Understanding *Pyrenopeziza brassicae* pathogen populations to improve control of light leaf spot in oilseed rape (*Brassica napus*)

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Abstract

This project aimed to improve understanding of host resistance against the light leaf spot pathogen *Pyrenopeziza brassicae* within oilseed rape (*Brassica napus*) by investigating factors contributing to disease development, studying specific host-pathogen interactions and by identifying quantitative trait loci (QTL) related to host genetic resistance.

Patterns of *P. brassicae* air-borne ascospore release in the whole year over three cropping seasons suggested two major periods of ascospore release: one in late autumn and a second one in early summer. Conversely, spores were absent in winter, but a small number of ascospores that were released in spring may be due to a sufficient number of apothecia produced in early spring. Optimal weather conditions for ascospore release were temperature (ranging between 15-17°C) and associated with rainfall. Next, controlled environment experiments using plants aged from one- to six-weeks-old suggested that four-weeks-old (with four to five true leaves) was the optimal age for disease development and a *P. brassicae* inoculum concentration of 10^5 spores/ml was optimal for inoculation without affecting comparable disease assessment between cultivars. Inconclusive results suggested that *P. brassicae* infection may affect host plant height in controlled environment, as plants inoculated with a greater *P. brassicae* inoculum concentration (10^5 spores/ml) were shorter than those inoculated with a smaller inoculum concentration (10^4 spores/ml). However, the findings could not be reproduced, suggesting that environmental conditions may have influenced the plant growth. Finally, greater light intensity promoted the development of light leaf spot symptoms in controlled environments compared to lower light intensity, suggesting light intensity is an important factor affecting the rate and severity of light leaf spot development.

Results from field experiments suggested that there are regional differences in *P. brassicae* pathogen races. Cultivars Poh Bolko, Castille and Yudal showed greater disease severity in Norwich, Norfolk than in Harpenden, Hertfordshire, suggesting *P. brassicae* pathogen races in Norwich were more virulent than those in Harpenden. However, cultivars Campus, Catana, Kielder and Acacia remained symptomless across all sites tested, suggesting stable resistances.

Similarly, disease severity was greater in Hereford, Herefordshire than in Huntingdon, Cambridgeshire for both moderately resistant cultivars Aquila and Flamingo, possibly due to increased rainfall in Hereford, compared to Huntingdon. Next, inoculation of single-spore *P. brassicae* isolates in glasshouse experiments suggested stable disease resistance in cultivar Imola, breeding line Q02 and line NPZ 06/22, while cultivars Charger, Bristol, Yudal and Barbados were susceptible to most of the isolates tested. Additionally, 24 single-spore *P. brassicae* isolates from six European countries, including the UK, were tested and showed differences in virulence between those countries; however, only few isolates per country were tested, so further work is needed to verify these findings. Next, *P. brassicae* was able to cross-infect between oilseed rape and kale, but oilseed rape-derived *P. brassicae* inoculum was more virulent than kale-derived *P. brassicae* inoculum. Finally, results showed that *P. brassicae* was able to continuously re-sporulate up to four times after removing the previous asexual sporulation until the leaf tissue fully senesced; investigating the inoculum produced by each re-sporulation cycle could provide further insight into the impact of light leaf spot secondary infections.

Segregation of resistance against *P. brassicae* was found within the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in two glasshouse experiments using two different *P. brassicae* populations (one from England and one from Scotland). Composite interval mapping analysis for eight quantitative traits identified 21 QTL distributed across nine linkage groups. Of these, QTL on linkage groups A02, A06, C01 and C02 overlapped with resistance QTL for control of two different diseases (phoma stem canker and light leaf spot) found in previous studies that used the same DY DH mapping population, suggesting that these QTL may be stable. Qualitative assessment of black necrotic flecking in different environments, on different cultivars and on different areas of the leaf suggested that this phenotype may be associated with disease pressure and may be isolate-specific, but its underlying mechanisms remain unknown.

This project improved understanding of host resistance against *P. brassicae* within oilseed rape by confirming existing knowledge and presenting novel findings that can be applied for light leaf spot disease management.

