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## **Project Report No. PR587**

# Investigating components of the oilseed rape light leaf spot epidemic responsible for increased yield loss to the UK Arable Industry

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# CONTENTS

<b>1.</b>	<b>ABSTRACT</b> .....	<b>1</b>
<b>2.</b>	<b>INTRODUCTION</b> .....	<b>2</b>
<b>3.</b>	<b>MATERIALS AND METHODS</b> .....	<b>3</b>
<b>3.1.</b>	<b>Work Package (WP) 1 Modelling and forecast development</b> .....	<b>3</b>
3.1.1.	WP1.1 Preparation of historic datasets and weather data.....	3
3.1.2.	WP1.2 Produce model and update with new data from the project .....	3
3.1.3.	WP1.3 Validate new model.....	5
<b>3.2.</b>	<b>WP2 Spore Trapping, qPCR and disease monitoring</b> .....	<b>5</b>
3.2.1.	Spore trapping .....	5
3.2.2.	qPCR of historic air samples.....	6
3.2.3.	Establish field experiments and disease assessments.....	6
3.2.4.	Meteorological data collection.....	7
<b>3.3.</b>	<b>WP3 Light leaf spot and phoma forecasts</b> .....	<b>8</b>
3.3.1.	WP3.1 Prepare new forecasts based on existing models each year with spring update for LLS .....	8
<b>3.4.</b>	<b>WP4 Reporting and Knowledge Transfer</b> .....	<b>8</b>
<b>4.</b>	<b>RESULTS</b> .....	<b>8</b>
<b>4.1.</b>	<b>WP1 Modelling and forecast development</b> .....	<b>8</b>
4.1.1.	WP1.1 Preparation of historic datasets and weather data.....	8
4.1.2.	WP1.2 Produce model and update with new data from the project. ....	9
<b>4.2.</b>	<b>WP2 Spore Trapping, qPCR and disease monitoring</b> .....	<b>13</b>
4.2.1.	Spore trapping .....	13
4.2.2.	qPCR of historic air samples.....	15
4.2.3.	Establish field experiments and disease assessments.....	19
4.2.4.	Meteorological data collection.....	22
<b>4.3.</b>	<b>WP3 Light leaf spot and phoma forecasts</b> .....	<b>22</b>
4.3.1.	WP3.1 Prepare new forecasts based on existing models each year with spring update for LLS .....	22
<b>4.4.</b>	<b>WP4 Reporting and Knowledge Transfer</b> .....	<b>24</b>
<b>5.</b>	<b>DISCUSSION</b> .....	<b>26</b>

6. REFERENCES .....31

## 1. Abstract

Extensive research over the past 40 years has investigated different aspects of the epidemiology of the winter oilseed rape disease light leaf spot caused by the fungus *Pyrenopeziza brassicae*. Growers follow a variety of best management practices on their farms. Despite these efforts, the incidence and severity of light leaf spot and subsequent yield loss have increased substantially in the UK in recent years. This project aimed to re-assess the importance of different components of the epidemic with respect to the additive steps that result in yield loss.

The main objective of the project was to develop a novel decision support tool with respect to light leaf spot epidemic onset in autumn by modelling the inter-crop development and maturation of the fungus using pre-defined parameters readily available from the literature. The aim was to provide growers and advisors with a “heads up” warning that the start of the epidemic was imminent, much like the phoma leaf spot forecast. The model predictions were validated by PCR-based spore analysis of pre-existing multi-site air samples from England and Scotland and with air samples collected during the course of the project from project partner field sites along with field data on disease development on varieties with different levels of host resistance against light leaf spot. Results from three years of field experiments indicated that there were differences in disease development on varieties across different geographic locations and also between seasons. This suggested that there were differences between populations of the *P. brassicae* fungus across the UK and that this had implications for breeders with respect to the development of commercial resistant varieties. In addition, the most striking result from the project was that spore sampling work indicated that large quantities of spores were produced from May onwards and were continuously released throughout the summer, much earlier in the season than previously reported. This observation not only indicates that crops can become infected and epidemics begin at any time following emergence of the new crop, but also negates the possibility of developing a date driven forecast system for light leaf spot since there is no effective “starting date” from which to model apothecial development and ascospore release. These results raise some important questions with regard to our current understanding of the epidemic cycle, such as, why are symptoms not seen earlier in the season (on cotyledons and young leaves, for example), are there as yet unknown resistance factors protecting young plant material and does the industry need to revisit autumn fungicide timing work targeted at light leaf spot control?

## 2. Introduction

Extensive research over the past 30-40 years has investigated different aspects of the epidemiology of the winter oilseed rape (WOSR) disease light leaf spot (LLS) caused by the fungus *Pyrenopeziza brassicae* (Boys *et al.*, 2007; Evans *et al.*, 2003; Karolewski *et al.*, 2006). Growers follow a variety of best management practices on their farms. Despite these efforts, the incidence and severity of LLS and subsequent yield loss have increased substantially in the UK in recent years (Anon, 2017). The increase is partly explained by failure to use varieties with improved levels of host resistance and the poor timing of fungicide applications targeted at controlling LLS. This responsive-mode project aimed to re-assess the importance of different components of the epidemic (earliness of epidemic onset, importance of physiological stunting and subsequent winter survival of plants, relevance of harsh winter conditions/snow cover to epidemic progression, importance of stem infection and contribution to subsequent floral and pod infection) with respect to the additive steps that result in yield loss.

The main aim of the project was to develop a novel decision support tool to predict LLS epidemic onset by modelling the inter-crop development and maturation of the fungus using pre-defined parameters readily available from existing literature (Gilles *et al.*, 2000; Gilles *et al.*, 2001b; Gilles, Fitt & Jeger, 2001). This would provide growers and advisors with a “heads up” warning that the start of the epidemic was imminent, much like the current phoma leaf spot forecast (Evans *et al.*, 2008). Model predictions were validated by PCR-based spore analysis of existing multi-site air samples from England and Scotland and with air samples and in-field meteorological data collected during the course of the project from partner project field sites. There were 4 primary objectives to the project:

1. Develop a LLS spore release/epidemic onset model for growers/advisors to more accurately guide on-farm decisions on light leaf spot disease control.
2. Improved understanding of meteorological factors that affect pathogen development with respect to spore release and the onset and development of the epidemic.
3. Continued updating and maintenance of current oilseed rape decision support tools during the course of the project.
4. Knowledge transfer of results through to industry through publication of reports, web pages/articles, social media and recommendations.

### 3. Materials and methods

#### 3.1. Work Package (WP) 1 Modelling and forecast development

##### 3.1.1. WP1.1 Preparation of historic datasets and weather data

###### *Year 1 (August 2013-July 2014)*

At the start-up meeting, Weather Innovations Consulting LP (WIN) requested that ADAS, Rothamsted and Scotland's Rural College (SRUC) provide historic LLS data and any corresponding qPCR/spore trapping data as detailed in the proposal. Data was requested to be provided by the end of January 2014 (end of Quarter (Q) 2) in order to allow WIN to begin modelling work (WP 1.2) during the second half of year 1.

##### 3.1.2. WP1.2 Produce model and update with new data from the project

Historical data provided by ADAS, Rothamsted and SRUC were used as a starting point to look at relationships between weather, spore release (where available) and disease onset to produce a model to pin point the onset of the epidemic. This was developed using regression techniques and used to build on the original weather-parameter-driven model developed by Evans *et al.* (2006 and 2008) that predicts the onset of the phoma epidemic. Using summer temperature and rainfall, the model predicts the date when WOSR plants at a specific geo-location could be expected to show 10% incidence of phoma leaf spotting, the current threshold that triggers a suggested fungicide application for control. It was envisaged that a new LLS model would be developed by the end of year 1 and that this would then be further tested and updated/refined during years 2 and 3 of the project.

Initial modelling work was based on publications by Tijs Gilles during a PhD study programme at Rothamsted during the 1990s. Dr Gilles used a mixture of glasshouse, controlled environment and field studies to look at aspects of the biology and development of the causal agent of light leaf spot, *Pyrenopeziza brassicae* (Gilles *et al.*, 2000; 2001b; Gilles, Fitt & Jeger, 2001). The following equations were used to model each stage of the epidemic using actual weather datasets and compared with disease data where possible.

Rates of apothecial maturation were calculated using the equation:

$$t(T) = 7.6 + 55.8 \cdot 0.839^T \text{ (Gilles, Fitt \& Jeger, 2001)}$$

Where t = time from onset of maturation until 50% of the maximum number of *P. brassicae* apothecia matured and T: temperature (hourly) with wet oilseed rape stubble debris (wet debris assumed when surface wetness > 50%). The relationship can be visualised in Figure 1.

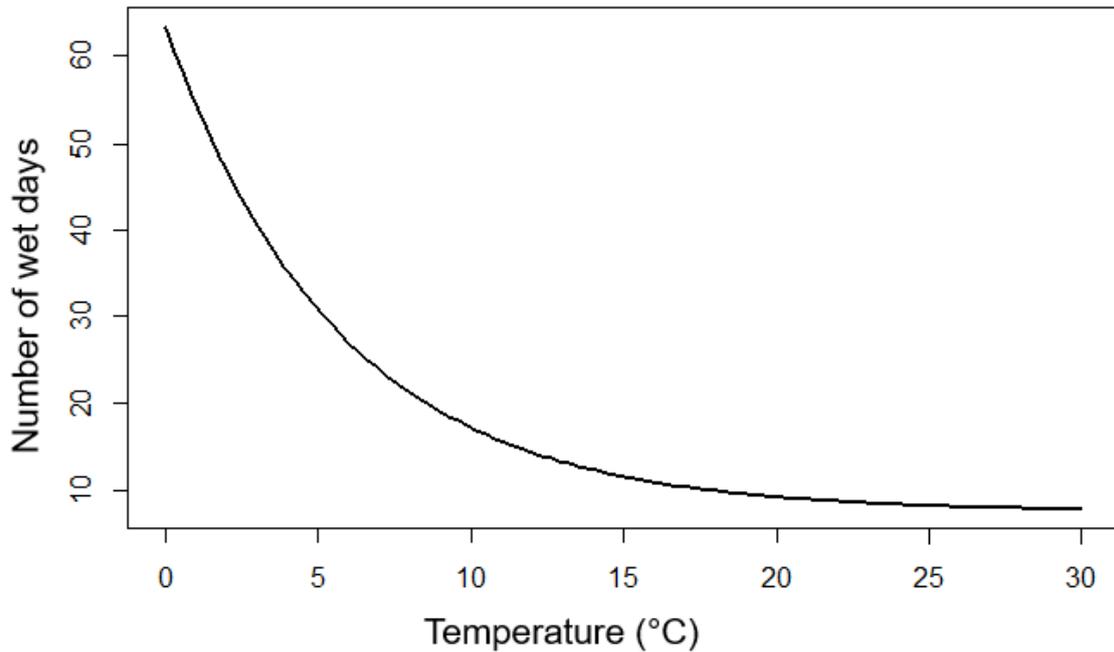


Figure 1. Schematic detailing the rate (time, in wetness days [ $\geq 12$  H stubble wet]) required for maturation of 50% of apothecia of the light leaf spot fungus *Pyrenopeziza brassicae* as a function of oilseed rape stubble debris wetness and temperature. As temperature increases, the period of time debris needs to be wet for maturation to occur decreases exponentially.

Using a function of leaf wetness and temperature, the following equation was used to calculate the resulting % area of leaf with sporulation from ascospore infection:

$$W \geq 6: c(T, W) = (3.65 + 7.02 \cdot T + 0.3 \cdot T^2) \cdot \exp(-\exp(-0.15 \cdot (W - (55.47 - 6.08 \cdot T + 0.21 \cdot T^2)))) \quad (\text{Gilles } et \text{ al., 2001b, equation 7}).$$

Where  $c$  = maximum percentage leaf area with sporulation,  $T$  = Temperature and  $W$  = daily hours of leaf wetness at time of infection. This relationship can be visualised in Figure 2.

