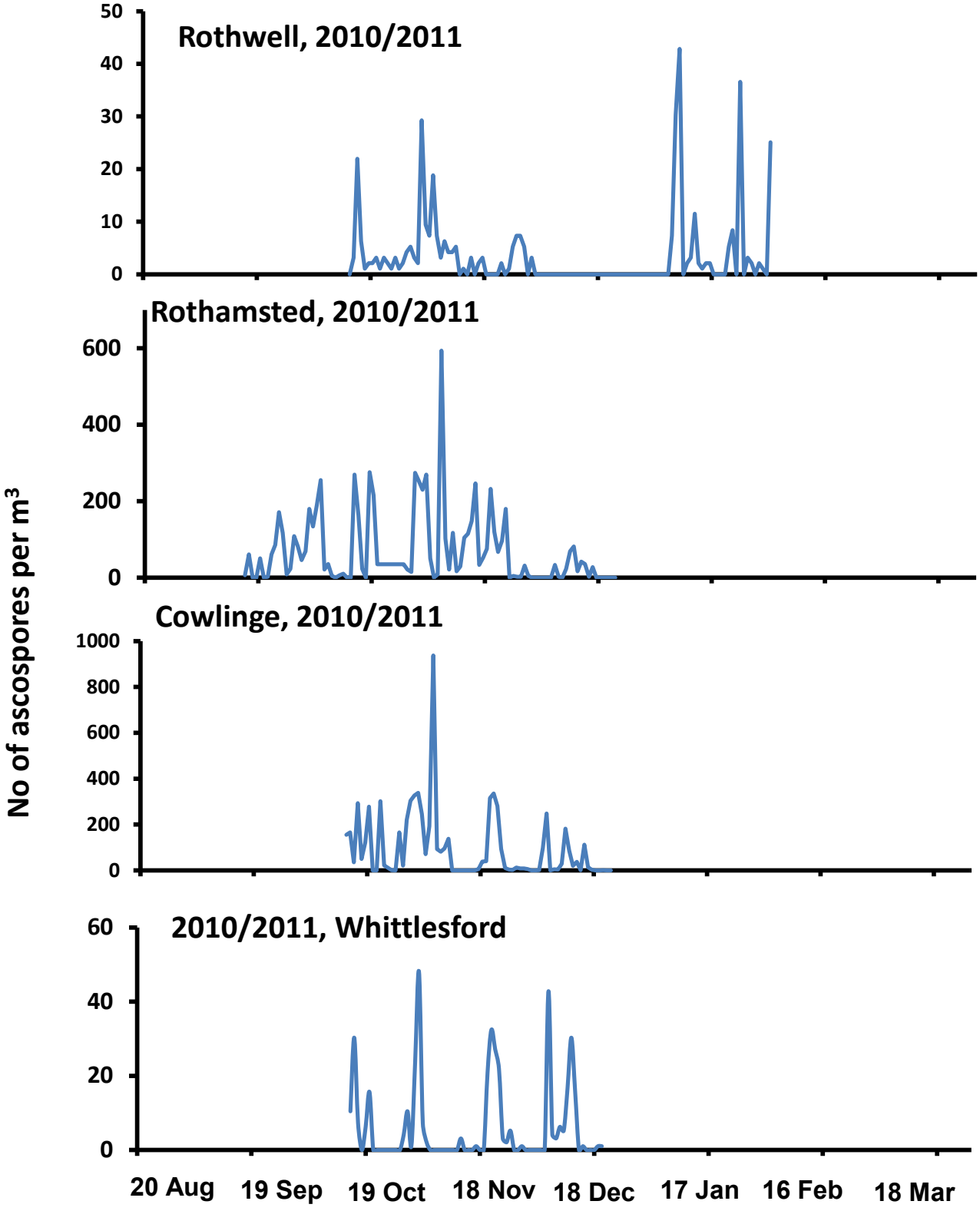


Fig. 4.11 Patterns of *Leptosphaeria maculans* ascospore release at four locations in the 2010/2011 winter oilseed rape growing season.

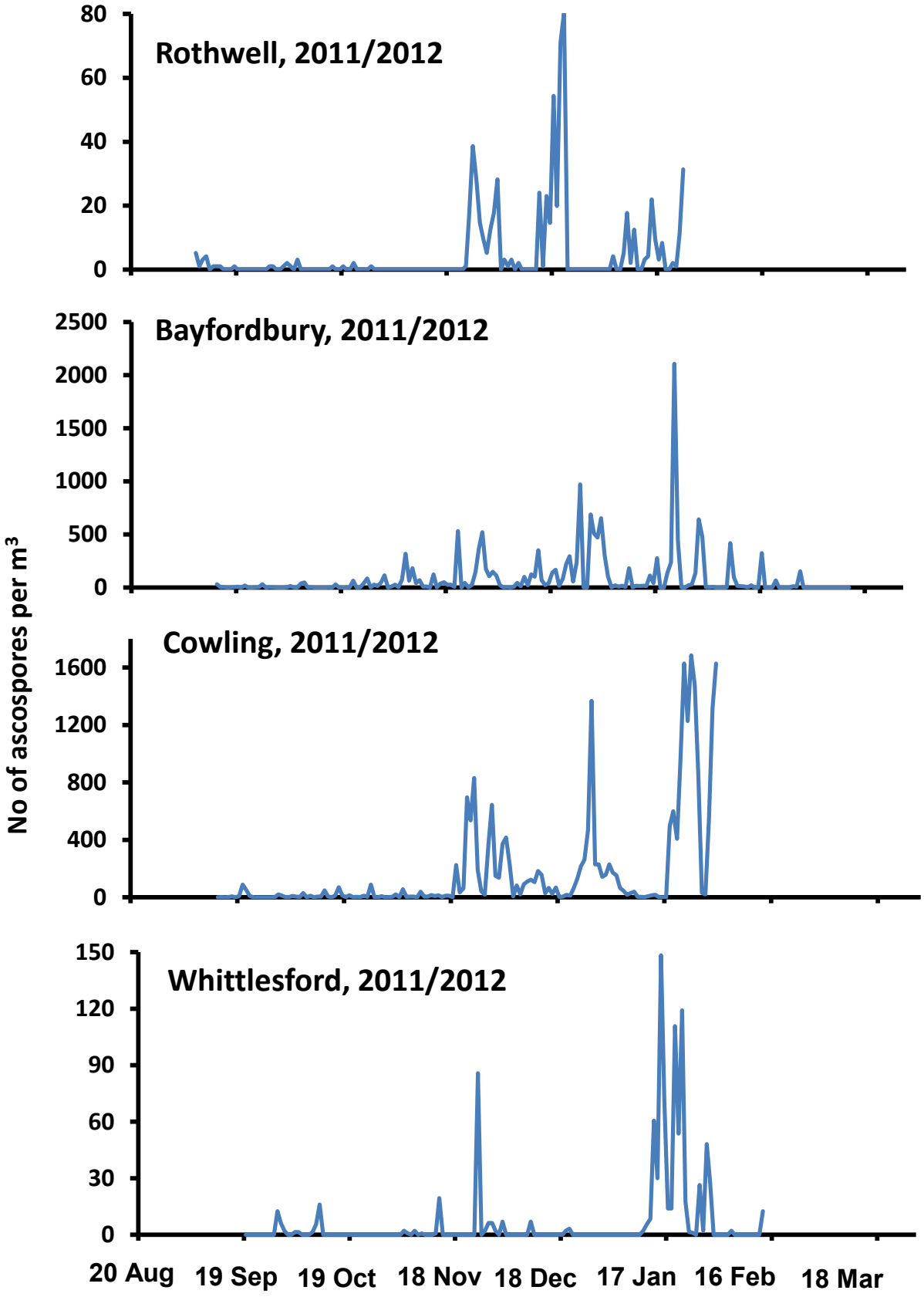


Fig. 4.12 Patterns of *Leptosphaeria maculans* ascospore release at four locations in the 2011/2012 winter oilseed rape growing season.

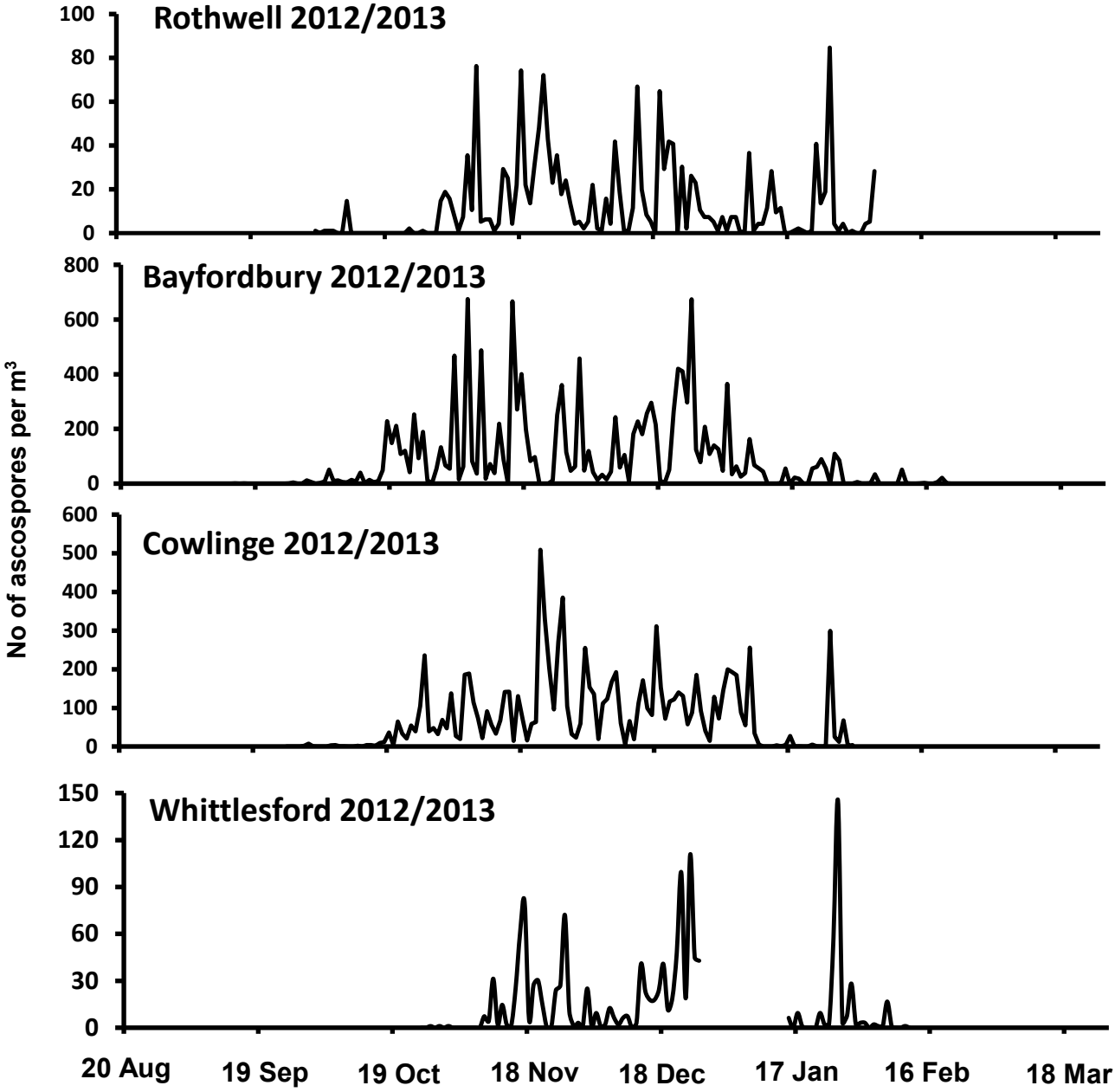


Fig. 4.13 Patterns of *Leptosphaeria maculans* ascospore release at four locations in the 2012/2013 winter oilseed rape growing season.

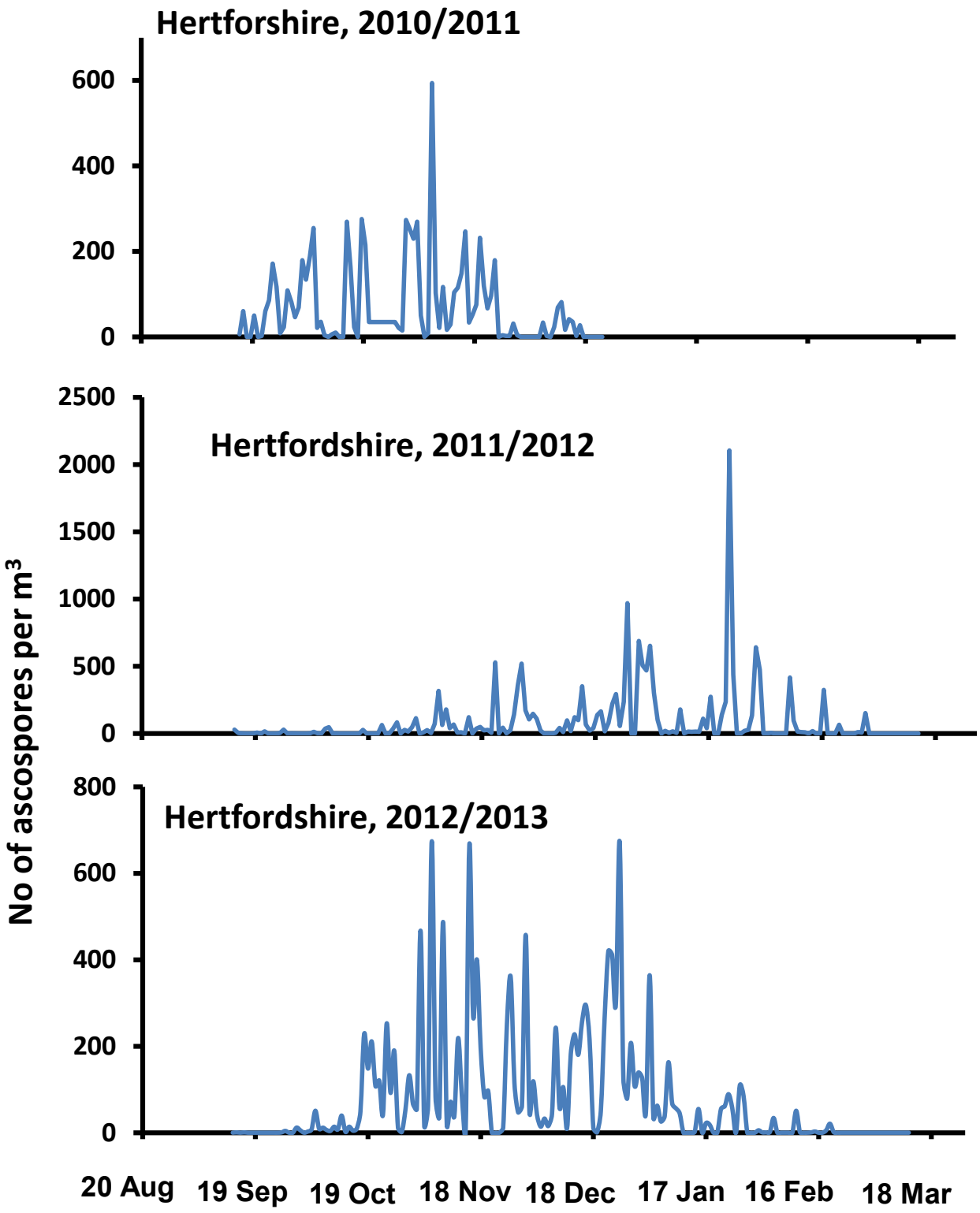


Fig. 4.14 Patterns of ascospore release at Rothamsted/Bayfordbury in Hertfordshire in the 2010/2011, 2011/2012 and 2012/2013 seasons.