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List of Publications, Presentations and Events

Publications

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Presentations

Internal

"Identification of host resistance to combat the increasing threat of light leaf spot in oilseed rape" – University of Hertfordshire Life and Medical Science Research conference, Hertfordshire, UK, June 2022 - Talk

"Mush-room for improvement: studying *Pyrenopeziza brassicae* pathogen races to manage light leaf spot in oilseed rape" – University of Hertfordshire Postgraduate Research Student conference 2023, Hertfordshire, UK, June 2023 – Flash-talk (Appendix B.4)

National

"Effects of plant age and inoculum concentration on light leaf spot disease phenotypes on oilseed rape" – Agri-Food Charities Partnership conference 2021, online, June 2021 – Conference paper (Appendix A)

"Effects of plant age and inoculum concentration on light leaf spot disease phenotypes on oilseed rape" – Agri-Food Charities Partnership student forum 2022, Cranfield, UK, March 2022 – Poster (Appendix B.2)

"Effects of plant age and inoculum concentration on light leaf spot disease phenotypes on oilseed rape" – International Organization for Biological and Integrated Control – Integrated Control in Oilseed Crops, online, May 2022 – Flash-talk (Appendix B.3)

"Effects of plant age and inoculum concentration on light leaf spot disease phenotypes on oilseed rape" – Cereals 2022, Hertfordshire, UK, June 2022 - Poster

"Identification of host resistance to combat the increasing threat of light leaf spot in oilseed rape" – UK *Brassica* Research Community/Oilseed Rape Genetic

Improvement Network StakeHolders Meeting 2022, Norwich, UK, October 2022 - Talk

“Investigating *Pyrenopeziza brassicae* pathogen races to combat light leaf spot in winter oilseed rape” – UK *Brassica* Research Community/Oilseed Rape Genetic Improvement Network StakeHolders Meeting 2023, Norwich, UK, October 2023 – Talk

“Mush-room for improvement: studying *Pyrenopeziza brassicae* pathogen races to manage light leaf spot in oilseed rape” – British Crop Production Council Diseases Review, Warwick, UK, October 2023 – Flash-talk

International

“Understanding host resistance to improve control of light leaf spot on winter oilseed rape in the UK” – British Society of Plant Pathology conference "Our Plants, Our Future" 2021, Birmingham, UK, December 2021 - Poster (Appendix B.1)

“Understanding *Pyrenopeziza brassicae* populations for effective control of light leaf spot in winter oilseed rape” – 12th International Congress of Plant Pathology 2023, Lyon, France, August 2023 – Abstract and Poster (Appendix B.5 and Appendix B.6)

“Investigating *Pyrenopeziza brassicae* pathogen races to combat light leaf spot in winter oilseed rape” – 16th International Rapeseed Congress 2023, Sydney, Australia, September 2023 – Talk (Appendix B.7)

Events

Internal

University of Hertfordshire Postgraduate Research Student conference 2020, Hertfordshire, UK, January 2020

University of Hertfordshire Postgraduate Research Student conference 2021, online, February 2021

University of Hertfordshire Life and Medical Sciences research conference 2021, online, June 2021

University of Hertfordshire Postgraduate Research Student conference 2022, Hertfordshire, UK, May 2022

University of Hertfordshire Life and Medical Sciences research conference 2022, Hertfordshire, UK, June 2022 (Appendix C.2)

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University of Hertfordshire Life and Medical Sciences research conference 2023, Hertfordshire, UK, June 2023

National

Cereals 2019, Lincolnshire, UK, June 2019

Agri-Food Charities Partnership conference 2021, online, June 2021

Cereals 2021, Lincolnshire, UK, June 2021

Agri-Food Charities Partnership forum 2021, Gloucester, UK, November 2021

Agri-Food Charities Partnership student forum 2022, Cranfield, UK, March 2022

International Organization for Biological and Integrated Control – Integrated Control in Oilseed Crops, online, May 2022

Cereals 2022, Hertfordshire, UK, June 2022

UK *Brassica* Research Community / Oilseed Rape Genetic Improvement Network StakeHolders Meeting 2022, Norwich, UK, October 2022 (Appendix C.3)

Agri-Food Charities Partnership/NIAB Joint Event, Cambridge, UK, February 2023

UK *Brassica* Research Community / Oilseed Rape Genetic Improvement Network StakeHolders Meeting 2023, Warwick, UK, October 2023

British Crop Production Council Disease Review 2023, Cambridge, October 2023 (Appendix C.6)