Latent period of infection ( $I(T)$ : representing the time from initial leaf infection to the production of new conidiospores) was calculated as a function of temperature using the equation:

$$I(T) = 48 - 3.87 \cdot T + 0.11 \cdot T^2 \quad (\text{Adapted from Gilles } et \text{ al., 2001b, equation 9}).$$

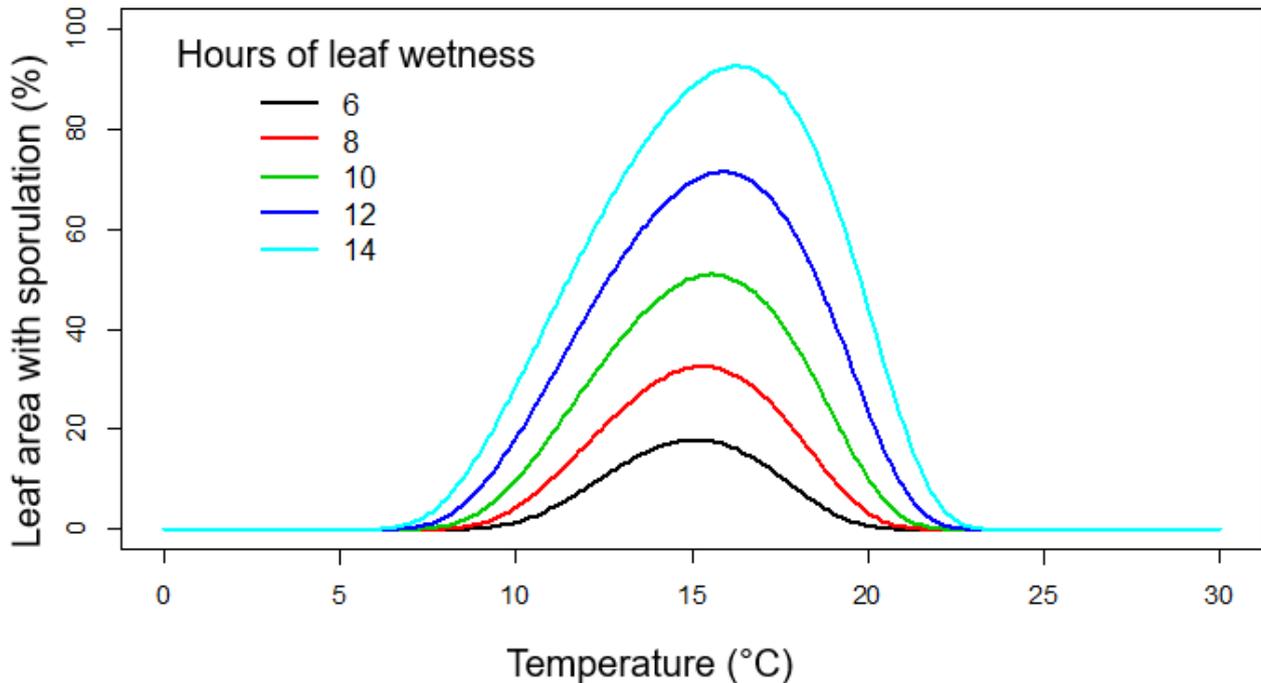


Figure 2. Percentage area of leaf with sporulation following an ascospore infection of winter oilseed rape by the light leaf spot fungus *Pyrenopeziza brassicae* as a function of leaf wetness duration and temperature.

### 3.1.3. WP1.3 Validate new model

Spore release and disease onset data produced during the project from WP2 was used to test and validate the model to test that predictions were robust at different locations. Once the project team were happy with the model and performance, the new date-driven predictive system was rolled-out alongside the current regional and site-specific LLS risk forecast model and the phoma leaf spot model.

## 3.2. WP2 Spore Trapping, qPCR and disease monitoring

### 3.2.1. Spore trapping

Burkard spore traps (7 day volumetric) were deployed at the three main disease monitoring sites (Aberdeen and Edinburgh, Scotland and near ADAS High Mowthorpe, North Yorks) (see 3.2.3 below) and at Rothamsted, where a field-deployed spore trap was also supplemented by a long-term roof-top deployed Burkard spore trap (Fig. 3). The protocol used to set-up and maintain spore traps was followed as set out in Appendix 1.

Spore traps were deployed from the start of September until late November during years 1 and 2, with the additional Rothamsted site (North Building Roof top) with 365 day monitoring per year.

Spore sampling was not done during year three due to budget restraints. Spore tapes were changed on a weekly basis with Scottish tapes being processed by SRUC in Edinburgh and tapes from ADAS sites being sent to Rothamsted to be processed in parallel with the samples taken at Rothamsted. Sample tapes were processed and analysed using the methods published by Karolewski *et al.* (2006).

### 3.2.2. qPCR of historic air samples

Historic air samples from previous years (10 seasons x 2 sites, Scotland [SRUC]; 8 seasons x 1 site, Hertfordshire) collected and stored in deep freeze storage were tested by SRUC and Rothamsted, respectively. Results, in association with corresponding field data were provided to WIN during year 1 for use in 3.1.2. to aid model development. As described previously, sample tapes were processed and analysed using the methods published by Karolewski *et al.* (2006).

### 3.2.3. Establish field experiments and disease assessments

Untreated replicated disease monitoring plots (to include one resistant, one intermediate and one susceptible variety) were established at ADAS Rosemaund, Boxworth and High Mowthorpe and at SRUC-maintained sites near Aberdeen and Edinburgh (Fig. 3). Disease progress (incidence and severity) was then monitored on a monthly basis from October to July each season. Disease assessments were done on 25 plants per plot after incubation of destructively sampled plants in late winter and early spring, on 25 plants in the field (non-destructive) monthly from early spring to July once disease symptoms were readily visible and assessments of stem disease (as the percentage of stem area affected) on 25 plants per plot prior to harvest (in July). A plot of susceptible WOSR was also sown near the field-deployed Burkard spore trap at Rothamsted and was monitored for LLS epidemic onset, but was not scored.

Seed of each variety was kindly provided by Bayer CropScience throughout the course of the project. Table 1 indicates which varieties were grown each season, with adjustments made to account for availability of seed and relevance to market-share.

Table 1. Variety of seed sown at 5 sites across the UK as monitor plots for the AHDB-Bayer CropScience funded light leaf spot epidemiology study 2013-2016.

Harvest year	Variety and resistance rating to light leaf spot		
2014	Cuillin (8)	Harper (6)	Patron (4)
2015	Cracker (7)	Harper (6)	Charger (4)
2016	Cracker (7)	Harper (6)	Charger (4)



Figure 3. Map indicating the positions of field experiments at 6 sites across the UK as monitor plots for the AHDB-Bayer CropScience funded light leaf spot epidemiology study 2013-2016.

#### 3.2.4. Meteorological data collection

Three meteorological stations were set-up at the three main LLS monitoring sites to gather in-field meteorological data during the course of each season. These were based at the two SRUC sites, Bush Estate, near Edinburgh and near the Craibstone Estate near Aberdeen and near ADAS High Mowthorpe in North Yorkshire. Each meteorological station consisted of a scaffold pole driven into the ground from which the following sensors were mounted:

1 x Adcon SEN-R Combisensor measuring temperature and relative humidity

1 x rain gauge measuring rainfall

2 x WIN leaf wetness sensors, one at 5 cm (rosette canopy level), one at 50 cm (mid-canopy after stem extension)

1 x Adcon addWAVE GPRS QUAD RTU (Remote Terminal Unit), collecting, storing and sending data

Data were collected every 5 minutes, polled and sent via GPRS to an Adcon Gateway server every 2 hours. Data were quality controlled and verified before being added to the WIN database. Data were then accessible in near real-time via mobile devices or could be download as .CSV files via an internet web link as hourly, daily or 15 minute increment files. Data were provided to WP1, along with disease monitoring data for use in model development.

### **3.3.WP3 Light leaf spot and phoma forecasts**

#### **3.3.1. WP3.1 Prepare new forecasts based on existing models each year with spring update for LLS**

Each autumn, in collaboration with Rothamsted and ADAS, WIN gathered relevant meteorological data and updated the regional and site-specific LLS risk forecast developed previously at Rothamsted by Welham *et al.* (2004) and also the phoma leaf spot forecast developed by Evans *et al.* (2008). The LLS risk prediction was also updated in the spring of each season to update the risk forecast to incorporate actual winter rainfall. The updated forecasts were delivered to industry via the Rothamsted website.

### **3.4.WP4 Reporting and Knowledge Transfer**

In addition to a project start-up meeting in early August 2013, regular project management meetings were held twice a year (a teleconference in January and then a summer face-to-face meeting approximately one month after harvest). These meetings were used to analyse and report on results and to decide on the most efficient route for the dissemination and knowledge transfer (KT) of information, utilising the resources of AHDB and the project partners through publication of reports, topic sheets, web pages/articles, social media and recommendations. These were mainly delivered through the farming press and at farm roadshows and trade events such as the annual “Cereals” event. Information was also distributed via the WOSR forecast “registered users” email distribution list and via tweets using the Twitter account @LeafSpot.

## **4. Results**

### **4.1.WP1 Modelling and forecast development**

#### **4.1.1. WP1.1 Preparation of historic datasets and weather data**

Rothamsted and SRUC were unable to supply any historical datasets for LLS. ADAS were able to supply some historic data which are summarised in Table 2. However, even ADAS historical data

were very old (with outdated varieties, e.g. Bristol, Jet Neuf, etc), often assessments started late in the season, or data were from demo plots rather than replicated trials and so were not detailed enough to be useful for modelling purposes. It was decided at the first annual project review meeting that to save time and effort, the data would be sidelined and modelling work would focus on data generated from the current project.

Table 2. Summary of historical datasets of light leaf spot incidence and severity provided by ADAS.

Harvest Year	Site	Assessments	Variety known?	Growth stage?	Incidence data	Severity data
1980	Cambridge	19/12/79 – 26/06/80*	Jet Neuf	Yes	Yes	No
1980	Cambridge2	19/12/79 – 26/06/80*	Jet Neuf	Yes	Yes	No
1987	Reading	30/10/86 – 05/02/87	Jet Neuf	No	Yes	Yes
1987	Bristol	30/10/86 – 05/03/87	Jet Neuf	No	Yes	Yes
1989	Spaxton	14/10/88 – 28/06/89*	Bienvenue	No	Yes	Yes
1990	Bristol	17/11/89 – 04/01/90	Jet Neuf, Ariana, Darmor	No	Yes	No
1991	Neston	27/11/90 – 17/07/91	Not known	Yes	Yes	Yes
1991	Bristol	21/11/90 – 19/12/90	Jet Neuf, Falcon, Ariana	No	Yes	No
1993	Boxworth	17/11/92 – 04/01/93	Envol	No	Yes	No
1993	Rosemaund	11/10/92 – 14/07/93	Envol	No	Yes <sup>a</sup>	Yes <sup>a</sup>
1995	Stamford	17/10/94 – 02/07/95	Not known	Yes	Yes	Yes
1996	Stamford	22/10/95 – 18/07/96	Apex, Nickel, Amber, Bristol	Yes	Yes	Yes
1997	Boxworth	19/10/96 - 06/06/97	Bristol, Capital	Yes	Yes	Yes
1999	Boxworth	23/10/98 – 30/06/99	Bristol, Capital	Yes	Yes	Yes

\* Assessed every two months only.