Table 4.6 Dates when the maximum numbers of ascospores were observed at different locations over the 2010/2011, 2011/2012 and 2012/2013 growing seasons.

Site	2010-2011	2011-2012	2012-2013
D-Rothwell	1 Nov 2010	21 Dec 2011	8 Nov 2012
H-Cowlinge	5 Nov 2010	24 Jan 2012	22 Nov 2012
I-Whittlesford	2 Nov 2010	19 Jan 2012	17 Nov 2012
K-Bayfordbury	6 Nov 2010	22 Jan 2012	5 Nov 2012

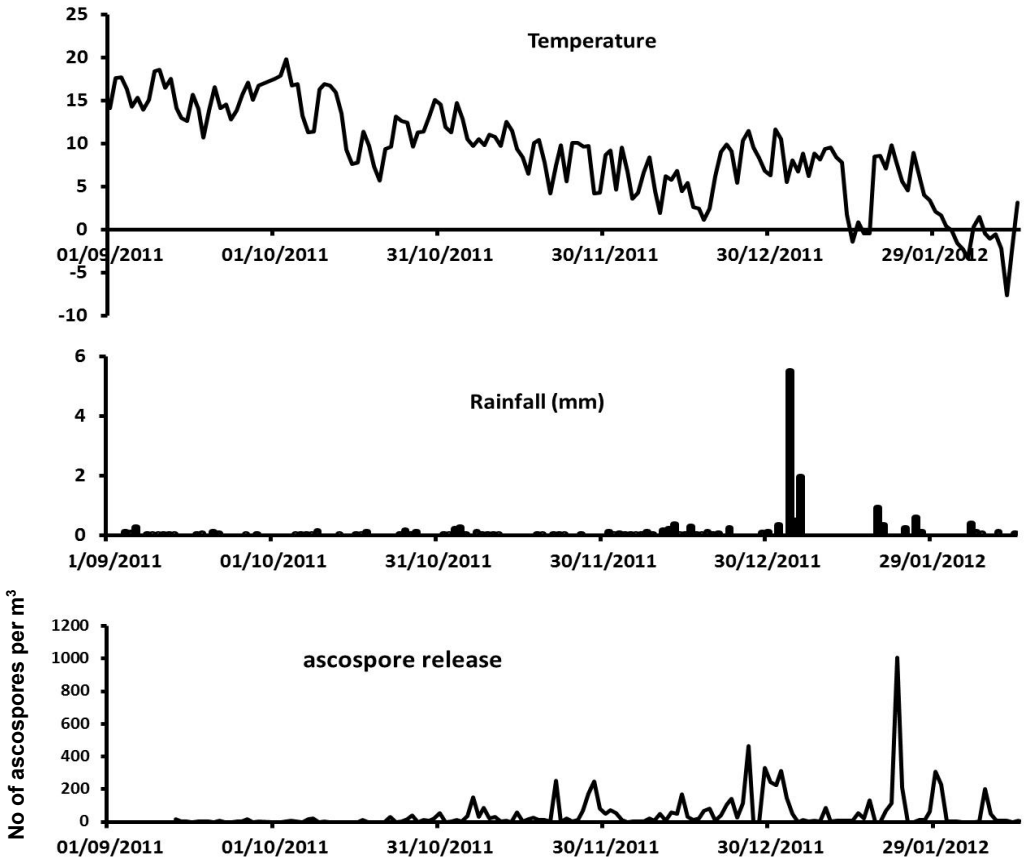


Fig. 4.15 Mean temperature, rainfall and number of ascospores released at Bayfordbury in Hertfordshire in the 2011/2012 season.

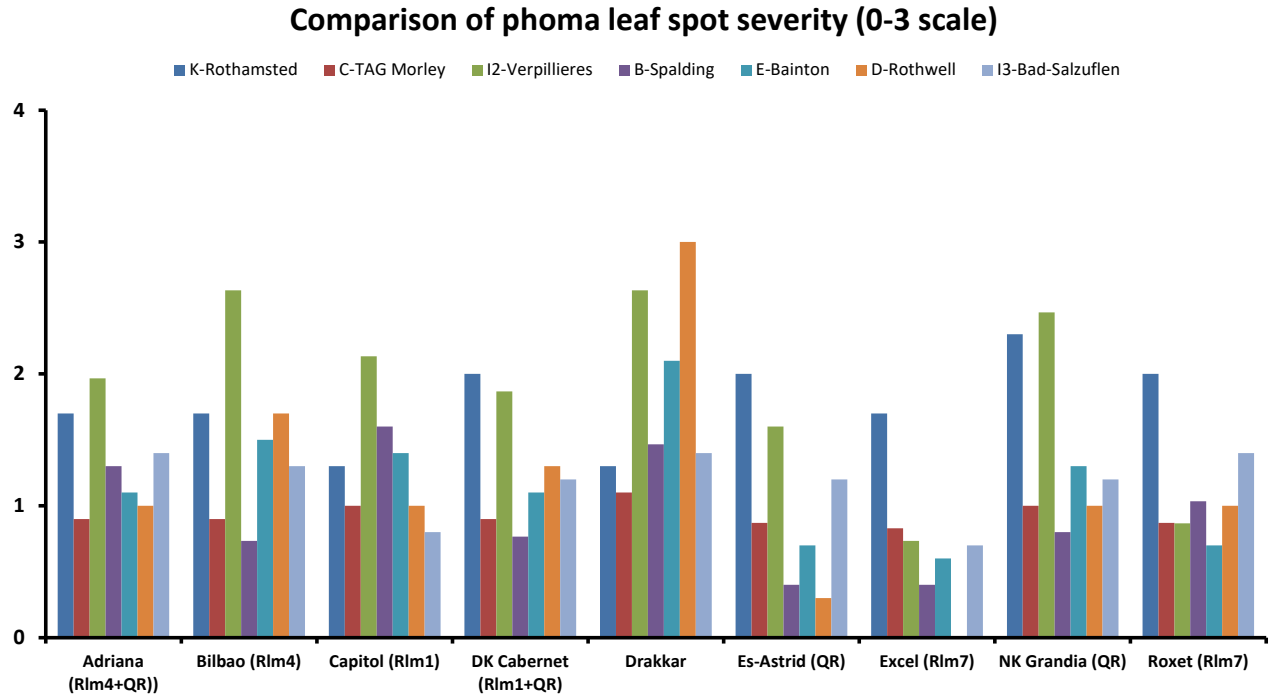


Fig. 4.16 Severity of phoma leaf spotting at different locations, assessed on a 0-3 scale (section 3.2.1) in the autumn in the 2010/2011 season.

In the 2011/2012 growing season, field experiments with 8 cultivars carrying different *R* genes in background with/without quantitative resistance were set up at the same 15 sites (Table 4.1). Since the phoma leaf spot epidemics started later than usual in this season, incidence of phoma leaf spot was very low at most of the locations. The severity of phoma leaf spots was only assessed on a 0-3 scale at 5 sites in the autumn/winter. There were differences in severity of phoma leaf spot between locations (Fig. 4.17). Phoma leaf spot was more severe in the field experiment at Rothwell than at other sites. Similarly to last season, there were also differences in severity of phoma leaf spot between cultivars. Cultivar Drakkar always had more phoma leaf spots than other cultivars at all the locations. The two cultivars with *Rlm7* (Roxet and Excel) had less phoma leaf spots than other cultivars. Cultivar Adriana (*Rlm4* + QR) generally had less phoma leaf spots than cultivar Bilbao (*Rlm4*) (Fig. 4.17).

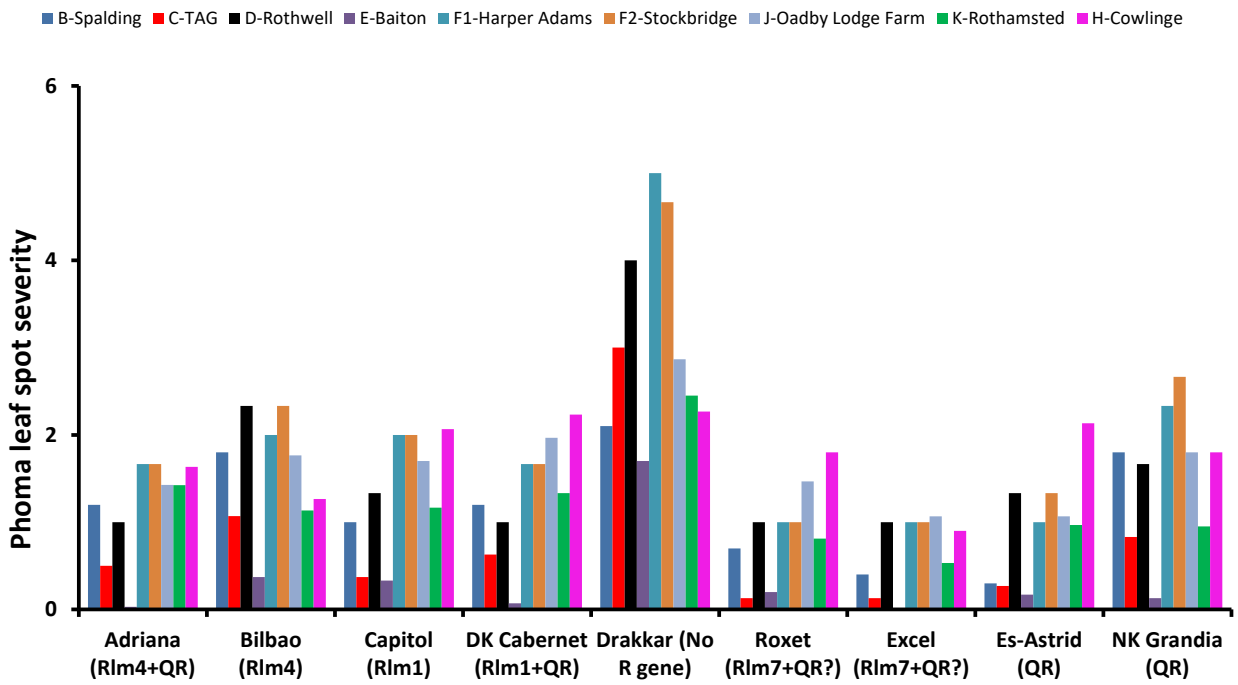


Fig. 4.17 Severity of phoma leaf spot at different locations assessed on a 0-3 scale (section 3.2.1) in the autumn in the 2011/2012 season.

In the 2012/2013 growing season, field experiments with 8 cultivars carrying different *R* genes in background with/without quantitative resistance were set up at the same 15 sites (Table 4.1), except at site J (Oadby Lodge Farm) because poor weather conditions occurred (field flooded). More *L. biglobosa* type phoma leaf spots (small dark lesions) were observed on Drakkar than in 2010/2011 (Fig. 4.18). Therefore the number of *L. maculans* (big grey lesions with pycnidia) and *L. biglobosa* type of lesions on five cultivars from five locations were assessed. More *L. maculans* lesions were observed at Cranwell than at other locations (Fig. 4.19). More *L. maculans* lesions were observed on Drakkar than on other cultivars, while fewer *L. biglobosa* lesions were observed on Drakkar than on other cultivars. Cultivars with resistance against *L. maculans* were generally more susceptible to *L. biglobosa* (Fig. 4.19).

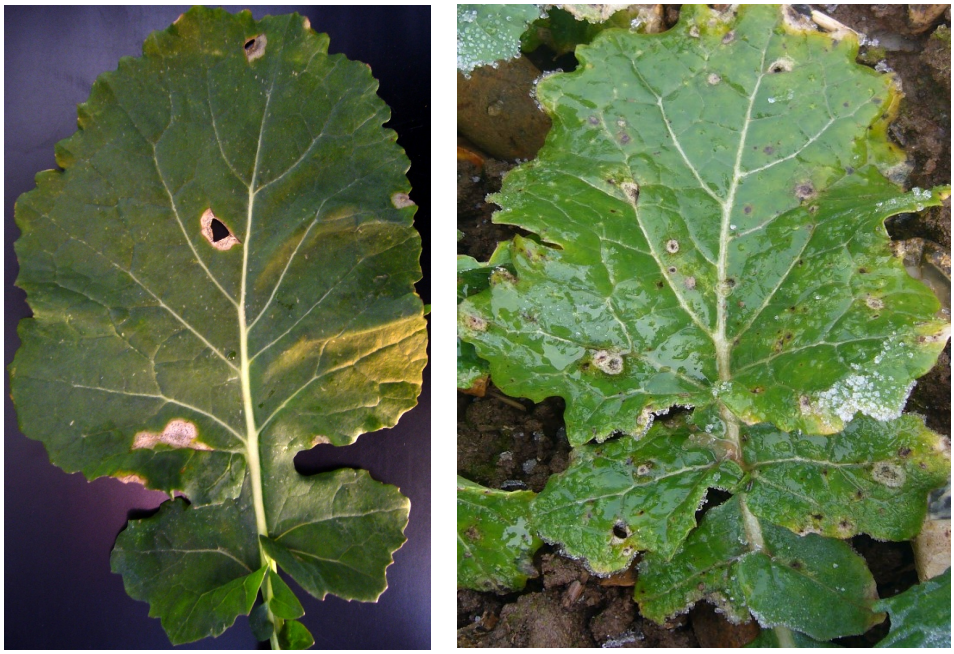


Fig. 4.18 Symptoms of phoma leaf spot on oilseed rape leaves at Rothamsted observed in December 2011 and December 2012.