Agri-Food Charities Partnership student forum 2024, Cambridge, UK, March 2024 (Appendix C.8)

International

British Society of Plant Pathology conference "Our Plants, Our Future" 2021, Birmingham, UK, December 2021 (Appendix C.1)

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Chapter 1 Introduction

1.1 Oilseed rape

1.1.1 Cultivation and significance

Oilseed rape (*Brassica napus* L.) is an arable crop of considerable economic importance. Global production of oilseed rape has significantly increased over the past few decades from 20 million tonnes in 1970 to nearly 100 million tonnes in 2022 (Figure 1.1) and it is currently the third largest source of vegetable oil, after soybean and palm (Parcell *et al*., 2018). Major producers are China, India, Canada, European Union and Australia (Carré and Pouzet, 2014). Two agronomically distinct groups of *B. napus*, winter and spring oilseed rape, are grown globally with the type sown based on climatic conditions. Winter oilseed rape is primarily grown in Europe (EU, UK and Ukraine) and Asia due to vernalisation requirements, whereas spring varieties that do not need vernalisation are cultivated in North America, Northern China, parts of Europe and Australia (Jankovska-Bortkevič *et al*., 2022; Zhang *et al*., 2014). Rapeseed production became established in the 1970s following the introduction of double low (00) cultivars with low erucic acid and low glucosinolates levels, making rapeseed oil viable for human consumption as an edible and widely used cooking oil (Abbadi and Leckband, 2011; Jankovska-Bortkevič *et al*., 2022). Rapeseed oil also has various industrial applications, such as in lubricants, cosmetics, detergents, jet fuels and biodiesel. Residues from rape oil production can then be used as valuable animal feed (rape meal) with high energy and high protein content. Furthermore, oilseed rape is used for crop rotation to improve soil properties and as a break crop with cereals for disease control (Jankovska-Bortkevič *et al*., 2022; Orlovius and Kirkby, 2003). Rapeseed is also being explored as a novel source of plant-based proteins for human consumption as an alternative to animal-derived products, in response to growing environmental concerns over animal protein production (van der Spiegel *et al*., 2013). Overall, oilseed rape production has been steadily increasing to meet rising demands for its many applications.