<sup>a</sup> Stem data only

#### 4.1.2. WP1.2 Produce model and update with new data from the project.

Due to lack of useful historical data and whilst data were generated during the first two seasons of the project, modelling work began by using real weather data to simulate the outbreak and continued progress of an epidemic. Using weather data from autumn 2009 for Aberdeen (corresponding to Aberdeen 2009 historic spore data, see 4.1.2 below) the epidemic could be defined as follows: assuming apothecial development began on the 1st August 2009, criteria for apothecial maturation were met by 31st August, just 30 days later. However, it was noted that this process was very weather dependant and the weather would affect the presence or absence of ascospores greatly. For example, in the same year, if apothecial development began on the 11th September, apothecia would not be mature until the 21st October (40 days). Conversely, if apothecial development began on the 30th September, apothecia would be mature by the 28th October, only 28 days later. Returning to the scenario where maturation began on 1<sup>st</sup> August, ascospores would potentially be released on 31<sup>st</sup> August. In 2009, the crop in Aberdeen was not

sown until 7th September and the first true leaves developed by the 21st September, which indicates that under the scenario, the crop is emerging into air that contains ascospores and there is the potential for infection straight away.

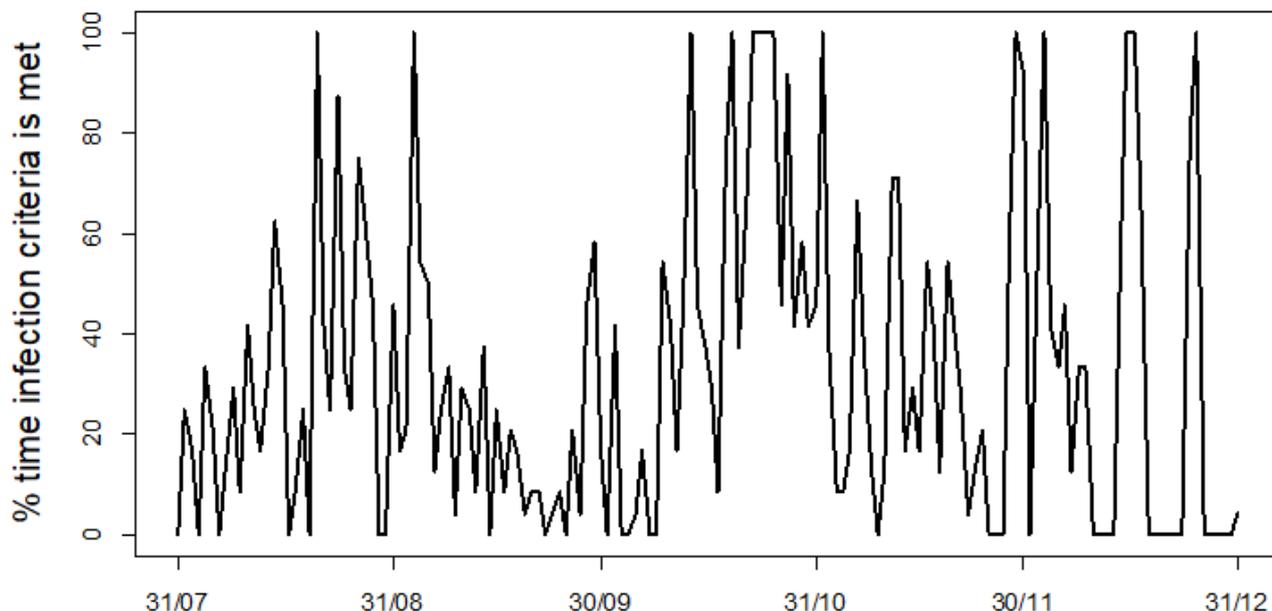


Figure 4. A plot indicating the percentage of time within each 24 hour period when *Pyrenopeziza brassicae* ascospore infection criteria (16 hours continual leaf wetness) were fulfilled for the autumn of 2009 for the Bush Estate, Edinburgh.

Figure 4 shows the percentage of time when infection criteria were fulfilled for the autumn of 2009 for Edinburgh and indicates that during September, infection criteria were met on average between 30-50%, but sometimes 100% of the time within each 24 hour period. Assuming the first true leaves were infected on the 21<sup>st</sup> September, the warm weather during September 2009 would drive the epidemic to produce a new flush of conidiospores on 12<sup>th</sup> October. If we then consider the days with rainfall after the production of the conidiospores on the 12<sup>th</sup> October (Table 3), there is rain every single day to varying degrees, so no shortage of potential rain splash events to spread infection from initial foci to new uninfected leaves and adjacent plants. The implications of this in light of results from spore trapping during the project, on the production of a date-driven model for onset of the LLS epidemic are further discussed in section 4.1.1. below.

As the project only produced a limited number of spore data sets (see 4.1.1. below; 2 x Rothamsted, 2 x High Mowthorpe), on the advice of statisticians, it was not possible to effectively adapt Gilles' sporulation model by regression modelling with weather data. With a limited number of datasets, rainfall/wetness and temperature were investigated, but there was no clear relationship with spore release. This was compounded by the result from the spore monitoring that indicated, for most datasets, sampling began too late and the main flush of ascospore had probably been missed. However, from this work, it was interesting to note the apparent negative relationship

between rainfall and spore release. It was observed that when there was hardly any rain, many spores were released. Conversely, when there was much rain, spore release stopped.

Table 3. Daily rainfall (mm) for a period during the autumn of 2009 from the meteorological station at the Bush Estate, Edinburgh.

<b>Date</b>	<b>Daily rain (mm)</b>
13.10.2009	9.4
14.10.2009	9
18.10.2009	1.8
19.10.2009	7
20.10.2009	1
21.10.2009	24.2
22.10.2009	74.2
23.10.2009	10.8
24.10.2009	19.6
25.10.2009	8
26.10.2009	2.8
27.10.2009	10
28.10.2009	8.2
31.10.2009	12.4

In order to present the relationship between rainfall and spore release more succinctly, cumulative rainfall and spore release (i.e. cumulative rainfall and spore counts from the start to the end of the observation period) were plotted together (Figures 5 and 6). Horizontal stretches of data in figures 5b and 6b indicate spore release without rainfall while vertical stretches indicate rainfall without spore release. Diagonal lines indicate simultaneous rainfall and spore release. Additional information can be taken from the vertical level of horizontal stretches: if they appear at low levels on the plot, major spore release happened without too much previous rainfall (since measurements started) (Figure 5). Conversely, horizontal stretches at high y-levels indicate spore release only happened after most of the rainfall during the observation period had already happened (Figure 6).

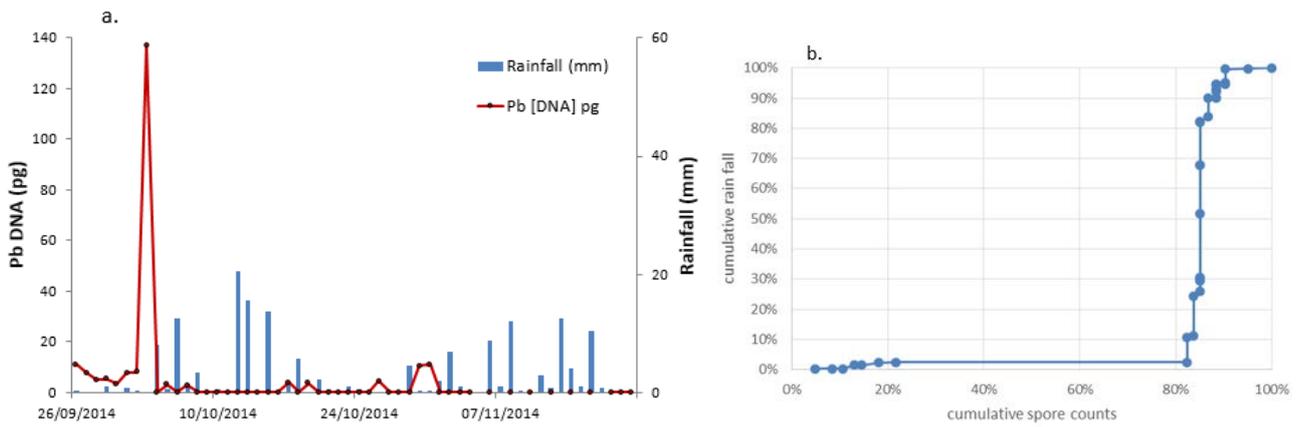


Figure 5. a) Amount of *Pyrenopeziza brassicae* DNA captured by a 7 day volumetric Burkard spore trap sited on the roof of the North Building, Rothamsted Research during 2014 plotted against rain events and b) cumulative percentage of total DNA captured plotted against cumulative percentage rainfall for the same observation period.

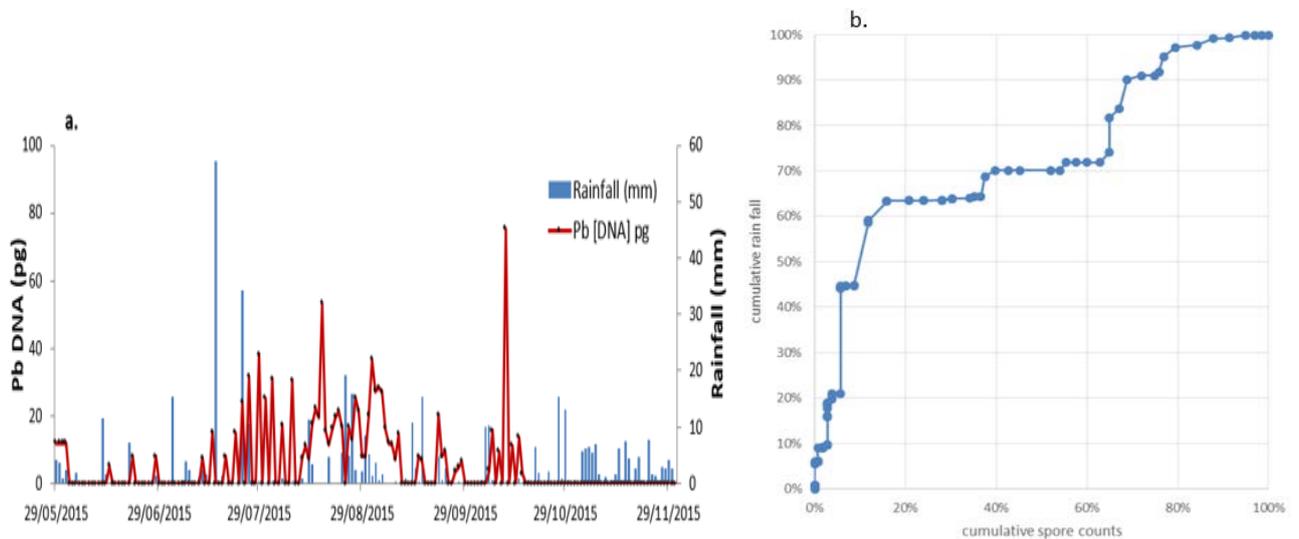


Figure 6. a) Amount of *Pyrenopeziza brassicae* DNA captured by a 7 day volumetric Burkard spore trap sited on the roof of the North Building, Rothamsted Research during 2015 plotted against rain events and b) cumulative percentage of total DNA captured plotted against cumulative percentage rainfall for the same observation period.