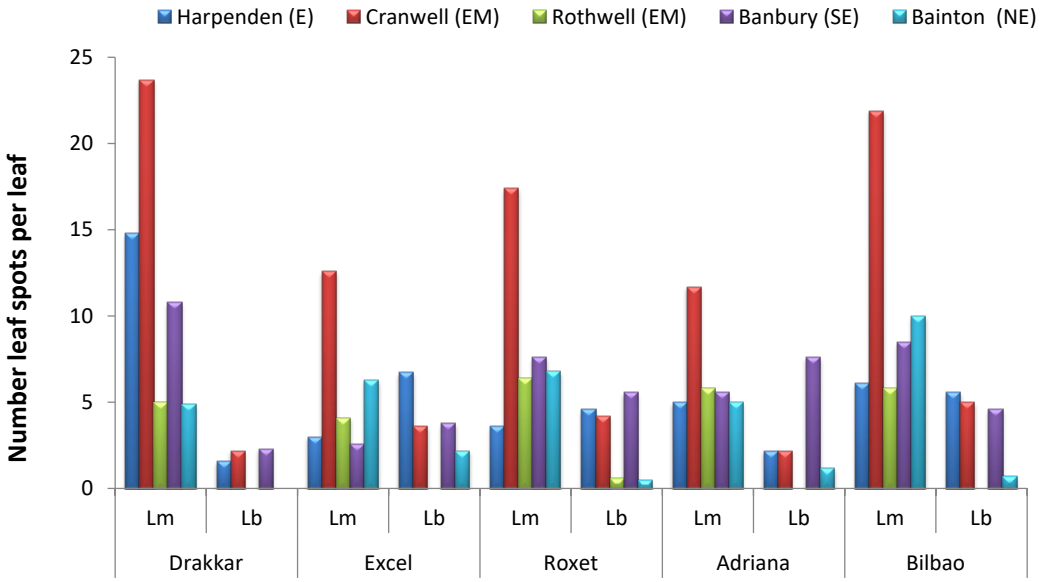


Fig. 4.19 Numbers of *Leptosphaeria maculans* and *L. biglobosa* lesions on leaves of five oilseed rape cultivars sampled from five different locations in autumn/winter of the 2012/2013 growing season.

4.2.1.2 Severity of phoma stem canker

In the 2010/2011 growing season, severity of phoma stem canker was assessed in 11 field experiments. There were differences between cultivars and between locations in severity of phoma stem canker (Fig. 4.20). Phoma stem canker was more severe at TAG Morley than at other locations. As expected, phoma stem canker was more severe on Drakkar than on other cultivar at all locations. The two cultivars with *Rlm7* (Roxet and Excel) generally had less phoma stem canker than other cultivars. For the cultivars with *Rlm1* or *Rlm4* in backgrounds with/without QR, cultivars DK Cabernet (*Rlm1* + QR) and Adriana (*Rlm4* + QR) generally had less phoma stem canker than cultivars Capitol (*Rlm1*) and Bilbao (*Rlm4*). For the two cultivars with only QR, cultivar Es-Astrid generally had less severe stem canker than cultivar NK Grandia (Fig. 4.20).

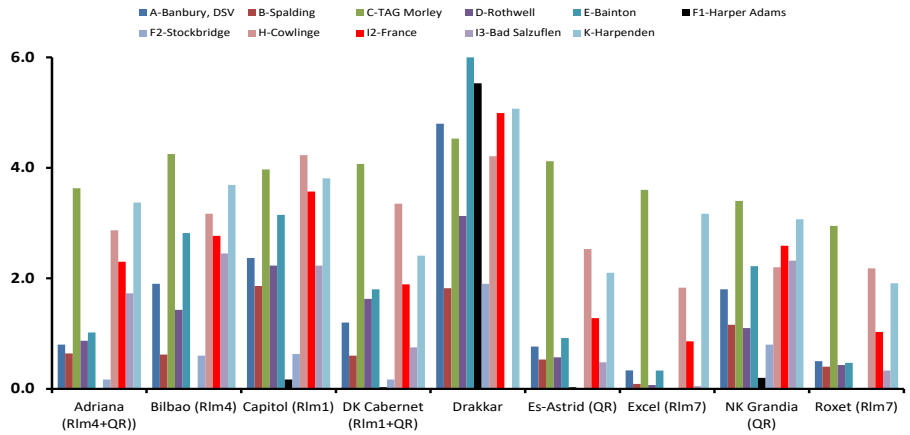


Fig. 4.20 Severity of phoma stem canker at different locations assessed on a 0-6 scale (section 3.2.1) in the 2010/2011 growing season.

In the 2011/2012 growing season, severity of phoma stem canker was assessed in 13 field experiments. There were differences between cultivars and between locations in severity of phoma stem canker (Fig. 4.21). Phoma stem canker was more severe at TAG Morley (Norfolk) and in France than at other locations. Drakkar had more severe phoma stem canker than other cultivars at all locations. The two cultivars with *Rlm7* (Roxet and Excel) generally had less severe phoma stem canker than other cultivars. For the cultivars with *Rlm1* or *Rlm4* in backgrounds with/without QR, cultivars DK Cabernet (*Rlm1* + QR) and Adriana (*Rlm4* + QR) generally had less severe phoma stem canker than cultivars Capitol (*Rlm1*) and Bilbao (*Rlm4*). For the two cultivars with only quantitative resistance, Es-Astrid had less severe stem canker than NK Grandia (Fig. 4.21). Although stem cankers developed on cultivars DK Cabernet (*Rlm1* + QR) and Adriana (*Rlm4* + QR), the symptoms were generally confined within the cortex. By contrast, the symptoms of stem canker had often spread into the stem pith of cultivars Capitol (*Rlm1*) and Bilbao (*Rlm4*) (Fig. 4.22). This suggests that *R* genes combined with quantitative resistance are more effective in control phoma stem canker than either alone.

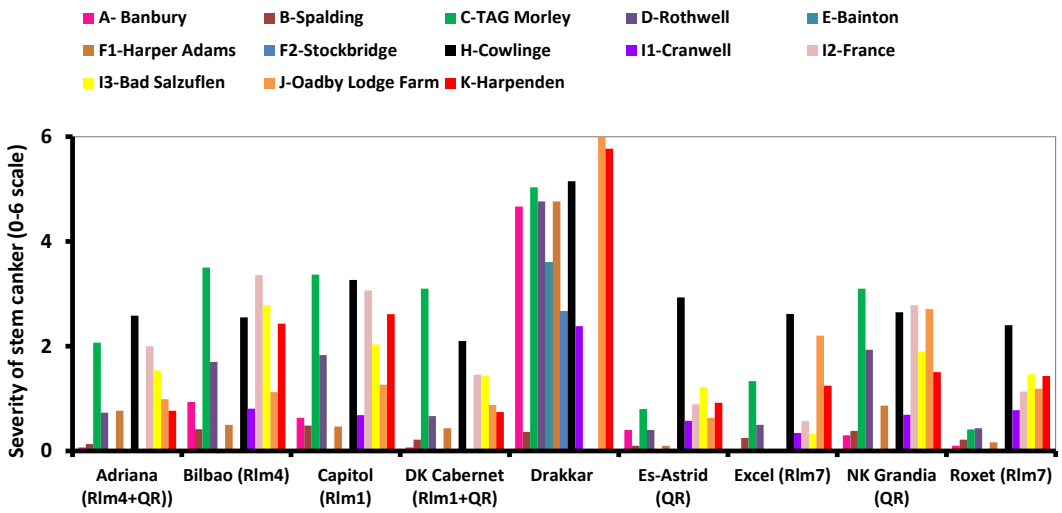


Fig. 4.21 Severity of phoma stem canker at different locations assessed on a 0-6 scale (section 3.2.1) in the 2011/2012 growing season. Site details in Table 4.2.

Fig. 4.22 Phoma stem canker symptoms on cultivars with different types of resistance observed in the experiment at TAG-Morley in July 2012.

In the 2011/2012 growing season, late release of ascospores (Fig. 4.14) led to severe upper stem lesions in the summer before harvest in the field experiment at Rothamsted (Fig. 4.23). Severe upper stem lesions even caused breakage of upper stems. Supported by an AHDB student bursary project, species-specific PCR analysis of these upper stem lesions showed that they were mainly caused by *L. biglobosa* (Huang et al., 2014).

Fig. 4.23 Symptoms of upper stem lesions observed in the winter oilseed rape field experiment at Rothamsted in July 2012.

In the 2012/2013 growing season, severity of phoma stem canker was assessed in 11 field experiments. There were differences between cultivars and between locations in severity of phoma stem canker (Fig. 4.24). Phoma stem canker was generally more severe at Rothamsted (Harpenden) than at other locations. As in previous seasons, Drakkar had more severe phoma stem canker than other cultivars at all locations. The two cultivars with *Rlm7* (Roxet and Excel) and the two cultivars with quantitative resistance generally had less severe phoma stem canker than other cultivars. Again, cultivars DK Cabernet (*Rlm1* + QR) and Adriana (*Rlm4* + QR) generally had less severe phoma stem canker than cultivars Capitol (*Rlm1*) and Bilbao (*Rlm4*). At Bainton, phoma stem cankers were particularly severe on cultivars Capitol and Bilbao.

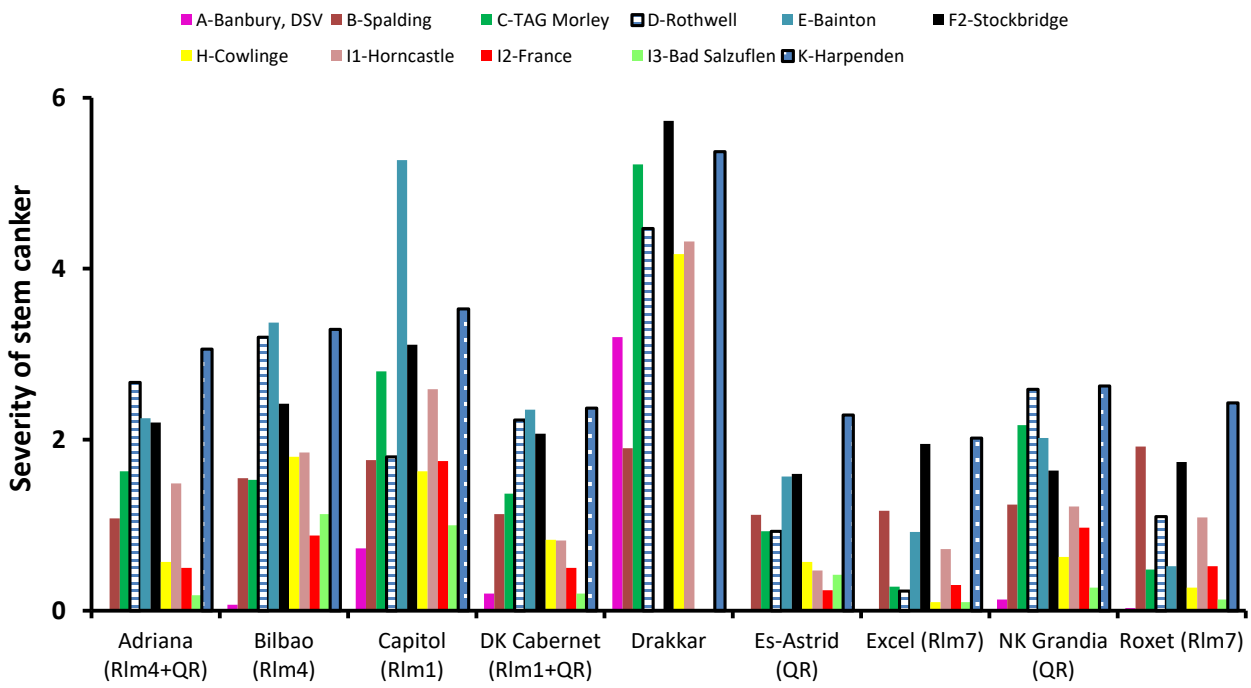


Fig. 4.24 Severity of phoma stem canker at different locations assessed on a 0-6 scale (section 3.2.1) in the 2012/2013 growing season.

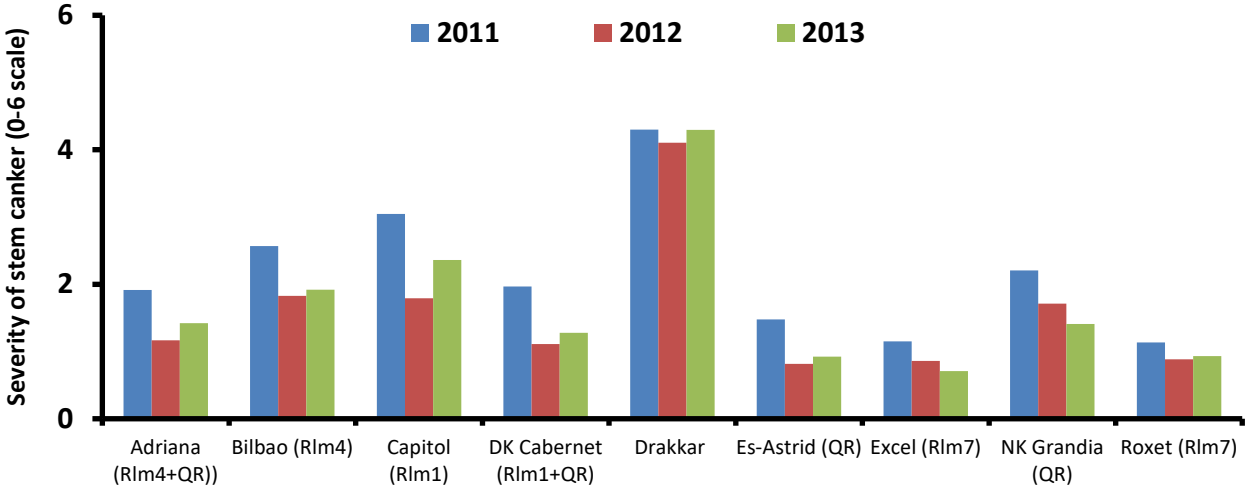


Fig. 4.25 Mean severity of phoma stem canker (0-6 scale, section 3.2.1) from different locations over the 2010/2011 (2011), 2011/2012 (2012) and 2012/2013 (2013) growing seasons.

Assessment of mean severity of phoma stem canker on different cultivars sampled from different locations in each growing season showed that the two cultivars with *Rlm7* (Roxet and Excel) had the least severe stem canker in all the three seasons (Fig. 4.25). For the two cultivars with only

quantitative resistance, Es-Astrid had less severe phoma stem canker than NK Grandia in all the three seasons. For the cultivars with resistance genes *Rlm1* or *Rlm4* in backgrounds with/without QR, cultivars with *Rlm1* or *Rlm4* in backgrounds with quantitative resistance (DK Cabernet, Adriana) had less severe phoma stem canker than cultivars with *Rlm1* or *Rlm4* in backgrounds without quantitative resistance (Capitol, Bilbao) in the three growing seasons (Fig. 4.25). These results suggest that there were effects of background quantitative resistance on the effectiveness of an *R* gene; the resistance genes *Rlm1* and *Rlm4* were more effective against *L. maculans* when they were in a background with quantitative resistance than in one without quantitative resistance.