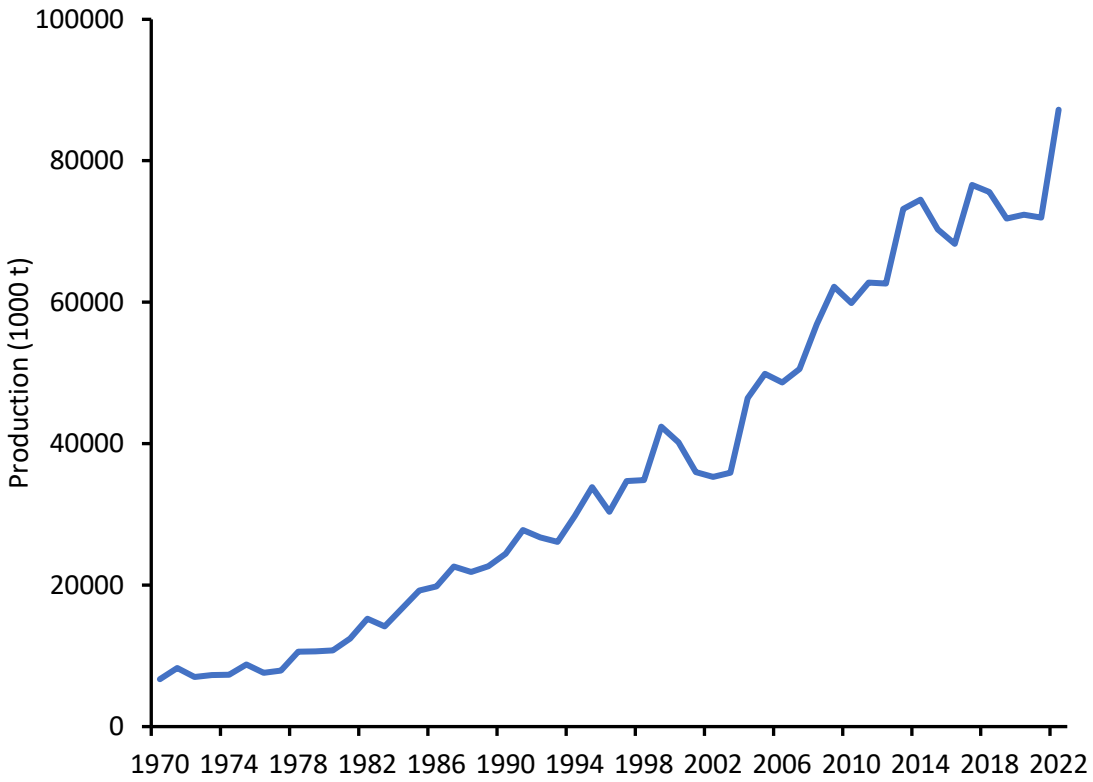

Figure 1.1: Global rapeseed production between 1970 and 2022.

Global rapeseed production has undergone substantial growth since the 1970s due to improvements in crop characteristics through selective breeding, leading to increased consumer demand (FAOSTAT; Accessed 16th April 2024).

In the UK, oilseed rape cultivation underwent significant growth from the 1970s and was maximum in 2012 with 755 thousand hectares (ha) grown. Production subsequently decreased by up to 50%, with only 307 thousand ha cultivated in 2021 (Figure 1.2). In 2013, the EU banned the usage of neonicotinoid seed treatments (EU Regulation No. 485/2013), which were previously the main method to control cabbage stem flea beetle (CSFB) infestations, over environmental concerns (Seimandi-Corda *et al*., 2024; Wood and Goulson, 2017). This ban, combined with CSFB populations developing widespread insensitivity to alternative insecticides such as pyrethroids (Willis *et al*., 2020), left farmers without efficient options to manage this major oilseed rape pest. As a result, oilseed rape cultivation decreased significantly after 2013, despite yield levels remaining relatively unchanged since the 1980s (Figure 1.2). Little improvement in commercial crop yields, despite regular introduction of better performing cultivars in variety trials, may be due to the combined effects of plant disease, increases in cropping frequency, poor selection and management of cultivars, and reduced nitrogen- and sulphur-use efficiency (AHDB, 2024d; Booth *et al*., 2005). Oilseed rape production value was maximum in 2011 at £1.1 billion and steadily decreased in the following decade down to £360 million in 2019. Prices recently increased again, however, with product value reaching up to £877 million in 2022 (Figure 1.3). Despite reduced UK rapeseed production, the demand for rapeseed-related products is increasing both domestically and globally, and in the past few years, the UK has been a net importer of rapeseed oilseed (USDA, 2022). Recent geopolitical events, namely Russia's invasion of Ukraine (a major rapeseed exporter) in 2022, negatively impacted UK imports, temporarily increasing domestic rapeseed prices; however, import levels are forecasted to return to standard values, and domestic rapeseed prices have been decreasing (AHDB, 2024b; USDA, 2022). Despite fluctuating production, oilseed rape remains the third most important arable crop in the UK only behind wheat and barley (Figure 1.4), establishing its economic significance in the UK farming industry.