## 4.2. WP2 Spore Trapping, qPCR and disease monitoring

### 4.2.1. Spore trapping

Year 1 (2013-2014)

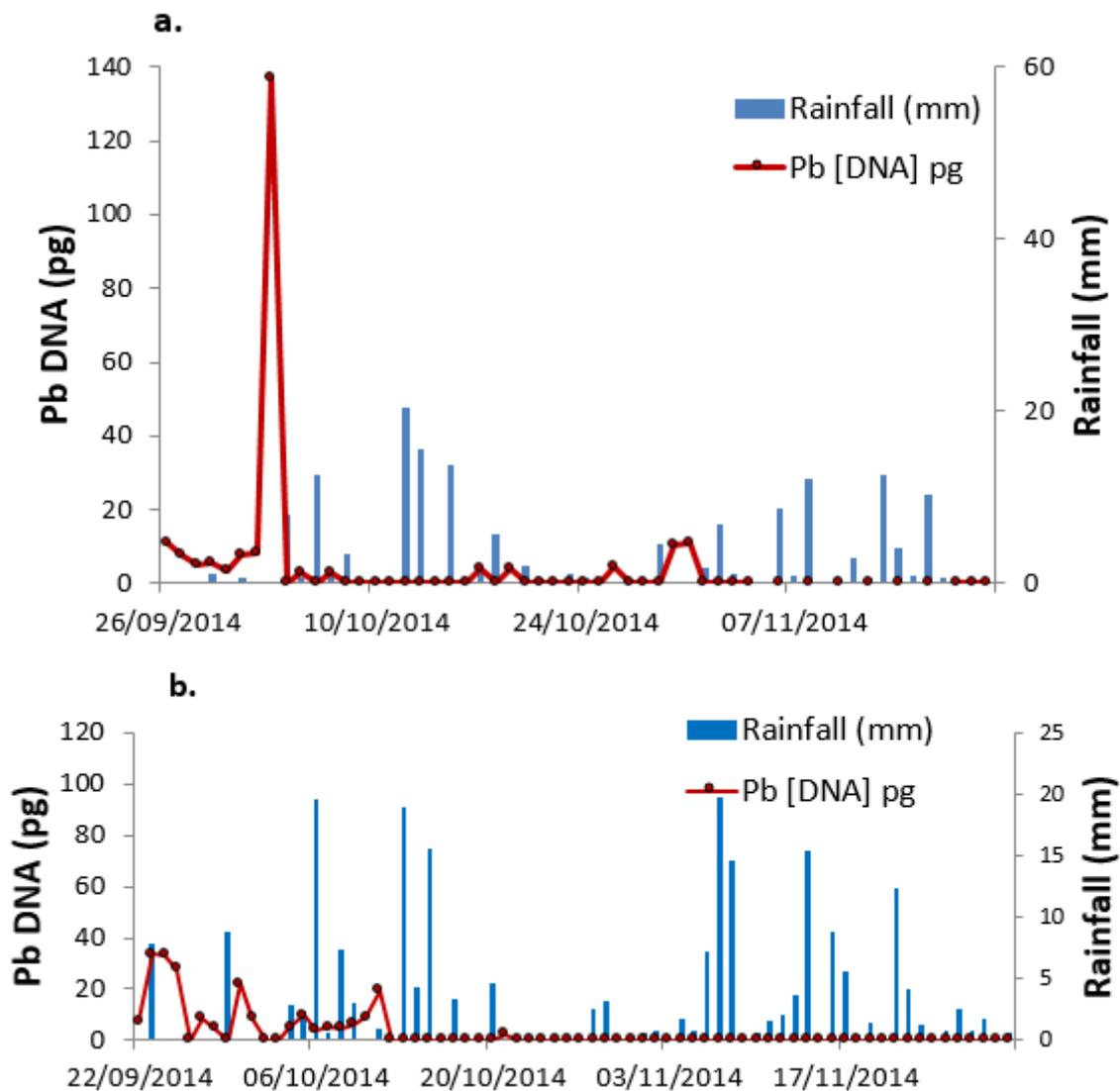


Figure 7. *Pyrenopeziza brassicae* DNA from spore traps situated a) Rothamsted Research, North Building Roof trap and b) ADAS High Mowthorpe for the 2013-14 season, with associated rainfall data. Plots are aligned, allowing comparison between sites.

Analysis of historic spore trap samples (see 4.1.2 below) tapes during year 1 indicated that masses of fungal material were observed in early August (SRUC, Aberdeen 2009 specifically and also SRUC Bush 2012). It was initially suspected at the year 1 project review meeting that this was probably released as mycelial matter in dust during harvest. However, subsequent ascospore releases were also observed in late August/early September much earlier than reported in earlier work (see discussion below). These results suggested that the epidemic may start earlier than previously thought. Sampling during year 2 was extended to the whole summer period for the Rothamsted trap (Figure 8).

Year 2 (2014-2015)

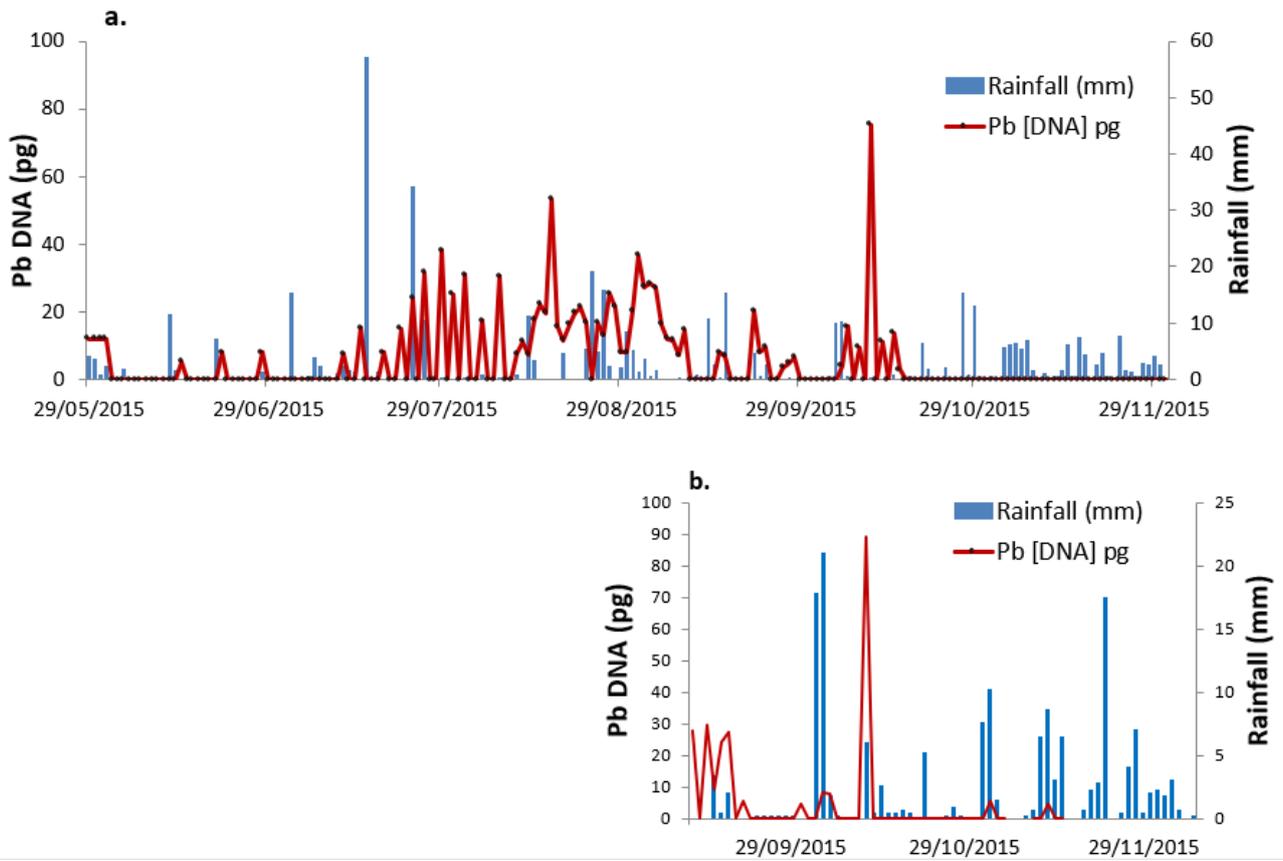


Figure 8. *Pyrenopeziza brassicae* DNA from spore traps situated a) Rothamsted Research, North Building Roof trap and b) ADAS High Mowthorpe for the 2014-15 season, with associated rainfall data. Plots are aligned, allowing comparison between sites.

Data from the most comprehensive spore data sets (Rothamsted and ADAS) were reviewed at the annual consortium meeting at the end of year 2 and the results are shown in Table 4. This indicated that each season, spores were usually detected as soon as spore sampling began, suggesting that spore release was happening earlier than previously thought from previous published work in this area.

Table 4. Summary of Rothamsted and ADAS spore sampling results (2006-2015).

2006	Spores detected on first date sampled 1/9/06 (Karolewski <i>et al.</i> , 2012)
2007	Spores detected on first date sampled 1/9/07(Karolewski <i>et al.</i> , 2012)
2008	Spores detected on first date sampled 23/9/08
2009	Spores detected on 25/9/09
2013	Spores detected on first date sampled 2/10/13 at High Mowthorpe and 24/9/13 at Rothamsted Research
2014	Spores detected on first dates sampled 22/9/14 at High Mowthorpe and 26/9/14 at Rothamsted Research
2015	Spores detected as early as 29/5/15 at Rothamsted Research

#### 4.2.2. qPCR of historic air samples

The following historic spore samples and rainfall sets were made available to the project:

SRUC Bush Estate, Edinburgh (Figure 9) and Craibstone Estate, Aberdeen (Figure 10) 2009

Rothamsted Research, North Building Roof trap (Figure 11), 2010

SRUC Bush Estate, Edinburgh (Figure 12), 2012

SRUC Bush Estate, Edinburgh (Figure 13), 2013

Rothamsted Research, Great Knott 3 Field (Figure 14) and the North Building Roof trap (Figure 15), 2013

The following data were used to inform project partners with regard initial and subsequent release patterns, but as detailed in section 4.2.1 above, the historic air sampling data were not particularly informative since sampling began too late each season to catch the initial release and probably only captured the latter half of the release curve with respect to numbers of spores captured. The data were also used to inform modellers regarding environmental parameters and/or patterns that affect apothecial development and subsequent spore release, although as detailed in section 4.1.2 and also due to the statement in the previous sentence, a satisfactory relationship could not be found.

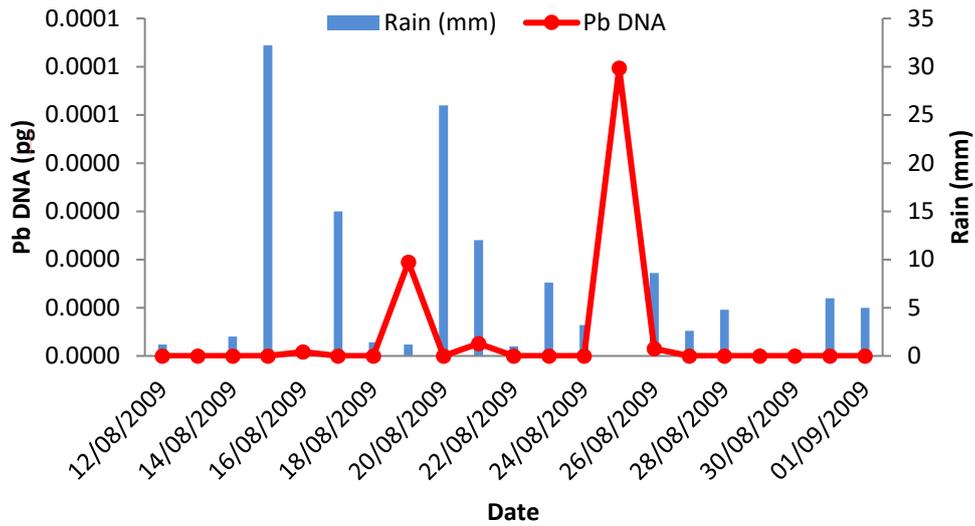


Figure 9. *Pyrenopeziza brassicae* DNA from spore traps situated at SRUC Bush Estate, Edinburgh, 2009 and associated rainfall data.