4.2.2. Effectiveness of *R* genes in backgrounds with/without quantitative resistance in leaf tissue in controlled environments at different temperatures

The cotyledon tests with cultivars carrying resistance genes *Rlm1*, *Rlm2, Rlm4, Rlm6, Rlm7* or *LepR3* at 20°C and 25°C showed differences between these *R* genes in their response to increased temperature (Table 4.7). The resistance mediated by *Rlm4* in Jet Neuf and by *Rlm7* in Hearty was not sensitive to temperature; it was stable when temperature increased from 20°C to 25°. However, resistance mediated by *Rlm1* in Columbus and by *Rlm6* in DarmorMX was sensitive to temperature. Resistance mediated by *Rlm2* in Tapidor was stable when temperature increased from 20°C to 25° (Fig. 4.26). By contrast, resistance mediated by *LepR3* in Surpass400 was not stable when temperature increased from 20°C to 25°. Since *Rlm2* and *LepR3* map in the same region of chromosome A10 (Larkan et al., 2015a) and are alleles of the same resistance gene (Larkan et al., 2015b), differences in their response to temperature may have been due to effects of host background resistance. Therefore, further experiments were done with cultivars/lines carrying *Rlm1* and *Rlm4* in backgrounds with/without quantitative resistance (Table 3.3).

Different cultivars with *Rlm1,* DK Cabernet (*Rlm1*+QR), Capitol (*Rlm1*) and Columbus (*Rlm1*+*Rlm3*) were inoculated with two *L. maculans* isolates each carrying the corresponding *Avr* gene *AvrLm1* at temperatures 20°C and 25° (Table 3.4). Similarly, cultivars with *Rlm4,* Adriana (*Rlm4* +QR), Bilbao (*Rlm4*) and Jet Neuf (*Rlm4*+QR) were inoculated with two *L. maculans* isolates each carrying the corresponding *Avr* gene *AvrLm4* at temperatures 20°C and 25°. At 20°C, there were no differences between cultivars, all the cultivars showed a resistant phenotype and the disease scores were all <3 (Fig. 4.27). For the disease score on the 0-9 scale, a score of 0-3 is classified as resistant, a score of 4-5 is classified as moderately resistant, a score of 6-9 is classified as susceptible. There were also no differences between the two isolates in disease score when they were inoculated onto the cultivar carrying the corresponding resistance gene.

Table 4.7 Phenotypic response of cultivars carrying different *R* genes inoculated with *Leptosphaeria maculans* isolates carrying the corresponding *AvrLm* genes at 20°C to 25°C (R, resistant; S, susceptible)

***R* gene**	**Cultivar**	**Isolate**	**20° C**	**25 ° C**	**Sensitive**
Rlm1	Columbus	ME24	R	S	Yes
Rlm2	Tapidor	99-79	R	R	No
Rlm4	Jet Neuf	99-79	R	R	No
Rlm6	DarmorMX	ME24	R	S	Yes
Rlm7	Hearty	ME24	R	R	No
LepR3	Surpass400	99-79	R	S	Yes

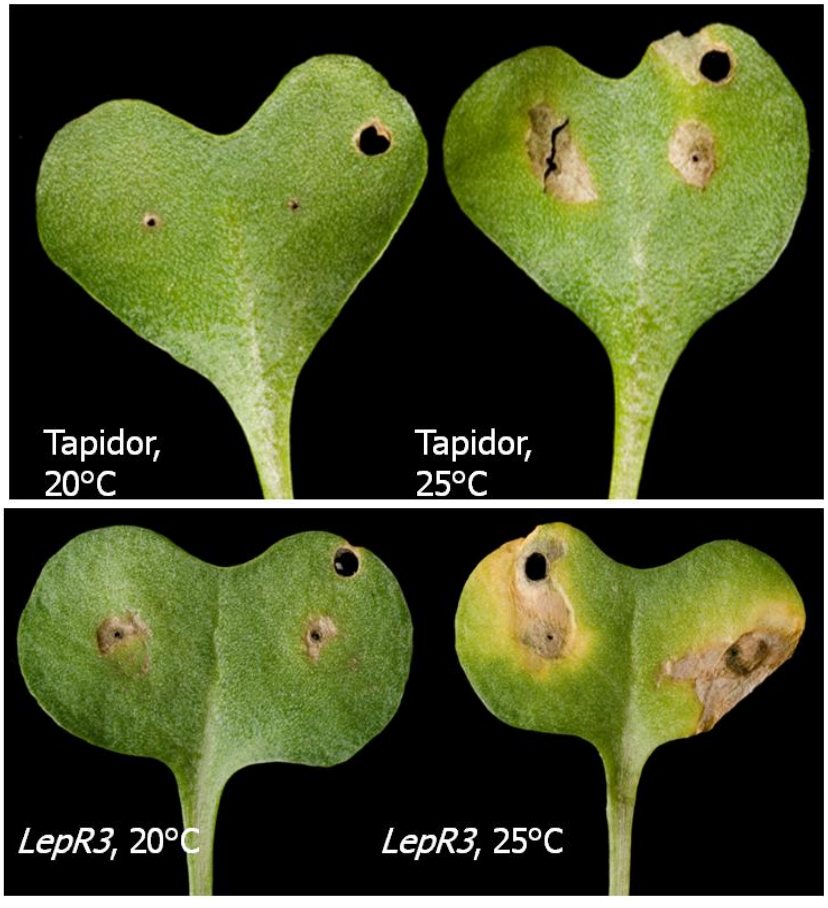


Fig. 4.26 Symptoms on cotyledons of Tapidor (*Rlm2*) and Surpass400 (*LepR3*) 18 days after inoculation with *Leptosphaeria maculans* at 20°C and 25°C.

However, at 25°C there were differences between cultivars in the resistance phenotype. For cultivars with *Rlm4,* Adriana (*Rlm4* +QR) was most resistant, Jet Neuf (*Rlm4*+QR) was moderately resistant and Bilbao (*Rlm4*) was susceptible (Fig. 4.27). For cultivars with *Rlm1,* DK Cabernet (*Rlm1* +QR) was moderately resistant, whereas Capitol (*Rlm1*) and Columbus (*Rlm1*+*Rlm3*) were susceptible at 25°C (Fig. 4.27). There were no differences between the two *L. maculans* isolates in disease score when they were inoculated onto cultivars carrying the corresponding resistance gene. To investigate whether leaf lesions produced at 25°C produced pycnidia, in a second experiment, cotyledons with lesions were detached and placed in Petri dishes lined with wet filter paper, then incubated at 20°C under alternating 12h light/12h darkness. After 3 days of incubation, numbers of mature pycnidia with pink cyrrhi (Fig. 3.2) were counted under a microscope. Results showed that there were differences between cultivars in number of pycnidia (Fig. 4.28).

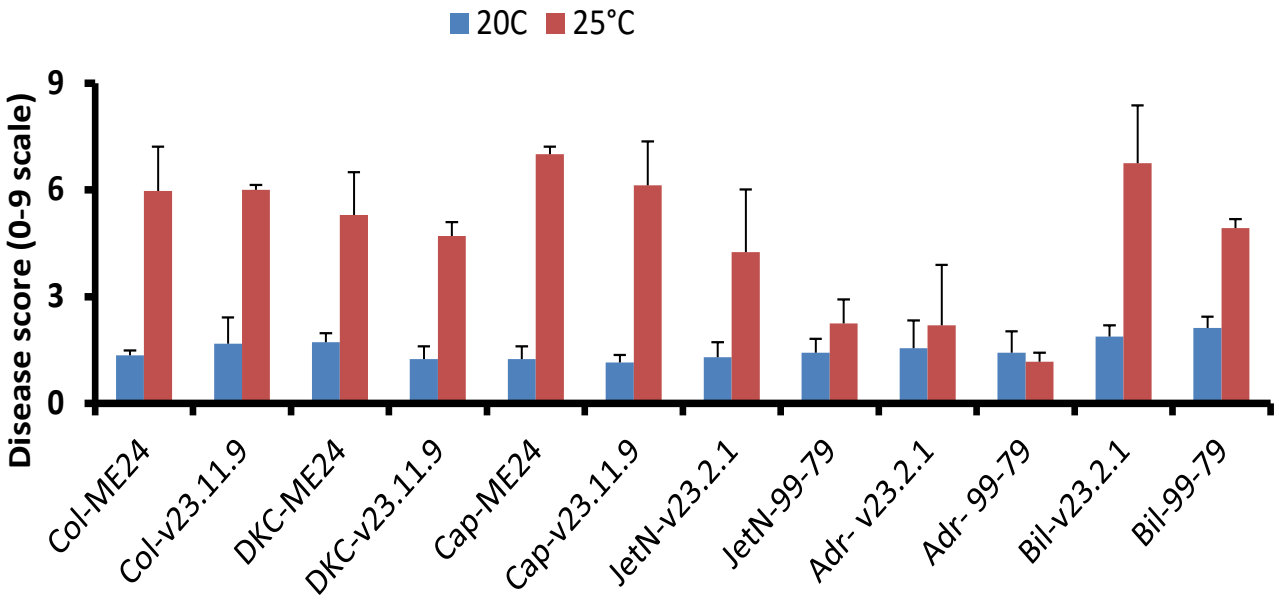


Fig. 4.27 The disease score on cotyledons of cultivars carrying different *R* genes in backgrounds with/without quantitative resistance (QR) inoculated with isolates of *Leptosphaeria maculans* carrying the corresponding *Avr* genes at 20°C or 25°C. Col-ME24: Columbus (*Rlm1*) inoculated with isolate ME24; Col-v23.11.9: Columbus inoculated with isolate v23.11.9; DKC: DK Cabernet (*Rlm1* +QR); Cap: Capitol (*Rlm1*); JetN: Jet Neuf (*Rlm4*+QR); Adr: Adriana (*Rlm4*+QR); Bil: Bilbao (*Rlm4*). Details of experiments are in Table 3.4. Data presented are means of two experiments.

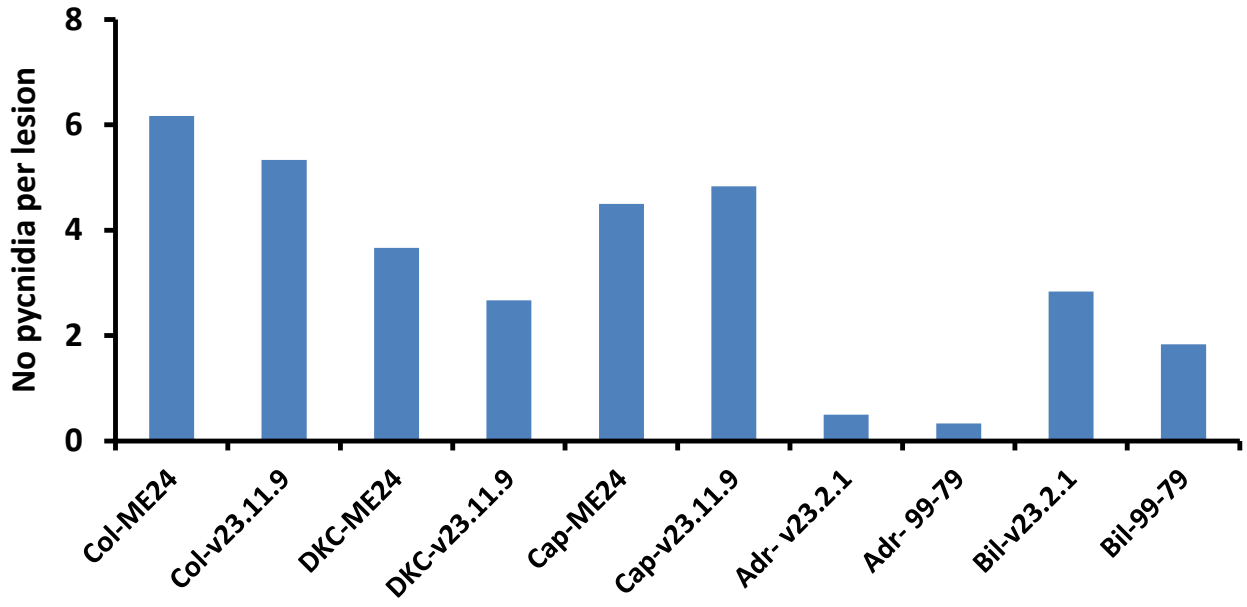


Fig. 4.28 Numbers of pycnidia produced on detached cotyledon lesions on different cultivars after incubation at 20°C with alternating 12h light/12h darkness for 3 days. These cotyledon lesions were produced on cultivars carrying different *R* genes in background with/without quantitative resistance (QR) inoculated with isolates of *Leptosphaeria maculans* carrying the corresponding *Avr* genes at 25°C. Col-ME24: Columbus (*Rlm1*) inoculated with isolate ME24; Col-v23.11.9: Columbus inoculated with isolate v23.11.9; DKC: DK Cabernet (*Rlm1* +QR); Cap: Capitol (*Rlm1*); Adr: Adriana (*Rlm4*+QR); Bil: Bilbao (*Rlm4*). Details of experiments are in Table 3.4.

4.2.3. Effectiveness of *R* gene and quantitative resistance in stem tissues in controlled environments at different temperatures

Symptoms were observed on petioles around the inoculation sites at 10 days after inoculation at 20°C and 25°C. By 18 days after inoculation, stem canker symptoms were observed at the leaf scars on the stems of the two DH lines (A30 and C119) without an effective *R* gene at both 20°C and 25°C. However, no symptoms were observed on cultivars with resistance genes *Rlm1* or *Rlm4* at either 20°C or 25°C (Fig. 4.29). By 49 days after inoculation, severe phoma stem cankers developed on stems of A30 at both 20°C and 25°C (Fig. 4.30). Stem cankers also developed on DH line C119 but were less severe than those on A30 at both 20°C and 25°C (Fig. 4.31a). At 20°C, for cultivars with *Rlm1* (Capitol and DK Cabernet) or *Rlm4* (Bilbao and Adriana) in backgrounds with/without quantitative resistance, no stem canker symptoms were observed or symptoms were only around the leaf scars of the inoculated leaf and the mean stem canker score was <1 (Fig. 4.31a). There was no difference between these four cultivars (Capitol, DK Cabernet, Bilbao and Adriana) in severity of stem canker. The severities of stem canker on these four cultivars were less than those on the two control DH lines (A30 and C119). For the two DH lines, the severity of stem canker on A30 was greater than that on C119 (Fig. 4.31a).