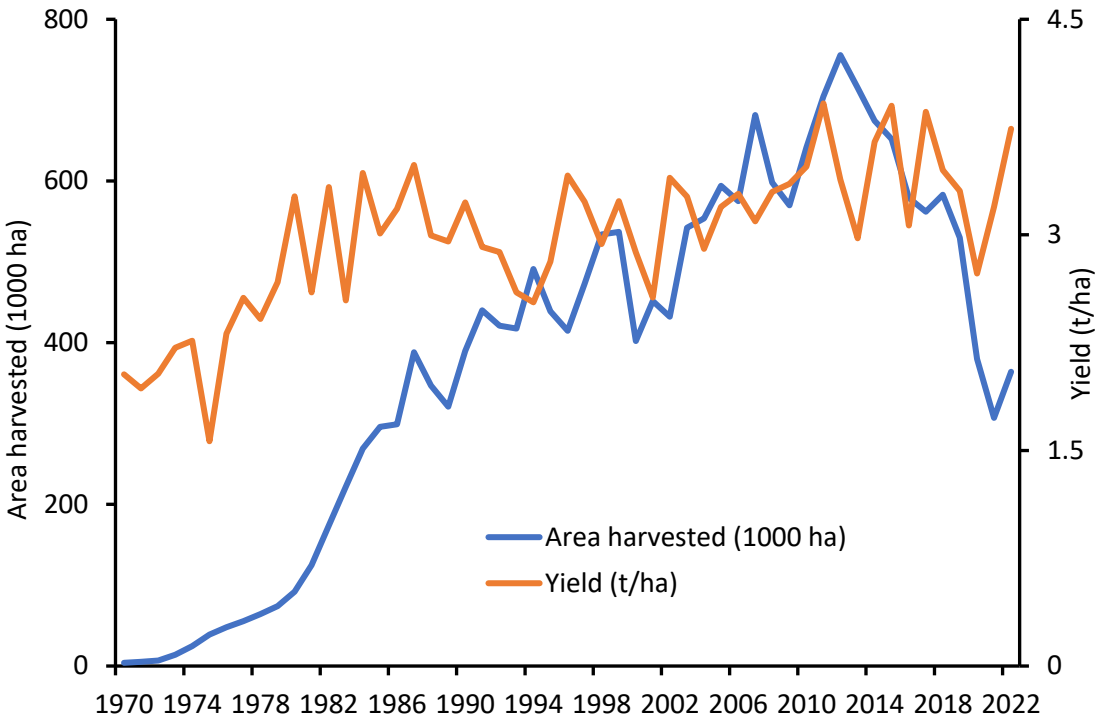

Figure 1.2: Oilseed rape area harvested and average yield in the United Kingdom between 1970 and 2022.

Oilseed rape cultivated area in the UK underwent substantial growth from the 1970s until c. 2013, when the EU ban on neonicotinoid insecicides, among others, drastically increased crop damage from cabbage stem flea beetle, contributing to reduced crop cultivation. Conversely, despite fluctuations in oilseed rape cultivation, yield has remained constant since the 1980s (FAOSTAT; Accessed 16th April 2024).

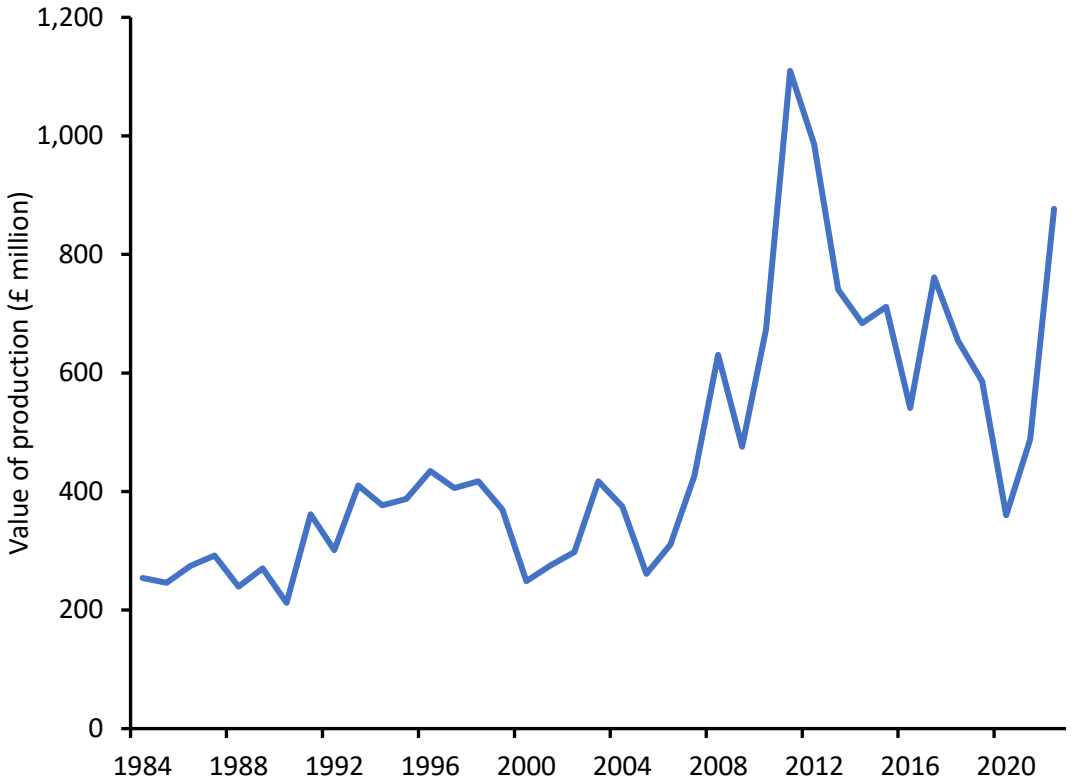

Figure 1.3: Value of oilseed rape production in the UK between 1984 and 2022.