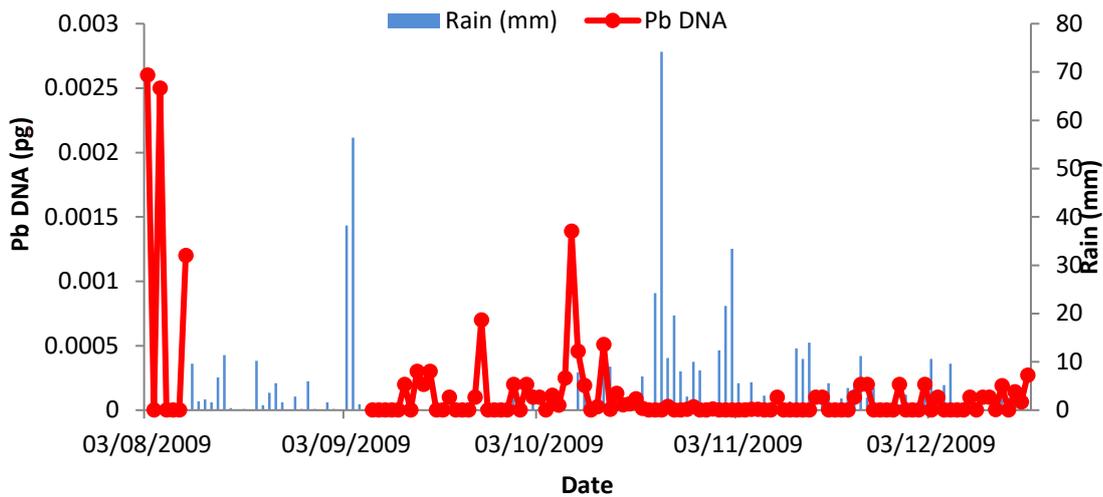


Figure 10. *Pyrenopeziza brassicae* DNA from spore traps situated at SRUC, Aberdeen, 2009 and associated rainfall data.

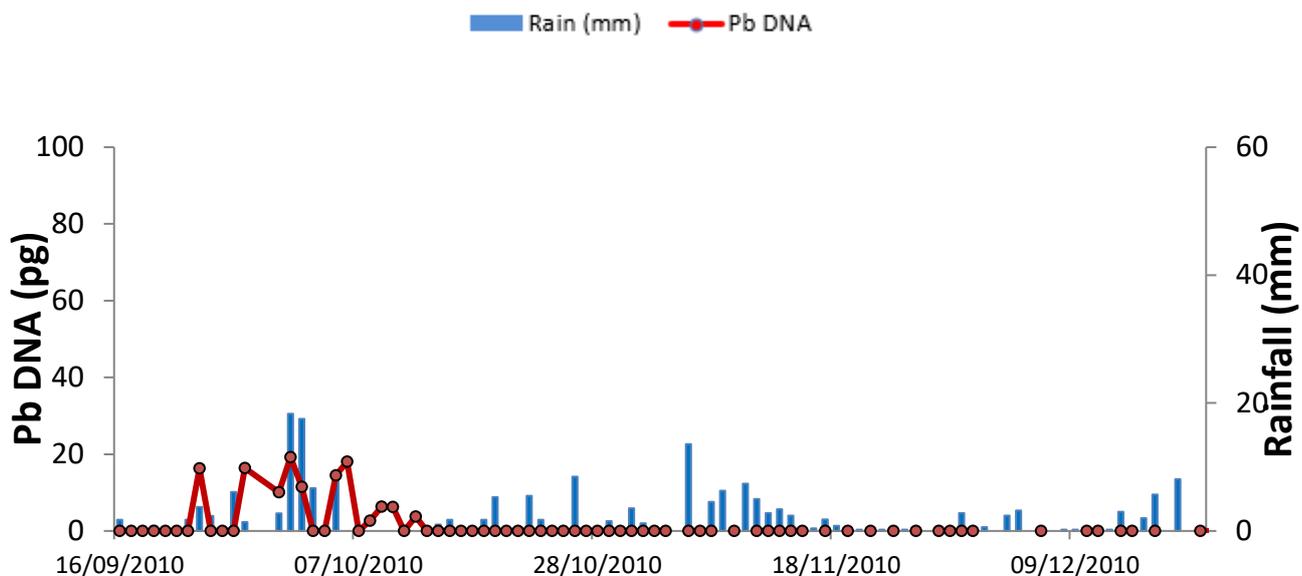


Figure 11. *Pyrenopeziza brassicae* DNA from spore traps situated at Rothamsted Research, North Building Roof trap, 2010 and associated rainfall data.

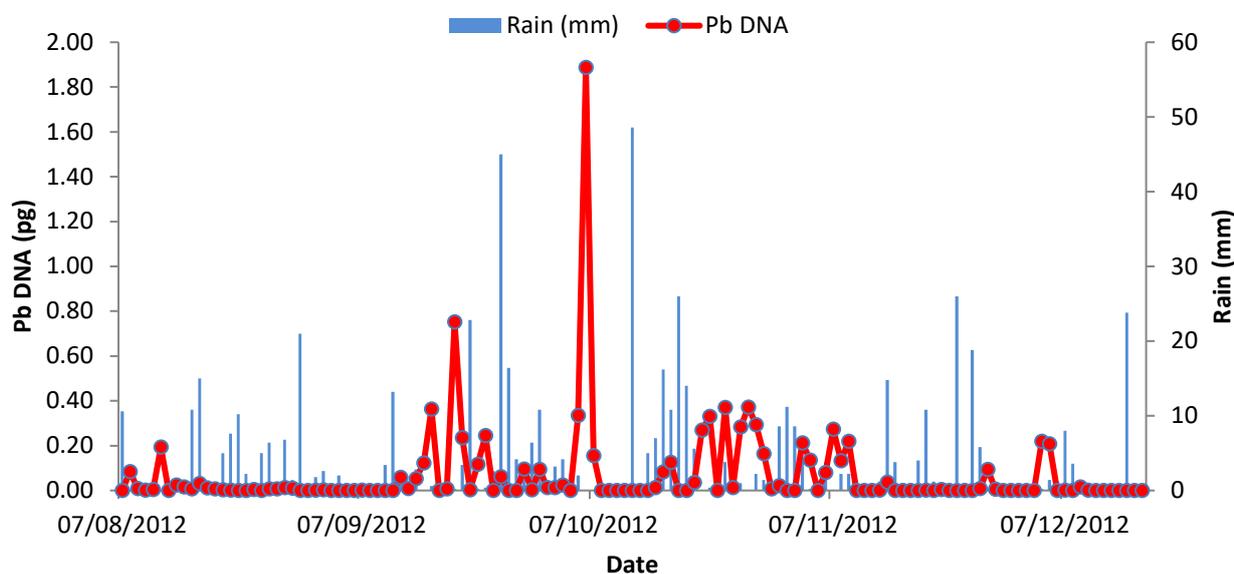


Figure 12. *Pyrenopeziza brassicae* DNA from spore traps situated at SRUC Bush Estate, Edinburgh, 2012 and associated rainfall data.

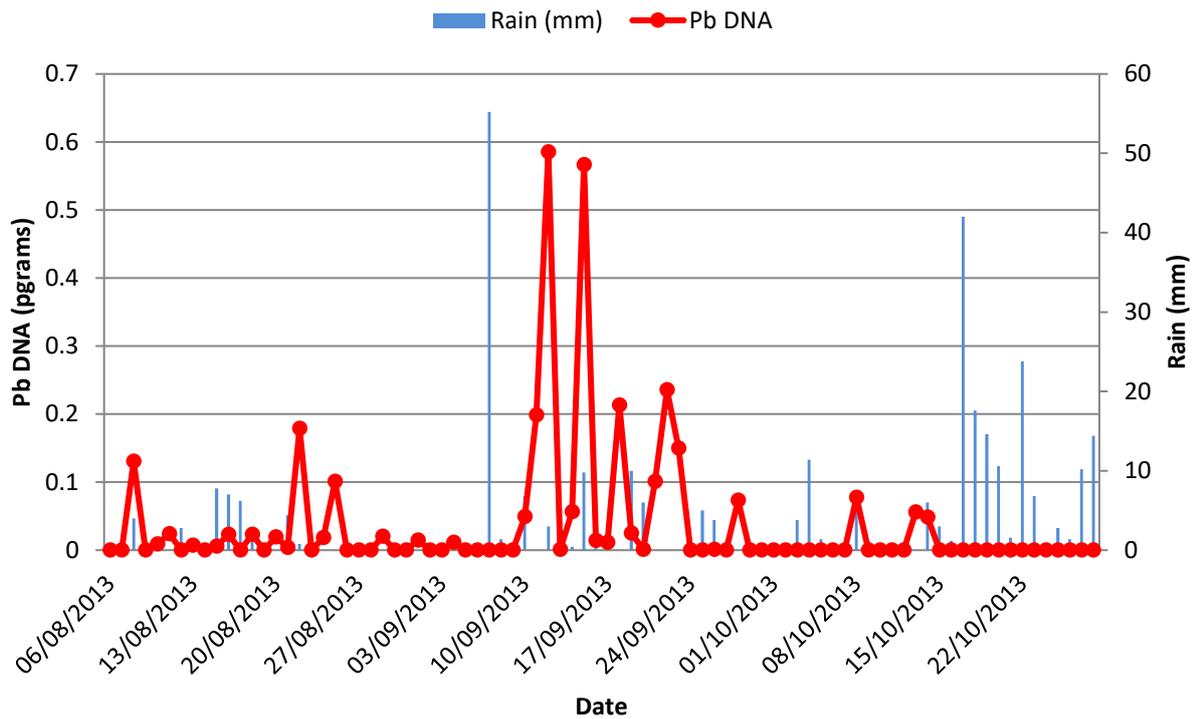


Figure 13. *Pyrenopeziza brassicae* DNA from spore traps situated at SRUC Bush Estate, Edinburgh, 2013 and associated rainfall data.

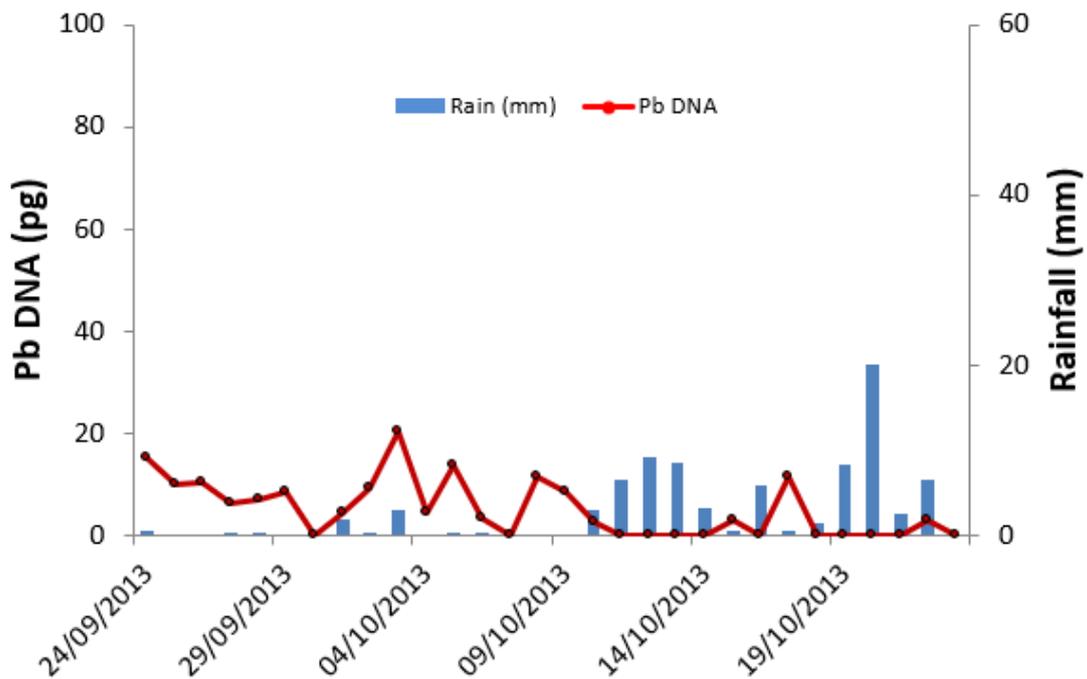


Figure 14. *Pyrenopeziza brassicae* DNA from spore traps situated at Rothamsted Research, Great Knott 3 Field, 2013 and associated rainfall data.