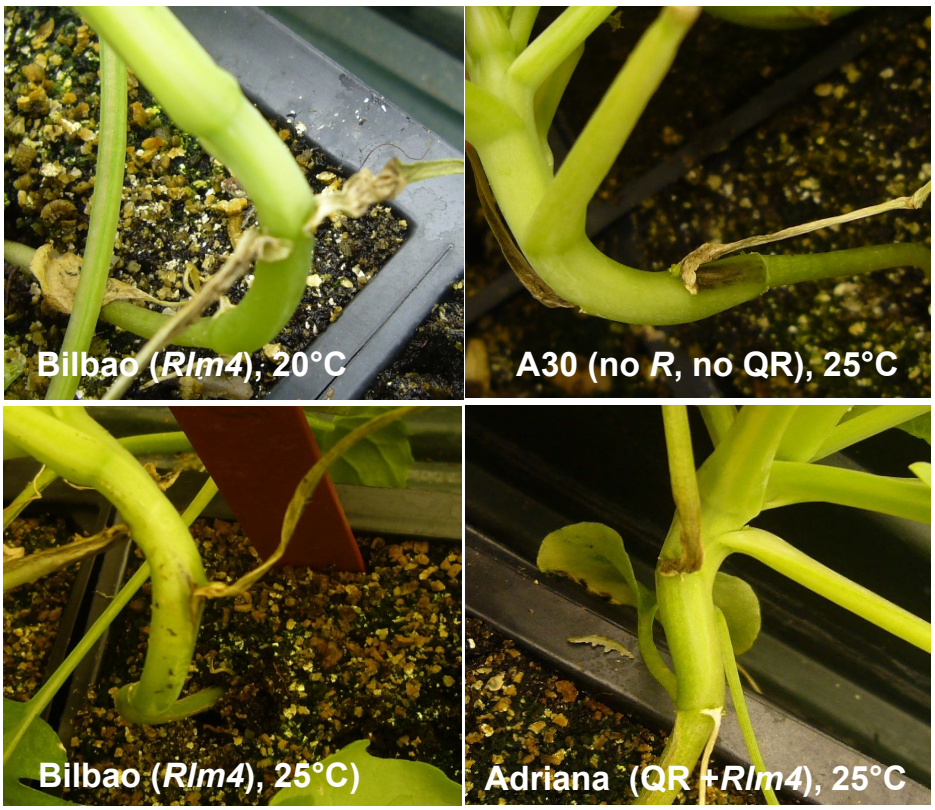


Fig. 4.29 Symptoms at the leaf scars on stems of cultivars Bilbao (*Rlm4*), Adriana (*Rlm4* + QR) and DH line A30 (no *R*, no QR) at 18 days after inoculation at 20°C or 25°C. Details of experiments are in Table 3.4.

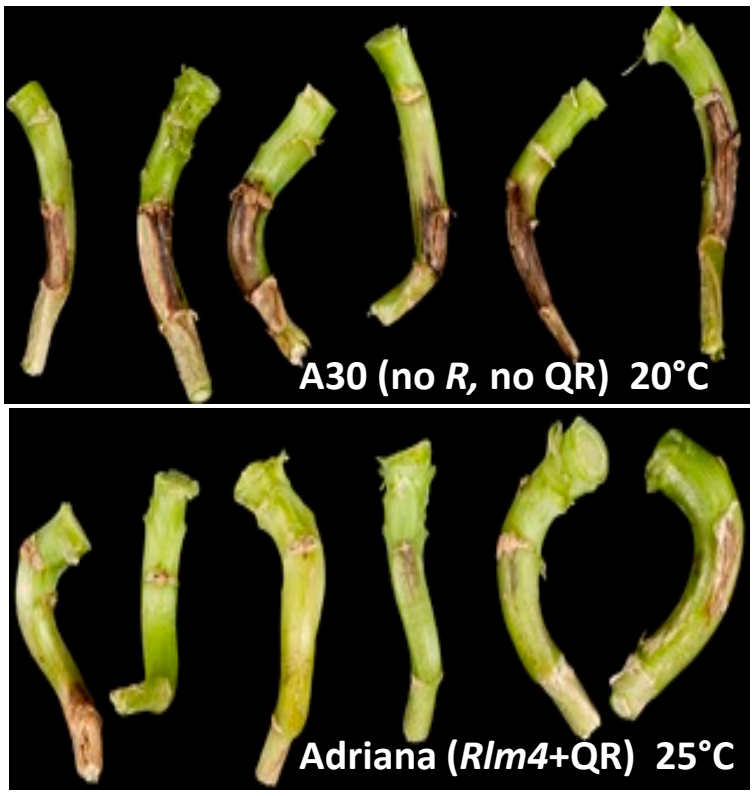


Fig. 4.30 Symptoms on stems of DH line A30 (no *R* gene, no QR) at 20°C and Adriana (*Rlm4* + QR) at 25°C at 49 days after inoculation. Details of experiments are in Table 3.4.

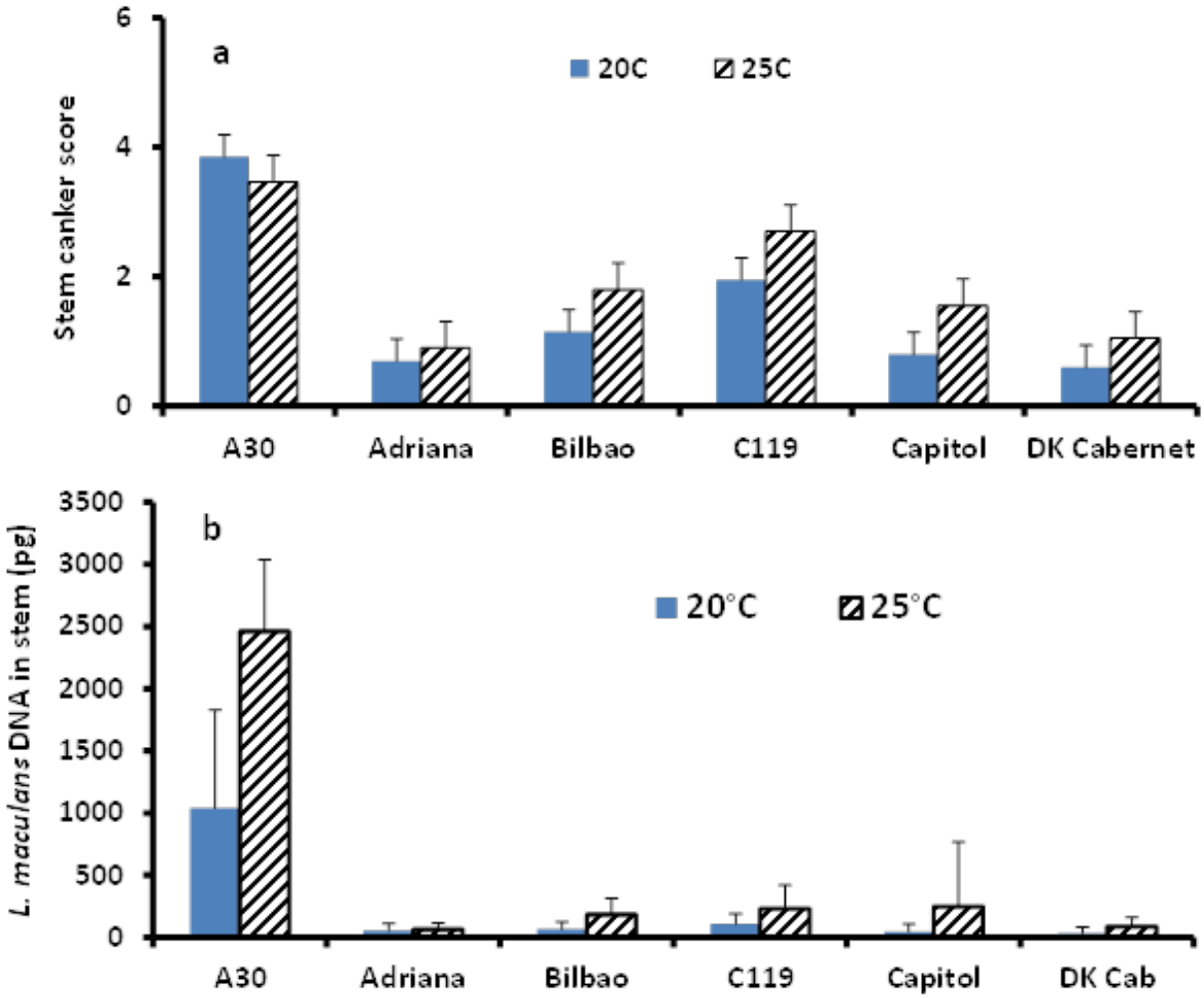


Fig. 4.31 The severity of phoma stem canker (a) and amount of *Leptosphaeria maculans* DNA (b) in stems of cultivars carrying different *R* genes in backgrounds with/without quantitative resistance (QR) at 20°C and 25°C. DK Cabernet (*Rlm1* +QR); Capitol (*Rlm1*); Adriana (*Rlm4*+QR); Bilbao (*Rlm4*); A30 (no *R* genes, no QR); C119 (QR). Leaf petioles were inoculated, and severity of stem canker was assessed on a 0-6 scale at 49 days after inoculation; the amount of *L. maculans* DNA was assessed using qPCR (Huang et al., 2014). Details of experiments are in Table 3.4.

However, at 25°C there were differences between these four cultivars (Capitol, DK Cabernet, Bilbao and Adriana) in severity of stem canker. There were no/limited stem canker symptoms on stems of DK Cabernet and Adriana at 49 days after inoculation at 25°C (Fig. 4.30). However, stem cankers had developed on Bilbao and Capitol, although mean stem canker severities on Bilbao and Capitol were less than those on A30 and C119 (Fig. 4.31a). Quantification of *L. maculans* DNA in stems of these cultivars using quantitative PCR showed that at 20°C there was very little *L. maculans* DNA detected in stems of the four cultivars (Capitol, DK Cabernet, Bilbao and Adriana) with resistance gene *Rlm1* or *Rlm4* compared to that in two DH lines (A30 and C119) without *R* genes (Fig. 4.31b). At 25°C, there was still very little *L. maculans* DNA detected in stems of Adriana and DK Cabernet; there was a small amount of *L. maculans* DNA detected in stems of Bilbao, Capitol and C119; there

was a large amount of *L. maculans* DNA detected in stems of A30 (Fig. 4.31b). Surprisingly, there were no differences between Bilbao, Capitol and C119 in the amount of *L. maculans* DNA detected in stems at 25°C even though there were differences between them in severity of stem canker (Fig. 4.31a).

4.3. Development of experimental systems to study mechanisms of operation of *R* gene-mediated resistance and quantitative resistance against *L. maculans*

4.3.1. Establishment of sets of material with different *R* genes in the same background or the same *R* gene in different backgrounds

During the life of the project, our collaborators in Canada (Dr Derek Lydiate, Dr Nick Larkan and Dr Hossein Borhan, Saskatoon Research Centre, Agriculture and Agri-Food Canada) introduced *LepR3* (from cultivar Surpass400), *Rlm2* (from Glacier), *Rlm4* (from Scoop) and *Rlm7* (from Roxet) into a common susceptible Topas DH background by back-crossing. Near-isogenic materials with *Rlm2, Rlm4, Rlm7* and *LepR3* in the same Topas background were obtained under a MTA agreement. Therefore, establishment of materials with different *R* genes in the same background was achieved through working with Canadian collaborators. Through collaboration with industrial partners and collaborators in France (Dr Thierry Rouxel, INRA-Bioger; Dr Regine Delourme, INRA-Rennes) and China (Prof Jinling Meng, Huazhong Agricultural University), different cultivars or breeding lines carrying the same *R* gene (e.g. *Rlm2*, *Rlm4, Rlm6* or *Rlm7*) were obtained.

4.3.2. Controlled environment experiments to investigate operation of *R* gene and quantitative resistance

Cotyledon inoculation experiments with Tapidor (*Rlm2*), Bristol (*Rlm2*) or Surpass400 (*LepR3*) at 20°C and 25°C showed that there were differences between these cultivars in their response to increased temperature. At 20°C, a resistant phenotype was observed on all these cultivars. However, when temperature was increased to 25°C, Surpass 400 (carrying *LepR3*) showed a susceptible phenotype. The two cultivars carrying the same *R* gene (*Rlm2*) showed different responses to temperature; Tapidor still showed a resistant phenotype while Bristol showed a susceptible phenotype (Fig. 4.32). This suggests that the host background resistance affects the effectiveness of *R* gene resistance at increased temperature.

To investigate host defence response and avoid host background effects, near-isogenic lines carrying the resistance gene *Rlm4* or *LepR3* in the same Topas background were tested at 20°C and 25°C. To avoid host defence response caused by wounding (normally cotyledons were wounded before inoculation), a new infiltration inoculation method was used (Fig. 4.33). A 10 µl drop of the conidial suspension was infiltrated into the centre of each cotyledon lobe from the under surface using a 1 ml syringe (Fig. 4.33a). Results from infiltration inoculation showed that

Fig. 4.32 Symptoms on cotyledons of Bristol (*Rlm2*) or Tapidor (*Rlm2)* inoculated with *Leptosphaeria maculans* isolates carrying the corresponding *AvrLm2* gene at 20°C and 25°C at 19 days after inoculation. Details of experiments are in Table 3.5.