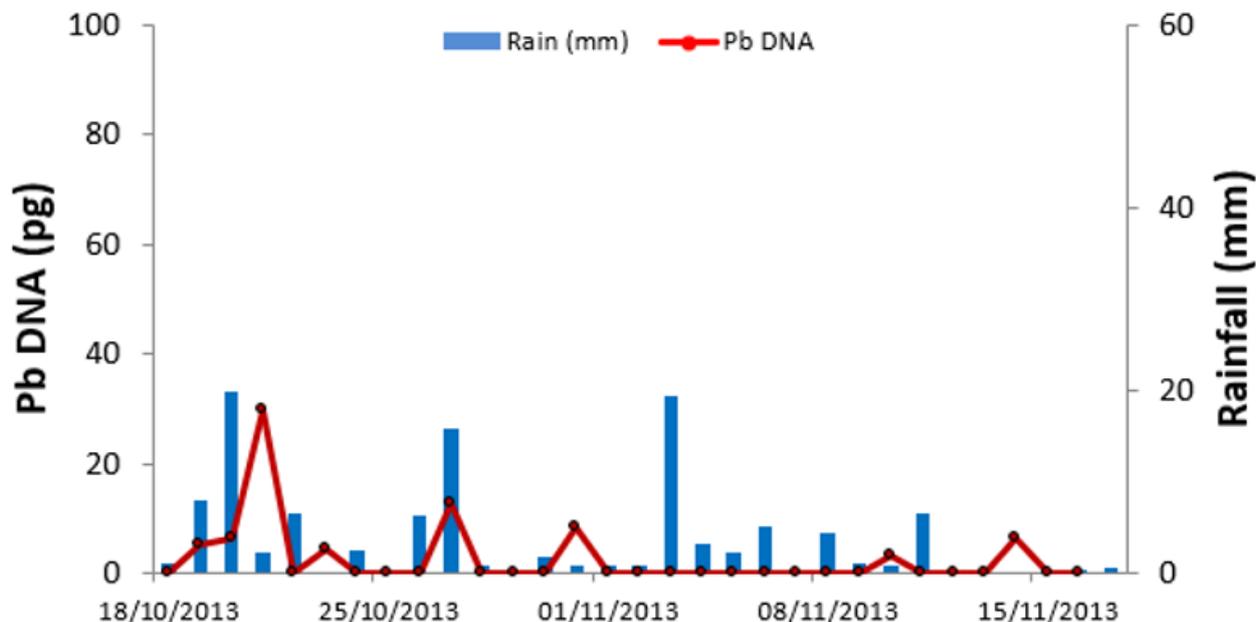


Figure 15. *Pyrenopeziza brassicae* DNA from spore traps situated at Rothamsted Research, North Building Roof trap, 2013 and associated rainfall data.

#### 4.2.3. Establish field experiments and disease assessments

##### Year 1 (2013-2014)

Monitoring plots indicated significant LLS infection levels across three varieties representing resistance ratings from 4 to 7 (Patron [4], Harper [5], Cuillin [7]). At the two Scottish sites, disease levels were low in Edinburgh (Figure 16b), and moderate in Aberdeenshire (Figure 16a) where all varieties were infected but Harper had less disease than the other varieties. Cuillin was only marginally better than Patron which would confirm that varietal resistance in this variety has been eroded. There was a similar trend at the ADAS sites (Figure 17). Onset was earliest at High Mowthorpe (Figure 17a), however, levels appeared to be lower than the other two sites and declined. There was significant lower leaf loss at this site over winter which may be a factor in this apparent decline of visible LLS symptoms. Stem area affected was highest at Rosemaund compared to Boxworth and High Mowthorpe, but generally levels were low (Figure 17d,e,f). Severity varied between site and variety. At High Mowthorpe (Fig 17d), stem infection was highest on Cuillin whereas at Rosemaund, little stem infection was reported on Cuillin (Fig 17e). At Boxworth, lowest stem severity scores were observed on Cuillin (Fig 17f).

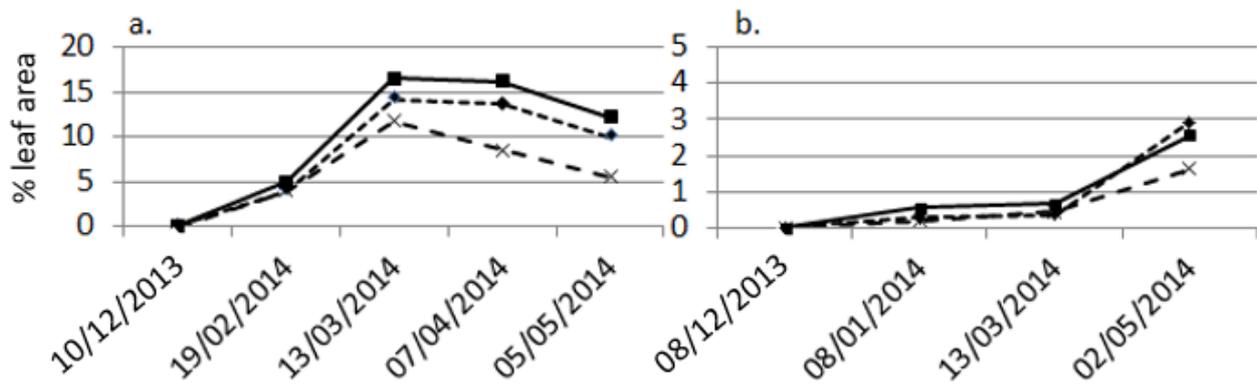


Figure 16. Percentage leaf area affected with light leaf spot in a) Aberdeenshire and b) Midlothian in Scotland during the 2013/14 season. (Varieties: Cuillin ----◆---- resistance rating 8, —■—, Harper ---x--- resistance rating 6, Patron resistance rating 4; Note difference in scale at each site indicating very different levels of disease at each site).

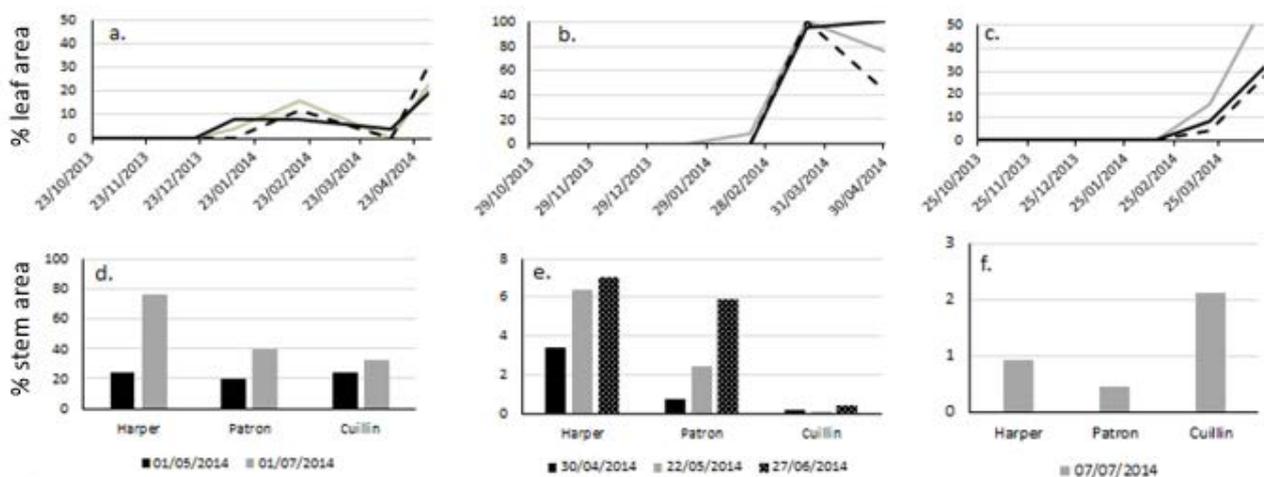


Figure 17. Incidence of leaves affected (a, b & c; Cuillin ----- resistance rating 8, Harper — resistance rating 6, Patron — resistance rating 4) and percentage stem area affected (d, e, and f) with light leaf spot at High Mowthorpe North Yorkshire (a & d), ADAS Rosemaund (b & e) and ADAS Boxworth (c & f) during the 2013/14 season. (Note difference in scale at each site indicating very different levels of disease at each site).

### Year 2 (2014-2015)

Monitoring plots indicated significant LLS infection levels across three varieties representing resistance ratings from 4 to 7 (Charger [4], Harper [6], Cracker [7]). At the three ADAS English sites, disease levels were high at ADAS Rosemaund, Hereford (Figure 18a), moderate at ADAS High Mowthorpe, North Yorkshire (Figure 18b) and low at ADAS Boxworth, Cambridgeshire (Figure 18c). All varieties were infected, with Cracker giving good control at ADAS Rosemaund (Figure 18a). Disease levels in Scotland were higher than in England (Figures 18d and e);

however, Cracker had only marginally less disease than Charger. These results suggest that there are differences in LLS populations between England and Scotland and could indicate that the varietal resistance in Cracker has been eroded with regard to northern populations of the LLS pathogen.

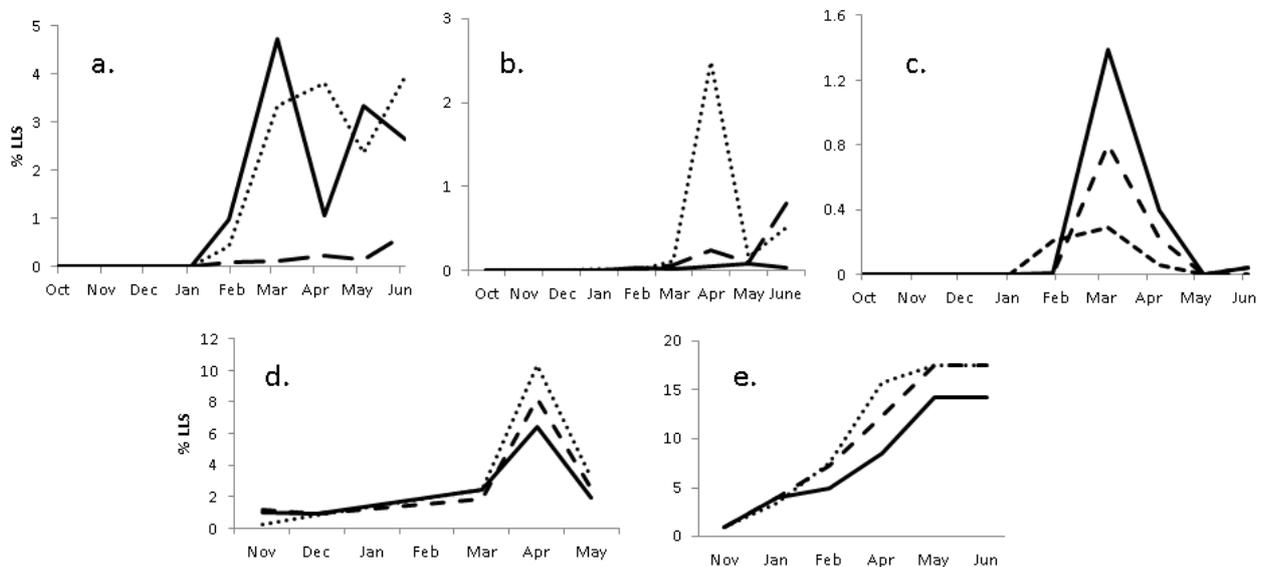


Figure 18. Percentage leaf area affected with light leaf spot at a) ADAS Rosemaund, b) ADAS High Mowthorpe, c) ADAS Boxworth, d) Bush, Edinburgh and e) Aberdeen [% infection on a plot scale] during the 2014/15 growing season (Varieties: Cracker - - - resistance rating 7, Harper — resistance rating 6, Charger ····· resistance rating 4; note difference in scale at each site indicating very different levels of disease at each site).

### Year 3 (2015-2016)

Monitoring plots indicated significant LLS infection levels across three varieties representing resistance ratings from 4 to 7 (Charger [4], Harper [6], Cracker [7]) as observed in the two previous seasons. For the second year in succession, Cracker gave good control at sites in England, but did not perform as well at Scottish sites (Figure 19). These results provide further information that there are differences in LLS populations between England and Scotland and also that varietal resistance in Cracker has been further eroded with regard to northern *P. brassicae* populations.

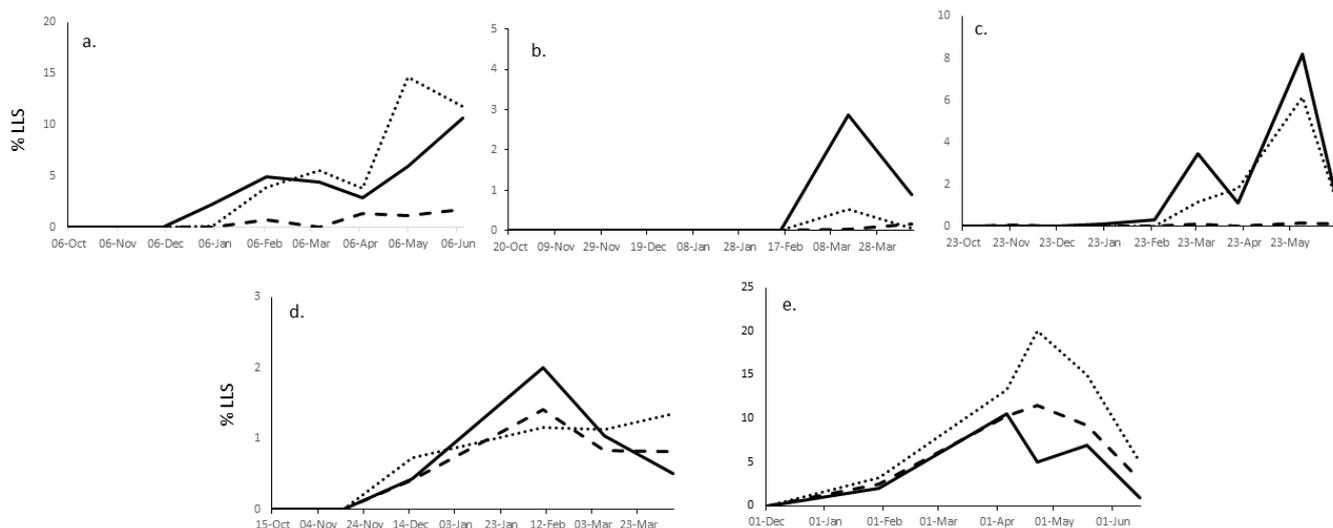


Figure 19. Percentage leaf area affected with light leaf spot at a) ADAS Rosemaund, b) ADAS High Mowthorpe, c) ADAS Boxworth, d) Bush, Edinburgh and e) Aberdeen [% infection on a plot scale] during the 2015/16 growing season (Varieties: Cracker - - - resistance rating 7, Harper — resistance rating 6, Charger ····· resistance rating 4; note difference in scale at each site indicating very different levels of disease at each site).

#### 4.2.4. Meteorological data collection

Data were collected at field experimentation sites for each successive season (harvest years 2014, 2015 and 2016) at Aberdeen, Edinburgh and High Mowthorpe and were used in conjunction with disease data and spore data for each site. Data are stored on the Weather Innovations archive data server and are available upon request.

### 4.3. WP3 Light leaf spot and phoma forecasts

#### 4.3.1. WP3.1 Prepare new forecasts based on existing models each year with spring update for LLS

Meteorological data from relevant daily synoptic and ground-based UK Meteorological Office stations were downloaded by Rothamsted Research in late September/early October each year and processed accordingly. Additional sites were added for ADAS (Rosemaund, Boxworth and High Mowthorpe) and AHDB Recommended List (RL) trial sites, subject to availability of data. LLS pod incidence data were kindly provided by colleagues from Fera from the Defra-funded oilseed rape Pest and Disease Survey as an input to the LLS forecast. All data were processed using

Genstat and new forecasts were published via the Rothamsted Research website as detailed in Table 5 below. The LLS risk forecast was also updated in March of each year, as detailed in Table 5, to include actual winter rainfall for each season. Each forecast update was also accompanied by a press release (in association with the AHDB press office) to highlight predicted disease risk and features of the forecast.

Table 5. Date when the light leaf spot regional risk forecast and the phoma leaf spot forecast decision support tools were updated 2013–2017.

Season	LLS risk and phoma leaf spot forecasts updated	LLS risk forecast updated to include winter weather
2013-14	1 <sup>st</sup> October 2013	4 <sup>th</sup> March 2014
2014-15	5 <sup>th</sup> November 2014	31 <sup>st</sup> March 2015
2015-16	8 <sup>th</sup> October 2015	15 <sup>th</sup> March 2016
2016-17	4 <sup>th</sup> November 2016	15 <sup>th</sup> March 2017

#### 4.4. WP4 Reporting and Knowledge Transfer

##### **Year 1 (2013-2014)**

###### Events

Project RD-2012-3814 mentioned during the HGCA Agronomists' Conference, 10th Dec 2013

HGCA/SRUC workshops Carfraemill 14th Jan 2014

HGCA/SRUC workshops Perth 15th Jan 2014

HGCA/SRUC workshops Aberdeen 22nd Jan 2014

Project demonstrated at Cereals (11-12th June 2014)

###### Conference presentations, papers or posters

SRUC advisers training day, Edinburgh 27th February 2014

###### Press articles

"Light leaf spot risk remains high", CPM, March 2014

"Spot a problem in OSR" CPM (From theory to field article), March 2014

"Giving the green light to light leaf spot treatment", Crops, Sept 2014

###### Other

45 tweets from @LeafSpot

##### **Year 2 (2014-2015)**

###### Events

Project mentioned during discussions whilst demonstrating Fungicide Response Experiments at Cereals 2015, June 2015 and SRUC/AHDB Disease roadshows in Jan 2015

###### Press articles

- Light leaf spot gets green light for treatment, Research in Focus, Crops, 6th September 2014

- Time to stop Phoma before stem hit, Farming UK. 7th Nov 2014

- Phoma disease alert for oilseed rape after recent rains, Farmers Weekly, 29th Sep 2015

- Press release alongside the publication of the 2014/15 phoma forecast

### Other

The project was mentioned in the 2014 Rothamsted annual report (available in autumn 2015).

2014/15 light leaf spot forecast and spring update

2014/15 phoma forecast

AHDB Phoma information sheet updated during year 2

"OSR emerges into a 'blitz' of disease", CPM article, 4th Sept 2015

60 tweets from @LeafSpot.

### **Year 3 (2015-2016)**

#### Events

Project featured in ADAS presentation ("The latest on light leaf spot") at the United Oilseeds presentation in Northampton on 17th March 2016

Project mentioned during discussions whilst demonstrating Fungicide Response Experiments at Cereals 2016, June 2016 and SRUC/AHDB Disease roadshows in Jan 2016

Project and spore data featured in ADAS Presentation at the Danish Agronomists' Conference on 23rd August 2016 ("Light leaf spot in the UK").

#### Press articles

- 11th Nov 2016, Light leaf spot disease risk high for most GB regions, says forecast.  
[https://www.farminguk.com/news/Light-leaf-spot-disease-risk-high-for-most-GB-regions-says-forecast\\_44782.html](https://www.farminguk.com/news/Light-leaf-spot-disease-risk-high-for-most-GB-regions-says-forecast_44782.html)
- 7th Oct 2016, Map: Mild autumn sparks oilseed rape phoma fears.  
<http://www.fwi.co.uk/arable/map-mild-autumn-sparks-oilseed-rape-phoma-fears.htm>
- 16th May 2016, iOSR grower group assesses latest thinking.  
<https://www.fginsight.com/news/news/iosr-grower-group-assesses-latest-thinking-12163>
- 2nd February 2016, Oilseed rape growers put on light leaf spot disease alert.  
<http://www.fwi.co.uk/arable/oilseed-rape-growers-put-on-light-leaf-spot-disease-alert.htm>
- 5th January 2016, Keeping ahead of light leaf spot.  
<https://www.fginsight.com/news/keeping-ahead-of-light-leaf-spot-8929>
- 5th November 2015, High risk of light leaf spot, says forecast,  
<http://www.nfuonline.com/sectors/crops/crops-news/high-risk-of-light-leaf-spot-says-forecast/>
- 30th October 2015, Why is phoma monitoring so essential this autumn?  
<https://www.fginsight.com/news/why-is-phoma-monitoring-so-essential-this-autumn-7282>

## Other

2015/16 light leaf spot forecast and spring update

2015/16 phoma forecast

CPM Theory to field, September 2015

## **5. Discussion**

The main aim of the project was to use a mixture of spore release data, crop disease data and weather data to effectively derive a date-driven forecast to inform growers that the onset of the annual LLS epidemic was imminent. A similar date-driven forecast developed previously for the onset of the phoma leaf spot epidemic (Evans *et al.* 2006: 2008), had proven popular with growers and their advisors and had helped growers target fungicide applications. The project aimed to revisit work previously published on environmental parameters (temperature and debris wetness) that drive the formation of apothecia, the subsequent release of ascospores and resultant infection cycles (Gilles *et al.*, 2000; 2001b; Gilles, Fitt & Jeger, 2001) and to tie these in with spore release patterns and field observations of disease onset to form a useable model for LLS. During the project, it was demonstrated that tracking fungal development and disease progress within a crop would be theoretically possible, as shown above (Section 4.1.2.). However, the development of a workable date-driven model for LLS under UK field conditions was not possible. Unlike the monocyclic disease phoma leaf spot and stem canker (*Leptosphaeria maculans*), the polycyclic nature of the LLS (*P. brassicae*) means ascospore production is a continual process from late spring, through the summer, so there is effectively no “start date” to the epidemic. Since ascospores are arriving in the crop from emergence onwards.