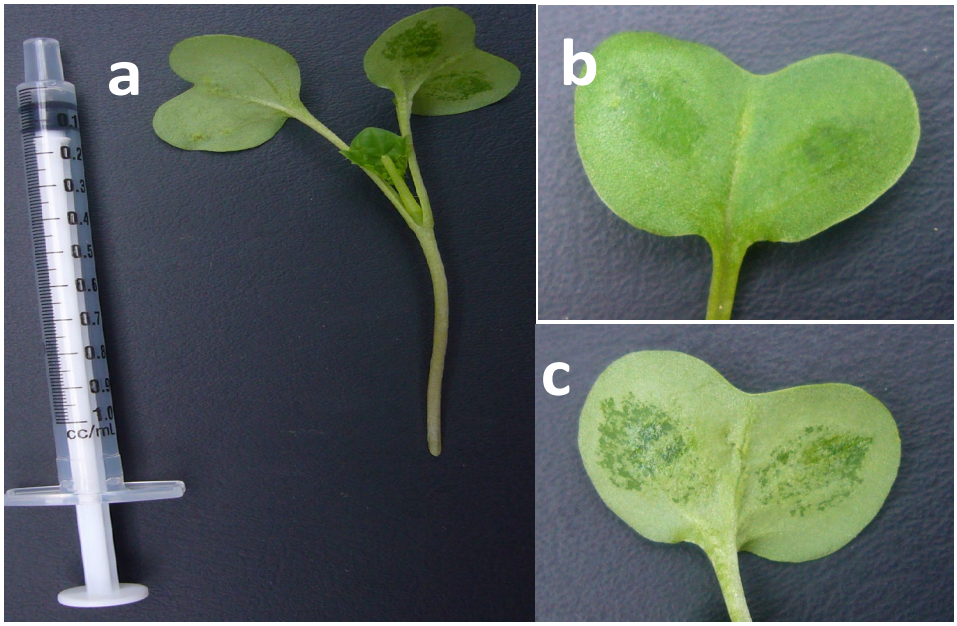


Fig. 4.33 Infiltration inoculation method. Cotyledons were inoculated by infiltrating 10 µl of the conidial suspension into the centre of each cotyledon lobe using a 1 ml syringe (a). The inoculated area is visible on the upper surface of the cotyledon (b) or from the under surface (c)

there were differences between *Rlm4* and *LepR3* in their effector triggered defence response. When Topas-*Rlm4* and Topas-*LepR3* were inoculated with isolates carrying the corresponding effector genes *AvrLm4* or *AvrLm1*, Topas-*Rlm4* produced a quicker, stronger defence response than Topas-*LepR3* at both at 20°C (Fig. 4.34) and 25°C (Fig. 4.35). At 25°C, the defence response of Topas-*Rlm4* was so strong that extensive cell death was observed at the inoculated site and a large lesion

developed. When Topas-*Rlm4* and Topas-*LepR3* were inoculated with isolates without the corresponding effector gene *AvrLm4* or *AvrLm1*, typical phoma leaf lesions were observed.

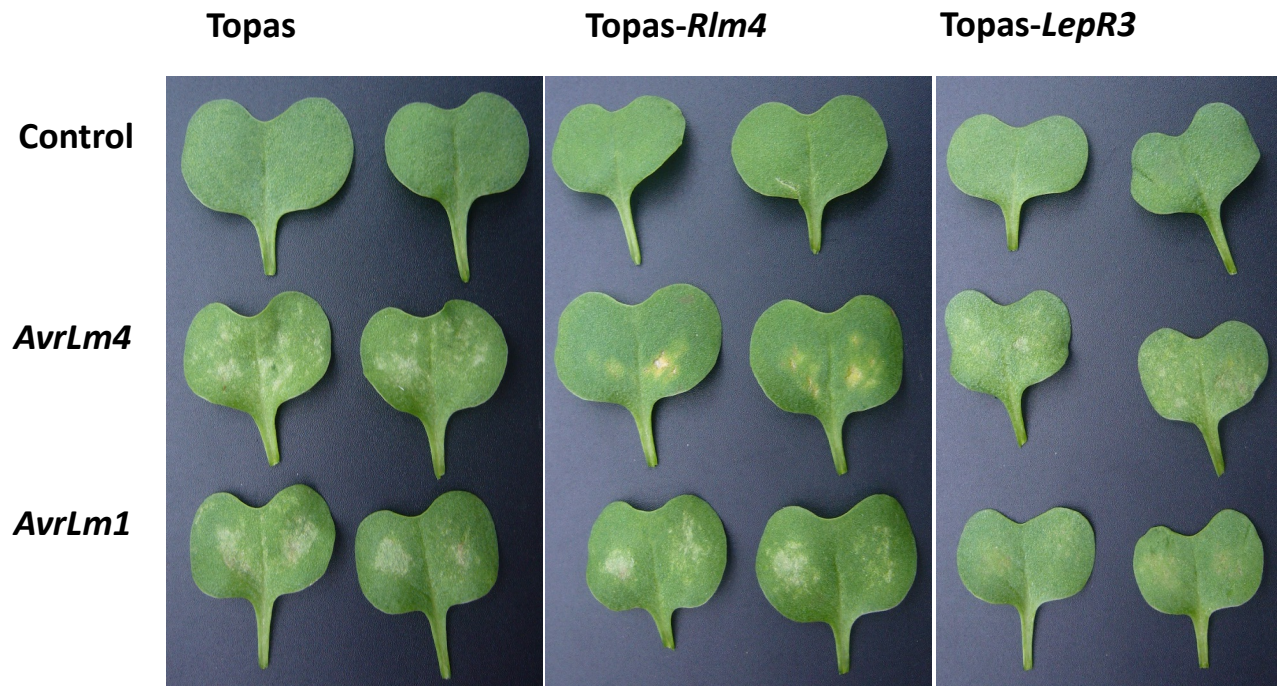


Fig. 4.34 Symptoms on near-isogenic lines Topas (no *R* genes), Topas-*Rlm4* (*Rlm4*) and Topas-*LepR3* 9 days after infiltration inoculation at 20°C. Topas-*Rlm4* was inoculated with *Leptosphaeria maculans* isolates with or without the corresponding effector gene *AvrLm4*; Topas-*LepR3* was inoculated with *L. maculans* isolates with or without the corresponding effector gene *AvrLm1.* Control plants were inoculated with sterile distilled water.

In petiole inoculation tests, stem cankers were observed at the leaf scars on the stems of the two controls A30 (no *R* genes, no QR) and C119 (QR) at both 20°C and 25°C. There was no difference between 20°C and 25°C in severity of stem canker on A30 but there were differences between 20°C and 25°C in severity of stem canker on C119, with more severe canker at 25°C than at 20°C (Fig. 4.36). There were no/limited stem canker symptoms observed on stems of Tapidor (*Rlm2*) or Surpass400 at 20°C. However, at 25°C phoma stem cankers developed on stems of Surpass400 but there were no/limited stem canker symptoms on Tapidor (*Rlm2*) with the mean stem canker severity score <1 (Fig. 4.36).

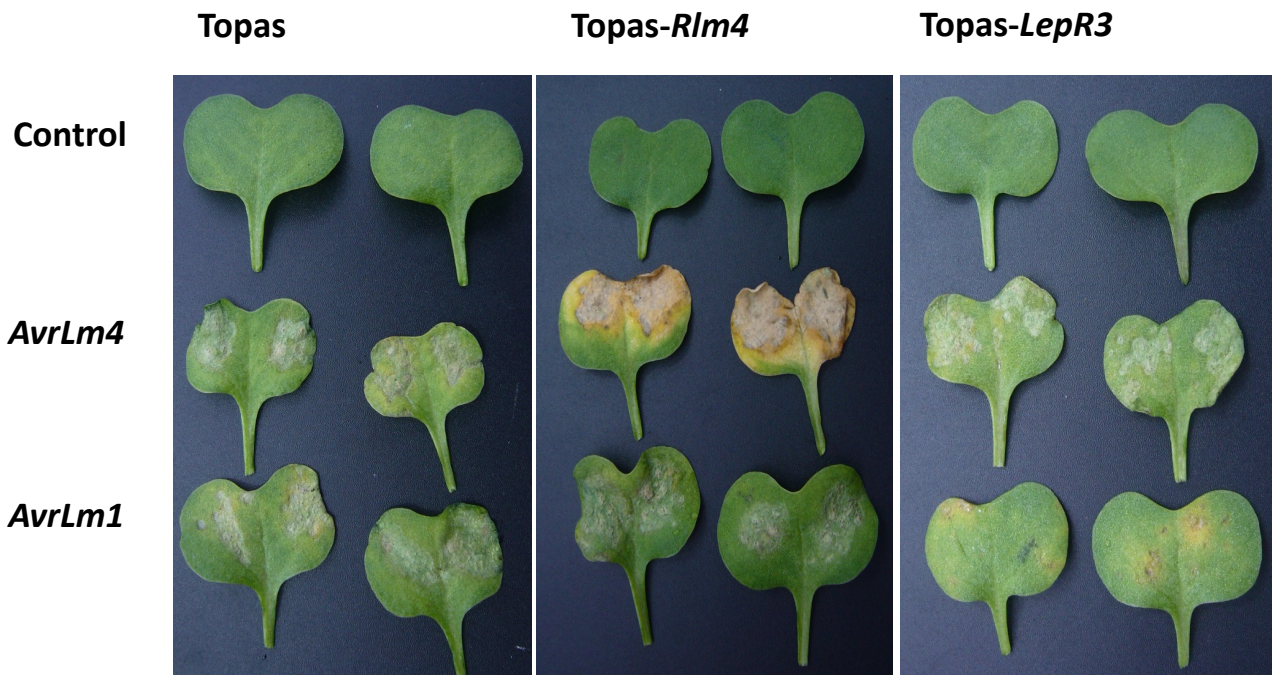


Fig. 4.35 Symptoms on near-isogenic lines Topas (no *R* genes), Topas-*Rlm4* (*Rlm4*) and Topas-*LepR3*) 8 days after infiltration inoculation at 25°C. Topas-*Rlm4* was inoculated with *Leptosphaeria maculans* isolates with or without the corresponding effector gene *AvrLm4*; Topas-*LepR3* was inoculated with *L. maculans* isolates with or without the corresponding effector gene *AvrLm1.* Control plants were inoculated with sterile distilled water.

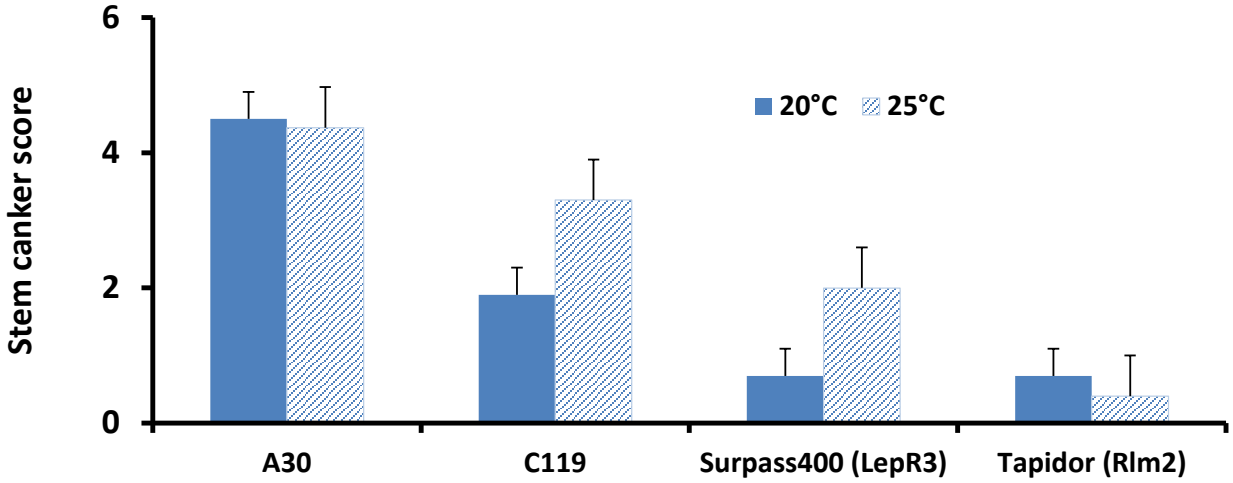


Fig. 4.36 The severity of phoma stem canker in stems of cultivars/lines with *R* gene-mediated resistance or quantitative resistance (QR) at 20°C or 25°C. DH line A30 (no *R* genes, no QR); DH line C119 (QR); Surpass400 (*LepR3*); Tapidor (*Rlm2*). Leaf petioles were inoculated and severity of stem canker was assessed on a 0-6 scale (section 3.2.1) at 49 days after inoculation. Details of experiments are in Table 3.5.

4.4. Exploitation and technology transfer

4.4.1. Exploitation and dissemination of new knowledge

The consortium for this project had 16 members, including nine breeding companies (DSV, Elsoms, Limagrain, Saaten Union, LS Plant Breeding, Monsanto, Pioneer, Grainseed and Syngenta), three farmer organisations (Co-operative Farms, AHDB Cereals & Oilseeds and NFU), three Agricultural Trusts **(**Felix Thornley Cobbold Agricultural Trust, Chadacre Agricultural Trust and the Perry Foundation) and one academic partner (University of Hertfordshire). The range of consortium members enabled good dissemination of the results to the agricultural industry. Consortium meetings were held 6-monthly and new results were disseminated to consortium members. The new knowledge was then disseminated directly to the arable sector of the agricultural industry during the course of the project through breeders' open days, workshops and conferences. Recommendations for growers were made through HGCA topic sheets (e.g. 'Are you on top of the spots?' June 2014) and events like Cereals' (this project was presented at Cereals' in 2012, 2013, 2014 through the AHDB stand). In addition, new information was disseminated to a wider audience through press releases. For example, information about pathogen population structure was published by Farmer's Weekly ('Phoma resistance genes in OSR under threat', Farmers Weekly, 13 December 2013). For all the press articles during the life of this project, please see section 4.4.2.3 (press articles).

4.4.2. Publications and conferences

New results were disseminated through publications and conference presentations. Three papers from this project and four papers related to this project have been published in peer-reviewed international journals since it started (Table 3.8; Appendix). After publication of research papers, the press and other media were used to highlight the benefits and novelty of the research. Findings were also communicated as papers at national and international conferences. Results from the project contributed directly to the current international debates on adaptation to climate change and food security and opportunities were taken to publicise the benefits of breeding crop cultivars with resistance that is effective at increased temperature at international conferences that addressed climate change/food security issues. For example, there was an oral presentation at a Food and Agriculture Organization (FAO) workshop on impacts of climate change on incidence of pests and diseases of agricultural crops at Santiago, Chile, 7-8 May 2014.

Table 4.8 Exploitation and technology transfer by publications, conferences and KT activities. Details are listed in the Appendix.

Publications in refereed international journals	
Papers from this project	4
Papers related to this project	3
Edited short conference papers	11
Conferences abstracts	38
Events/KT Activities and conference presentations	28
Press articles etc.	15

5. Discussion and recommendations

This work has identified a number of ways to decrease the risk of severe phoma stem canker epidemics on UK winter oilseed rape crops, both in the short-term and the long-term. These will be discussed in the context of a series of recommendations, which could be implemented by different sections of the UK oilseed rape industry, Including farmers, the AHDB (Cereals and Oilseeds), crop advisors and the breeding industry.

5.1. Short-term benefits to farmers

5.1.1. To guide regional deployment of oilseed rape cultivars with different resistance genes, there is a need to monitor regional populations of *Leptosphaeria maculans,* especially in relation to virulence at the *AvrLm1, AvrLm4* (populations currently differ between regions) and *AvrIm7* (to decrease risk of breakdown of *RIm7* cultivar resistance) loci.

Differences between regions in frequencies of avirulent *AvrLm1* and *AvrLm4* (Fig. 4.2, Fig. 4.8) suggest that cultivars with these two *R* genes can be used in one region but not in others. Normally, a cultivar with a given *R* gene is effective in protecting a crop when the frequency of the corresponding avirulent *AvrLm* allele in *L. maculans* population is >70% (reference). There were differences between growing seasons in frequencies of *AvrLm1* and *AvrLm4*. Compared to the 2010/2011 growing season, the frequencies of both AvrLm1 and AvrLm4 decreased in 2011/2012 (Fig. 4.7), suggesting that there is a need to monitor changes in *L. maculans* races each season.

Differences between regions in frequencies of avirulent *AvrLm5* and *AvrLm6* (Fig. 4.3, Fig. 4.8) suggest that virulent alleles are present, although no commercial cultivars carrying these two R

genes have been used in the UK. Breeding cultivars with these two *R* genes is not recommended. There were no significant differences between regions in frequencies of *AvrLm2, AvrLm3* and *AvrLm9*. The UK *L. maculans* populations were 100% virulent at AvrLm2 and AvrLm9 loci and nearly 100% virulent at the AvrLm3 locus, suggesting that cultivars with these three *R* genes cannot be used to control phoma stem canker in the UK.

By contrast, the UK *L. maculans* populations were 100% avirulent at AvrLm7 locus in 2010/2011 and only 3% of isolates were virulent (*avrLm7*) in 2011/2012 even though cultivars with *Rlm7* have been used in the UK for more than 10 years (Clarke et al. 2011). This indicates that the cultivars carrying resistance gene *Rlm7* are still effective in protecting the crop in the UK. The resistance gene *Rlm7* is a good source of resistance for breeding but there is a need to continue monitoring the pathogen population so that this *R* gene can be effectively deployed, because even if a small number of virulent isolates were detected there is a risk that this gene may become ineffective.

High throughput methods have been proposed to monitor frequencies of virulent/avirulent alleles of *AvrLm1*, *AvrLm6* and *AvrLm4* effector genes using quantitative PCR or pyrosequencing on samples of air-borne ascospores (Van de Wouw et al. 2010; Van de Wouw and Howlett 2012). Sampling populations of air-borne ascospores has many advantages over sampling phoma leaf spot lesions at a specific time in autumn, obtaining isolates of *L. maculans* and then testing them on a differential set of brassica cultivars/lines with different *R* genes. Firstly, sampling air-borne ascospores gives a much better representation of the population than sampling leaf spots. Furthermore, it is much more reliable since it is less dependent on timing of sampling; for a busy colleague finding the exact time to sample the leaf spots is often difficult, and sometimes samples rot whilst they are in the post to the laboratory for isolation. Secondly, it requires much less time to do a quantitative PCR assessment (1 week) than to do a cotyledon assay with a differential set of brassicas (3 months).

It is recommended that AHDB deploys a set of spore samplers at some of sites of Recommended List winter oilseed rape trials in different regions to monitor races present in *Leptosphaeria maculans* populations each growing season to detect frequencies of virulent alleles by using PCR of samples of air-borne spores. Such information could then be made available to farmers on the AHDB web-site to guide their choice of cultivars for the next growing season.

5.1.2. There is a need to develop a web-based scheme for guiding regional deployment of winter oilseed rape cultivars with different types of resistance.

This work shows that not only are there differences between different *R* genes in oilseed rape cultivars in their effectiveness for control of phoma stem canker, depending on the races of *L. maculans* present in local populations, but also there are effects of background quantitative

resistance (QR) on the effectiveness of these *R* genes, as in the work done in France by Brun et al. (2010) with *Rlm6* in a background with or without quantitative resistance. For the cultivars with *Rlm1* or *Rlm4* in backgrounds with/without quantitative resistance, cultivars DK Cabernet (*Rlm1* + QR) and Adriana (*Rlm4* + QR) generally had less phoma stem canker than cultivars Capitol (*Rlm1*) and Bilbao (*Rlm4*) over the three seasons suggesting that these two *R* genes are more effective when they are integrated in the host background with quantitative resistance than in the background without quantitative resistance. For the two cultivars with only quantitative resistance, Es-Astrid generally had less severe stem canker than NK Grandia over the three seasons, suggesting that there are differences in effectiveness of control of phoma stem canker between sources of quantitative resistance.

Web-based schemes to advise farmers on choice of oilseed rape cultivars, depending on their combination of *R* gene-mediated and quantitative resistance and the local races of *L. maculans* present in their area have been developed in France and Australia. CETIOM (now renamed to TerresInnovia; http://www.terresinovia.fr) recommendations in France involve knowledge of the *Rlm* genes of currently used cultivars and their classification into three groups (group I: high level of quantitative resistance with ineffective or partially effective *Rlm* genes; group II: cultivars with effective *Rlm* genes; group III: cultivars with low level of resistance. It is recommended by CETIOM not to use cultivars from group III and, in order to maximise the durability of group II cultivars, to alternate group II with group I cultivars. “Blackleg Management Guide FACT SHEETS” are produced by the Australian government, in which the disease “risk” is estimated by taking into consideration the regional density of oilseed rape, the rainfall and the stem canker severity in current/previous cropping season. Depending on the “risk” level estimated and classifications of cultivars into groups (A to G), based on their resistance, farmers are advised from which group to choose cultivars for the following cropping season

There is a need to develop similar web-based schemes to provide UK farmers with advice about appropriate choice of cultivars.

5.1.3. Information on timing of *Leptosphaeria maculans/L. biglobosa* ascospore release in autumn can be used to improve accuracy of autumn forecasts of severity of stem canker epidemics to guide fungicide timing.

In these experiments, the patterns of ascospore release differed between locations and between cropping seasons. Although the patterns of ascospore release differed between locations, the dates in autumn when the maximum ascospore release occurred were similar between the four locations within each season. There was only one to five days difference in this date between locations in

most normal seasons. This suggests that information on timing of ascospore release from one region can be used as a reference for another region for guiding the timing of fungicide sprays in autumn.

The 2011/2012 season was unusual, in that ascospores were released very late. For example, in Hertfordshire, the date when the first major ascospore release occurred in the 2011/2012 season was more than 2 months later than that in the 2010/2011 season. In normal seasons, the first major ascospore release is in late October or early November, but in the 2011/2012 season the first major ascospore release was not observed until January 2012. The late release of ascospores in this season was mainly due to a period with little or no rainfall in the summer/autumn of 2011. Previous work showed that rain-days in August and September affect the maturation of ascospores and their date of release (Huang et al. 2007; Evans et al. 2008).

Comparison of observed dates (this project) and predicted dates (weather-based forecasting, (http://www.rothamsted.ac.uk/phoma-leaf-spot-forecast/phoma-forecast) when 10% of plants have phoma leaf spotting (often used as a spray threshold for guiding spray timing) showed that the predicted dates were 5 to 17 days earlier than the observed dates in normal seasons (e.g. 2012/2013 and 2013/2014) at Rothamsted in Hertfordshire. However, in the unusual 2011/2012 season, the forecasted date was 93 days earlier than the observed date. While the predicted dates of first major ascospore release was only 5-6 days earlier than the observed dates in normal seasons (e.g. 2012/2013 and 2013/2014) at Rothamsted, it was 30 days earlier in the unusual 2011/2012 season. This suggests that use of the date of first major ascospore release to forecast the date when 10% of plants have phoma leaf spotting would be more accurate than purely weather-based forecasting.

There is a need to continue monitoring ascospore release to provide better guidance for timing of fungicide applications. This evidence suggests that deployment of a small number of spore samplers (for example at sites of a few AHDB Recommended List winter oilseed rape trials) would be sufficient to provide such guidance. **It is likely that a new generation of spore samplers will be developed that can provide 'real time' information about the timing of release of specific spores (West and Kimber 2016) but even if it is necessary to send weekly tapes to a laboratory for quantitative PCR analysis, results could improve the accuracy of guidance for spray timing.**

5.2. Long-term benefits to farmers

Whilst some recommendations could be implemented quite quickly to decrease the risk of phoma stem canker for UK farmers, others are more long-term, since they need to be implemented by oilseed rape breeders in the breeding of new cultivars for use by farmers in the future.

5.2.1. *R* gene-mediated resistance should always be combined with quantitative resistance to improve its effectiveness in different environments

Results of field experiments with eight cultivars carrying different *Rlm* genes in backgrounds with/without quantitative resistance showed that there were differences in severity of phoma stem canker between locations and between seasons. Comparing the 11 locations in the UK over the three cropping seasons, phoma stem canker was generally more severe at TAG Morley (Norfolk), Cowlinge (Suffolk) and Rothamsted (Hertfordshire) than at other locations. For the eight cultivars, there were significant differences in severity of stem canker between the three seasons, with more severe canker in 2010/2011 than in the other two seasons. There were also differences in weather conditions between sites and seasons. Previous work suggests that such differences in weather affect different stages in the development of phoma stem canker epidemics and ultimately affect severity of epidemics and yield loss (Evans et al. 2008; Butterworth et al. 2010). Furthermore, this work suggests that, whilst the timing of leaf spotting in autumn is dependent solely on weather factors (rainfall and temperature), the growth of the pathogen through leaf stalk and stem tissues and thus severity of stem canker is dependent on accumulated temperature and cultivar resistance factors.

In these experiments, Drakkar had more severe phoma stem canker than other cultivars at all locations. The two cultivars with *Rlm7* (Roxet and Excel) generally had less severe phoma stem canker than other cultivars, suggesting that the resistance gene *Rlm7* is effective in protecting the crop. For the cultivars with *Rlm1* or *Rlm4* in backgrounds with/without quantitative resistance (QR), cultivars DK Cabernet (*Rlm1* + QR) and Adriana (Rlm4 + QR) generally had less severe phoma stem canker than cultivars Capitol (*Rlm1*) and Bilbao (*Rlm4*) over the three seasons, suggesting that these two *Rlm* genes are more effective when they are integrated into backgrounds with quantitative resistance than in backgrounds without quantitative resistance. This agrees with previous study on the resistance gene *Rlm6* (Brun et al. 2010) and with evidence from Australia, when there were severe epidemics within three seasons of introduction of a new *Rlm* gene into a cultivar with a susceptible background because it had rapidly selected for virulence in the *L. maculans* populations (Sprague et al. 2006).

Results of petiole inoculation of cultivars/lines with different types of resistance (e.g. *Rlm* gene-mediated resistance or quantitative resistance) showed that quantitative resistance can reduce the severity of stem cankers. This is consistent with previous studies showing that quantitative resistance

against *L. maculans* cannot prevent plant infection but can reduce severity of stem canker (Delourme et al. 2006; Huang et al. 2009; Huang et al. 2015)

To provide farmers with cultivars that have effective resistance against the stem canker pathogen at different locations and in different growing seasons, it is essential that *Rlm* gene-mediated resistance is incorporated into backgrounds with good quantitative resistance that is environmentally stable (Huang et al. 2015).

5.2.2. Relationships between temperature-resilience and effectiveness of resistance should be investigated

Results of controlled environment experiments suggest that there were differences between different *Rlm* genes (e.g. *Rlm1, Rlm2, Rlm4, Rlm6, Rlm7* and *LepR3*) in their response to increased temperature at the leaf spot stage. The resistance mediated by *Rlm4* (e.g. in Jet Neuf) and *Rlm7* (in Hearty) was temperature-resilient; the resistance was stable when temperature increased from 20°C to 25°. However, the resistance mediated by *Rlm1* (in Columbus) and *Rlm6* (in DarmorMX) was temperature-sensitive and was ineffective at the higher temperature. This is consistent with previous work on *Rlm6* (Huang et al. 2006) .

These experiments also suggest that background quantitative resistance affects the effectiveness of *R* gene-mediated resistance at the phoma leaf spot stage. For example, *Rlm2* and *LepR3* are alleles of the same resistance gene (Larkan et al., 2015b); however, *Rlm2* (in Tapidor) and *LepR3* (in Surpass400) differed in their response to temperature. When temperature increased from 20°C to 25°, Tapidor was still resistant while Surpass400 became susceptible. The differences between Tapidor and Surpass400 may have been due to the host background resistance. Further evidence for this conclusion is provided by the results from experiments with cultivars carrying *Rlm1* and *Rlm4* in backgrounds with/without quantitative resistance. There were no differences between cultivars with *Rlm1* (DK Cabernet with *Rlm1* + QR, Capitol with *Rlm1*) or with *Rlm4* (Adriana with *Rlm4* + QR, Bilbao with *Rlm4*) at 20°C in their resistance phenotype. However, at 25°C there were differences between cultivars; Adriana (*Rlm4* + QR) was still resistant but Bilbao (*Rlm4*) was susceptible. Similarly, DK Cabernet (*Rlm1* + QR) was moderately resistant but Capitol (*Rlm1*) was susceptible at 25°C.

Temperature and host background quantitative resistance affected the effectiveness of *R* gene-mediated resistance at the stem canker development stage. Results of petiole inoculation experiments at 25°C suggest that there were differences between cultivars in severity of stem canker. For example, at 49 days after inoculation at 25°C, there were still little or no stem canker symptoms on stems of DK Cabernet (*Rlm1* + QR) and Adriana (*Rlm4* + QR), whereas severe stem

cankers developed on Bilbao (*Rlm4*) and Capitol (*Rlm1*). Further investigation using quantitative PCR showed that the amount of *L. maculans* DNA was less in stems of Adriana and DK Cabernet than that in stems of Bilbao and Capitol.

If factors that influence the effectiveness of resistance (differences between *Rlm* genes; background quantitative resistance) are associated with the temperature-resilience of that resistance, then breeders could screen potential cultivars/lines for temperature-resilient resistance as a means of selecting for effective resistance. This would save time and expense by comparison with field experiments at multiple locations over several seasons that are currently used to select for effective, environmentally stable resistance. Such screening could be used in initial selection stages, although the best material would then need to be tested in field experiments.

5.2.3. There is a need to understand interactions between the two pathogens (*Leptosphaeria maculans* and *L. biglobosa*) and cultivar *R* gene-mediated resistance/quantitative resistance to identify cultivars with resistance against both pathogens.

Results of these experiments suggest that there are interactions between *L. maculans* and the related coexisting species *L. biglobosa* in relation to oilseed rape cultivar *Rlm* gene-mediated/quantitative resistance. It is clear that, although *L. maculans* and *L. biglobosa* are almost identical morphologically, they attack the oilseed rape plant in different ways (Williams and Fitt 1999; Shoemaker and Brun 2001; Huang et al. 2003). On artificial media and dead plant tissues (e.g. stems at the end of the growing season), *L. biglobosa* generally grows faster than *L. maculans,* whereas in living plant tissue *L. maculans* may penetrate more deeply than *L. biglobosa* and there are clearly differences in susceptibility of oilseed rape cultivars to the two species (Fitt et al. 2006a; Fitt et al. 2006b).