Previous research had reported that, in the UK, the highest number of the ascospores of *P. brassicae* was recorded in April and May (McCartney & Lacey, 1990) or in June and July (Gilles *et al.*, 2001a) but, consistent with the results from the current study, these earlier studies also detected spores of the pathogen in UK air samples in the autumn (Welham *et al.*, 2004). A more recent publication again reported ascospores were collected during the autumn, but sampling only began in September (Karolewski *et al.*, 2012). However, close examination of the data for UK spore capture in 2006 and 2007 (Figure 2; Karolewski *et al.*, 2012) indicates that significant amounts of *P. brassicae* DNA were collected on 1<sup>st</sup> of September which suggests ascospore release began prior to this date. The current study appears to be the first study where spore trapping was done throughout the whole of the summer and autumn period and that also analysed data from some earlier years that had not been previously described. Early analysis of the data available after year 1 of the project indicated spore production began much earlier than September and that large quantities of spores were often found around harvest time (which led to the publication of the Headline “OSR emerges into a 'blitz' of disease” in a CPM article at the time). This was seen for early and mid-August 2009 for Aberdeen (Figure 10) and Edinburgh (Figure 9)

data and early August in seasons 2012 (Figure 12) and 2013 (Figure 13) at Edinburgh, respectively. The most significant result from the spore trapping work done during the project was seen during the second year of sampling when a spore trap was operated throughout the spring through to late autumn on the roof of the North Building at Rothamsted (Figure 8a). The data indicated that *P. brassicae* spores were caught on the first day of sampling on 29<sup>th</sup> May and that release continued throughout the summer, peaking during August. Also of note is that, for the majority of datasets analysed in this study, major production of ascospores appears to finish by late October or in some seasons early November. The main conclusion from this is that ascospores appear to be released right through the summer and therefore could potentially cause infections as soon as the new crop emerges in late August/early September. So, in effect, we understand all processes of the epidemic, but the spore trapping indicates that, unlike for the monocyclic phoma epidemic model, for LLS, there is effectively no “starting date” since spores are produced throughout the summer and autumn period.

The lack of a start date for apothecial maturation and subsequent ascospore release is compounded by the polycyclic nature of the *P. brassicae* epidemic. Crops are subject to different risks of infection depending on proximity and distance to infected stubble of a previous crop field and previous reports suggest that initial ascospore infections are quite rare and are randomly distributed at the field level (Evans *et al.* 2003). Secondary conidial infections then occur locally through rain-splash (Fatemi & Fitt, 1983), which eventually results in patches of light leaf spot at the field level, since distribution becomes aggregated (Evans *et al.*, 2003). With initial ascospore infections presumably happening all the time through the autumn period and accounting for canopy induced micro-climate effects, the relative development and maturation of the pathogen on the plant leads to different latent periods and subsequent secondary infection from those conidia. The authors suggest that the situation at the field scale becomes “chaotic” (Figure 20) and the epidemic is therefore impossible to model due to differential time for disease “cycles” to occur.

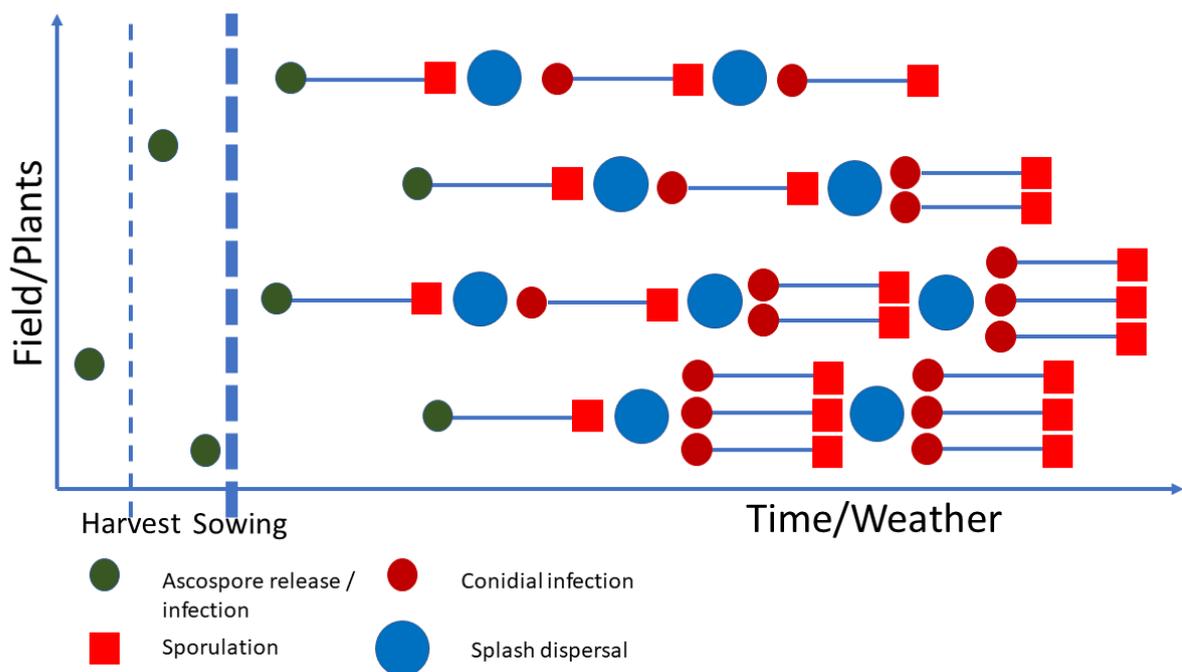


Figure 20. Schematic showing a proposed “chaotic” development of a light leaf spot epidemic on winter oilseed rape in the UK in late autumn, early winter.

The results of the study raise some interesting questions. The main one of which is, with seemingly unlimited ascospore inoculum from emergence, why are symptoms of LLS often not seen until mid to late winter? The long symptomless phase associated with LLS has been reported widely ever since the first paper describing the pathogen (Rawlinson *et al.*, 1978), but a definitive answer has not as yet been proven. The answer may be a variety of abiotic and biotic interactions with factors such as cuticle/wax thickness/topography in different varieties and at different growth stages of the plant (Boys *et al.*, 2007; Koch *et al.*, 2005) or specific weather events, such as the need for a frost event causing damage to the cuticle (Baierl *et al.*, 2002). *P. brassicae* is peculiar in that there appears to be subtle interactions between the pathogen, the *B. napus* host and the immediate environment. For example, Boys *et al.* (2007) highlight the “stealth-like” interaction between the *P. brassicae* pathogen and the *B. napus* host early in the interaction, where the pathogen grows biotrophically between the cuticle and the host epidermal cells (Boys *et al.* 2007; 2012). Boys *et al.* (2007) initially reported that a hypersensitive response was seen for some *B. napus* varieties bred using R gene “Imola” resistance and later reported that, whereas pure biotrophic interactions are usually killed by cell death, qPCR and electron microscopy of infected leaf material indicated that in the case of the hemibiotrophic/necrotrophic pathogen *P. brassicae*, pathogen growth was only stopped or delayed (Boys *et al.*, 2012). They also indicated that this suggested that R gene-mediated resistance in this case was fungistatic rather than fungitoxic for 13-36 days post inoculation. Boys *et al.* (2012) also concluded that most resistance conferred in current winter oilseed rape varieties was minor-gene mediated and that also, in crops, disease escape and

tolerance are also observed. It is clear that there are many factors involved in the *B. napus* host - *P. brassicae* pathogen - environment interaction that are not clear and require further investigation.

An investigation into the relationship between weather events (i.e. rainfall) and ascospore release was inconclusive. This was mainly because work from the latter years' results (and some of the historic spore samples analysed for the first time) indicated that in most seasons, the spore sampling began too late and that the main peak of ascospore release had already happened (June/July, as reported by Gilles *et al.*, [2001b]). By September, only the tail end of the release was being captured. Also, there was no correlation between the amount of rain and the number of spores released within a set period. Plotting accumulated rainfall against accumulated total spore release produced interesting results that indicated an apparent negative relationship between rainfall and spore release. Rainfall (debris wetness) was obviously required for continued maturation of apothecia. However, it was observed that when there was hardly any rain, many spores were released. Conversely, when there was much rain, spore release stopped. This is explained by the observation that many airborne particles (dust, spores, pollen) are washed from air during heavy rain events (Jonathan West, Pers comm.). These observations are in general agreement with the first report on the aerobiology of *P. brassicae* ascospores by McCartrney & Lacey (1990), who reported that ascospore release was associated with rain, but most ascospores are released after rainfall when the crop debris bearing apothecia were wet.

There have been previous reports that light leaf spot epidemics are more severe in earlier sown crops, with later crops out of the ground later than peak ascospore production (Welham *et al.*, 2004). This was recently observed in the field in the UK during the 2016-17 season when many early sown crops suffered significant LLS infection in contrast to later sown crops (Faye Ritchie, Pers comm.). The results from this project indicated that peak ascospore production can occur from before August to October, depending on the season, with little or no spore release from mid-October onwards. This suggests that ascospore infection later in the season is not a driving factor for epidemic development (see Figure 20) and that the potential for more cycles of sporulation and rain-splash on early sown infected crops are the main concern. In any season, in addition to utilising varietal resistance, additional fungicidal control is key. Given that AHDB fungicide performance trials regularly achieve between 40% to 60% control with fungicides, it is clear that two spray programmes on susceptible varieties are insufficient to control the disease and alternative strategies are required. There is evidence from previous studies to suggest that earlier fungicide applications e.g. October are less effective at controlling light leaf spot than those applied in November and December (Faye Ritchie, Pers Comm.). Typically, a single fungicide application for light leaf spot control is recommended, usually in late autumn/early winter, however, whether two sprays would offer a better option, particularly in years where the epidemic starts early, remains to be determined. Product choice is key, since insensitivity has recently been reported

with the identification of two mutations within the sterol 14 $\alpha$ -demethylase gene of some isolates of *P. brassicae* that confer decreased azole sensitivity (Carter *et al.*, 2014). As detailed in the latest AHDB publication on oilseed rape fungicide performance trial work (AHDB, 2016), field control of light leaf spot requires a careful balance of cultural practise (for example, sowing later), varietal selection and careful fungicide choice and timing of application. With this regard, the current oilseed rape decision tools (LLS risk forecast and the phoma leaf spot forecast) remain key components for the control of LLS and phoma leaf spot/stem canker.

In the monitoring series, the foliar epidemic was tracked until the end of February. There were differences in the levels of LLS observed on stems at the end of the season at the English sites, suggesting that the disease had continued to cycle in plots beyond the original monitoring period. There were differences between sites, however, the general trend was for more susceptible varieties to have more light leaf spot on stems. The exception was Yorkshire, where the most resistant variety, Cuillin, had the highest levels of light leaf spot. Results from Scotland also suggested that varietal resistance in Cuillin had been eroded. This suggests that there may be differences in light leaf spot populations by region and that this may also include their ability to infect different cultivars and supports the conclusions of other recent AHDB-funded studies (Klöppel *et al.*, 2015).

#### Suggestions for future work

Future research should focus on understanding this earlier start to the epidemic therefore monitoring spore production far earlier. All previous modelling work was done on susceptible variety Bristol, therefore the effect of varietal resistance on slowing the light leaf spot epidemic on more resistant varieties has not been considered. Spore trapping from April onwards combined with weekly monitoring of crops with different disease resistance ratings would help to pinpoint exactly when infection occurs and what weather criteria occurred prior to this.

A few small experiments have identified that using more resistant varieties can offer flexibility in fungicide timings and also protect yield, however, this has not been demonstrated on a large scale (Faye Ritchie, Pers Comm.). In addition, recent seasons have seen a large difference in LLS severity between earlier sown and later sown crops, so it may be worth re-visiting the importance of sowing date, particularly with respect to newly introduced resistant varieties. Given that growers are being encouraged to use integrated approaches and the difficulty of controlling light leaf spot at present, this would be the next step to identifying strategies to improve light leaf spot control in UK crops.

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