In this work, although *L. maculans* populations sampled from the trap cultivar Drakkar (with no *Rlm* genes) in the first growing season were 100% avirulent against *Rlm7*, phoma leaf spots were observed on cultivars carrying the resistance gene *Rlm7* (Excel and Roxet). However, the phenotypes of phoma leaf spots were different from those on the susceptible cultivar Drakkar; the lesions on Excel and Roxet were smaller than those on Drakkar and many of them had dark margins (no dark margins around lesions on Drakkar, Fig. 4.4). Results of further work on isolation/identification (e.g. whether those lesions on the *Rlm7* cultivars were caused by *L. maculans* or *L. biglobosa*) and characterisation (e.g. whether the isolates were avirulent or virulent against *Rlm7*) showed that small dark lesions on *Rlm7* cultivars were mainly caused by *L. biglobosa*, while larger lesions with dark margins were mainly caused by *L. maculans* (Table 4.3). Surprisingly, most

of the *L. maculans* isolates from the *Rlm7* cultivars were still avirulent against *Rlm7* cultivars in cotyledon tests (Fig. 4.6). By contrast, *L. maculans* isolates from cultivars with *Rlm1* (Capitol and DK Cabernet) were 100% virulent against *Rlm1* in cotyledon tests (Fig. 4.5). Furthermore, differences in frequency of *AvrLm7* alleles between isolates obtained from Roxet and Excel suggest that there are differences in selection pressure on the pathogen between cultivars with different genetic backgrounds. In addition, further work showed that cultivars with *Rlm7* were more susceptible to *L. biglobosa* than cultivars with no effective *Rlm* genes against *L. maculans*.

Furthermore, although *L. maculans* is generally regarded as a more damaging pathogen than *L. biglobosa*, there is evidence that *L. biglobosa* was the dominant pathogen, responsible for severe stem base and upper stem lesions, in the 2011/2012 growing season (Huang et al. 2014). **There is a need for further work to understand interactions between the two pathogens (*Leptosphaeria maculans* and *L. biglobosa*) and cultivar *Rlm* gene-mediated resistance/quantitative resistance to identify cultivars with resistance against both pathogens.**

5.2.4. There is a need to exploit new genomic information and genetic resources to improve our understanding of the operation of resistance against *Leptosphaeria* species and other extracellular pathogens

This project has used new materials with different *Rlm* genes in the same genetic background or same *Rlm* gene in different genetic backgrounds that became available during the life of the project through collaboration with international collaborators or industrial partners. Near-isogenic materials with *Rlm2, Rlm4, Rlm7* and *LepR3* in the same Topas background were obtained under MTA from Canada. Different cultivars or breeding lines carrying the same *R* gene (e.g. *Rlm2, Rlm4, Rlm6* or *Rlm7*) were obtained through collaboration with industrial partners and collaborators in France and China. These materials are valuable sources for investigating mechanisms of interactions between *R* genes and quantitative resistance, on which preliminary work has been done during the course of the project.

During the course of the project, the genome of the oilseed rape host *Brassica napus* (Chalhoub et al. 2014), together with those of the two related pathogens *L. maculans* and *L. biglobosa* (Rouxel et al. 2011; Grandaubert et al. 2014), have been published. **There are now unprecedented opportunities to exploit this novel genomic information through bioinformatics and biocomputational methods, together with the new host materials that have become available to improve our understanding of resistance against the phoma stem canker pathogens. This new understanding can be exploited by breeders to develop new cultivars with more effective, durable, temperature-resilient resistance for the benefit of farmers and the whole agricultural industry in the UK.**

Exploitation and technology transfer

This project has improved our understanding of risks to current AHDB Recommended List cultivars from phoma stem canker to guide improved strategies for breeders and farmers to maintain yields of oilseed rape to contribute to national food security and avoid unnecessary fungicide use to improve farmer gross margins. Because phoma stem canker is not only an important disease problem on oilseed rape in the UK but also a global disease on oilseed rape and brassica vegetables, results from this project will contribute to international food security. Results from this project were published in international peer reviewed scientific journals to benefit the academic community; four papers from this project and three papers from related projects were published during the course of the project. Results were also presented at conferences; there were 11 short edited conference papers from this project published. Results of this project were presented at 26 national and international conferences or commercial events with 38 conference abstracts published. Improved understanding of resistance against *L. maculans* will benefit not only breeders but also growers, especially if recent/proposed EU legislation leads to a decrease in fungicide types available and means that there is a greater need to produce cultivars with durable disease resistance.

Benefits to breeders and the academic community

This project has improved our understanding of effects of temperature on effectiveness of different *Rlm* gene-mediated resistance and effects of host background quantitative resistance on *R* gene-mediated resistance against *L. maculans*. This information is valuable to geneticists and breeders for selection of pre-breeding material and for guiding their breeding strategies for development of suitable cultivars for different environments. There is currently little information on variation in temperature response between different types of resistance to plant pathogens; results of this research will be of interest to all researchers working on resistance in brassicas, epidemiology, genetics and climate change. The results are also of generic relevance to scientists working on other pathosystems involving *Rlm* gene-mediated resistance and quantitative resistance. Results from this project on effectiveness of combinations of different *Rlm* genes with quantitative resistance in different environments are also generic to the understanding of factors influencing effectiveness of host resistance. It is possible that there may be associations between the temperature-resilience and the durability of *Rlm* genes; if this is proved it would provide a rapid method for screening *Rlm* genes for durability.

This project provides opportunities for international collaboration in production of new materials and new molecular markers. Sets of materials with different *Rlm* genes in the same background or the

same *Rlm* gene in different backgrounds produced in the project are ideal to study how environmental factors (e.g. temperature) influence operation of resistance to plant pathogens. These materials are also valuable for distinguishing contributions to temperature responses from *R* gene resistance and background quantitative resistance. This work is of generic benefit to scientists studying host-pathogen interactions, genetics and host resistance mechanisms.

Benefits to growers

This project improved understanding of risks to current AHDB Recommended List (RL) cultivars from phoma stem canker (potential basis for a scheme for deployment of cultivars with different resistant genes, as in France; http://www.terresinovia.fr) so that yields are maintained to contribute to national food security and avoid unnecessary fungicide use (reducing costs for growers). Improved understanding of operation of host resistance and knowledge about current *L. maculans* races will help to develop a scheme for regional deployment of cultivars with different combinations of *Rlm* genes and background quantitative resistance, so that growers can choose suitable cultivars and avoid unnecessary fungicide use, especially if recent EU legislation leads to a decrease in fungicide types available. Therefore, results of this project can help growers to use resistance more effectively for more sustainable and profitable control of phoma stem canker in oilseed rape. It will also decrease costs of National List and AHDB RL trials, if more resistant material can be selected more easily. It will also provide recommendations for industry/government on effective use of resistance in developing strategies for adaptation to climate change. Results of this project (e.g. frequency of avirulent alleles of different *Avr* effector genes in *L. maculans*) provide strategic guidance for growers and advisors (e.g. long-term business planning, cropping and disease control strategies) and AHDB Cereals and Oilseeds (e.g. RL system, investment of resources in research and development). Improved guidance on selection and deployment of resistance will reduce fungicide use and avoid disastrous epidemics like those that occurred in Australia. Furthermore, improved resistance for efficient disease control in oilseed rape crops will reduce the use of fungicide which will benefit the environment.

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Appendix

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Huang YJ, Balesdent M, Van de Wouw A, Stonard J, Evans N, West J, Rouxel T, Fitt B (2011). Effects of *Leptosphaeria maculans* race structure and fitness cost of virulence on management of phoma stem canker of oilseed rape. *Proceedings of the 13th International Rapeseed Congress*, June 5-8, 2011, Prague, 1181-1184.

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Huang YJ, Hood J, Rossall S, Ashworth M, King G, Fitt BDL (2011). Effects of fungicide on growth of *Leptosphaeria maculans* and *L. biglobosa* (phoma stem canker) in oilseed rape. *Proceedings of the 13th International Rapeseed Congress*, June 5-8, 2011, Prague, 1177-1180

Events/ KT Activities and conference presentations

Oral presentation at Felix Cobbold Trust conference 'Making a difference', Otley, Suffolk, 26 November 2015 (**Bruce Fitt**)

Poster presentation at the Hutchinsons Winter Farmer Technical Conference in Peterborough, 19 November 2015 (**Bruce Fitt** and **Yongju Huang**)

Oral and poster presentations at the BSPP presidential meeting, Bristol, UK, 13-15 September 2015 (**Georgia Mitrousia, Bruce Fitt and Yongju Huang)**

Oral and poster presentations at the 14th International Rapeseed Congress, 5-9 July 2015, Saskatoon, Canada (**Bruce Fitt, Yongju Huang** and **Georgia Mitrousia)**

Seminar at University of Goettingen, Germany, 13 May 2015 (**Bruce Fitt**)

Poster presentation at the Molecular Biology of Plant Pathogens meeting, Bristol, UK, 8-9 April 2015 (**Georgia Mitrousia)**

Oral presentation at International Conference on Agriculture and climate change: 'Arable crop disease control, environmental change and food security' **Bruce Fitt, Aiming Qi** and Fay Newbery (given by Fay Newbery on 16 Feb) Amsterdam, the Netherlands, 15-17 February 2015

Oral and poster presentations at the AAB conference 'Crop Production in Southern Britain: Precision Decisions for Profitable Cropping', 25-26 November 2014, Peterborough Arena, Peterborough, UK **(Yongju Huang** and **Georgia Mitrousia)**

Oral presentation at career event of Society of Biology - Beds, Herts & Essex branch, University of Hertfordshire, 11 November 2014 **(Bruce Fitt)**

Oral and poster presentations at the 11th Conference of the European Foundation for Plant Pathology, 8 – 13 September 2014, Kraków, Poland (**Bruce Fitt , Yongju Huang** and **Georgia Mitrousia)**

On 4 September 2014, Bruce represented UH at a meeting co-ordinated by the Government Chief Scientist Sir Mark Walport to coordinate across government departments bids to the next Coordination of UK Government spending round (2015) for work on animal and plant diseases. Attended by representatives from BIS, BBSRC, Defra, DFID and a group of independent 'experts' on plant or animal diseases **(Bruce Fitt)**

Poster presentation at the 16th International Congress on Molecular Plant-Microbe Interactions, 6-10 July 2014, Rhodes, Greece **(Yongju Huang** and **Georgia Mitrousia).**

Oral presentation at the AAB conference ' Breeding Plants to Cope with Future Climate Change', 16-18 June 2014, University of Leeds, Leeds, UK **(Aiming Qi)**

Cereals' 2014 at Chrishall Grange, Cambridgeshire, 11 – 12 June, the LINK project was presented at the HGCA stand (**Bruce Fitt** and **Yongju Huang)**

Oral presentation at the Food and Agriculture Organization (FAO) workshop on impacts of climate change on incidence of pests and diseases of agricultural crops, Santiago, Chile, 7-8 May 2014 **(Bruce Fitt)**

Oral presentation at 19th Crucifer Genetics Workshop, 30 March – 2 April 2014, Wuhan, China (**Yongju Huang)**

Oral presentation at HGCA Agronomist conference, 10 Dec 2013 **(Bruce Fitt)**

Poster presentation at HGCA Crop Research Conference, 25 Sept 2013, London **(Georgia Mitrousia)**

Oral and poster presentation at the 10th International Congress of Plant Pathology, 25-30 August 2013, Beijing, China (**Bruce Fitt , Yongju Huang** and **Georgia Mitrousia)**

Cereals' 2013 at Boothby Graffoe, Lincolnshire, 12 -13 June 2013, the LINK project was presented at the HGCA stand (**Bruce Fitt and Yongju Huang**)

Poster presentation at AgriFood Charities Partnership (AFCP) - Student Forum, 20 March 2013, NIAB, Cambridge **(Georgia Mitrousia)**

Poster presentation at the BSPP presidential meeting, Norwich, UK, 16-18 December 2012 (**Georgia Mitrousia)**

Poster presentation at the Crop Protection in Southern Britain, Association of Applied Biologists, Peterborough, UK, 27-28 November 2012 (**Georgia Mitrousia)**

Oral and poster presentations at the Plant Resistance Sustainability 2012 International Conference, Nice, France, 16-19 October 2012 (**Yongju Huang** and **Georgia Mitrousia)**

Oral presentations at University of Hertfordshire School of Life Sciences (May 2011), Cranfield University (October 2011) and EU Endure (Brussels) (November 2011) meetings (**Bruce Fitt)**

Oral presentations at Cranfield University (October 2011) and EU Endure (Brussels) (November 2011) meetings **(Bruce Fitt)**

Oral presentation at the IOBC-Working group meeting**,** Integrated Control in Oilseed Crops, Gottingen, Germany, 4-6 October 2011 (**Yongju Huang)**

Oral presentations at the 13th International Rapeseed Congress, Prague, Czech Republic, 5-9 June 2011 (**Yongju Huang**, **Bruce Fitt)**

Press articles, papers, etc.

Farming trust showcases wide range of projects it's supporting. East Anglian Daily Times, 28 November 2015.

Qi A, Fitt BDL (2014). Can crop disease control cope with climate change? *Outlooks on Pest Management (December 2014)* (DOI:10.1564/v25_dec_05)

'Phoma pathogen could pose additional threat', Farmers Guardian, 28 June 2014.

Topic sheet 'Disease management for oilseed rape - Are you on top of the spots? ', HGCA topic sheet, 10 June 2014 on HGCA website.

'New research into disease resistance', News Letter, 31 May 2014.

'Understanding disease resistance genes in crops to secure future food production', Science Daily, May 27, 2014.

'Professor probes crop diseases', Herts Advertiser (Harpenden Advertiser), 13 February 2014.

'Young crop research', Farmers Guardian, 07 February 2014.

'Developing new methods to assess resistance to disease in young oilseed rape plants', Science Daily, 27 January 2014.

University focus on crop killer', Hertfordshire Life, 1 January 2014.

Phoma resistance genes in OSR under threat', Farmers Weekly, 13 December 2013.

'University of Hertfordshire charts serious threat to Chinese crops', Business Weekly (Eastern England), 5 December 2013.

'Protecting vital crops in China', Science Daily, 28 November 2013.

'New research will protect vital crops', Bangla Mirror, 9, September 2011.

'New form of resistance to light leaf spot', Farmers Guardian, 9 September 2011

' £1.3 million Plant Pathology Research Project', UH School of Life Sciences Newsletter in May 2011.