Value of oilseed rape production (£ million) in the UK increased until c. 2013, when the EU ban on neonicotinoid insecticides, among others, greatly increased crop damage from cabbage stem flea beetle, contributing to reduced crop cultivation and value. Prices increased in 2021, partially due to reduced imports related to geopolitical conflicts, but were expected to decrease again subsequently (DEFRA; Accessed 16th April 2024).

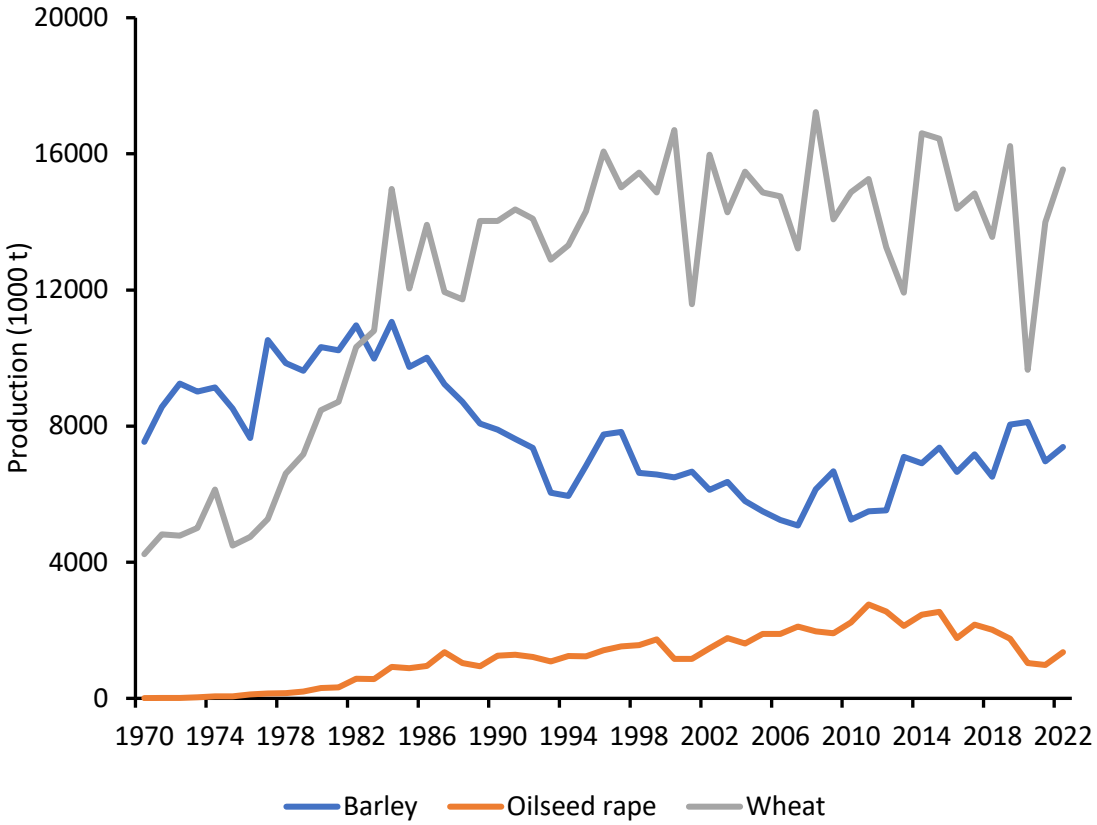

Figure 1.4: Production of three major arable crops (wheat, barley and oilseed rape) in the United Kingdom between 1970 and 2022.

Oilseed rape production has increased little over the past five decades compared to wheat and barley, but it remains the third most important arable crop in the UK (FAOSTAT; Accessed 16th April 2024).

1.1.2 Genetics of oilseed rape

Oilseed rape (*B. napus*, AACC genome, n = 19) is an allotetraploid crop plant belonging to the *Brassica* genus in the Brassicaeae (or Cruciferae) family, together with a number of commercial crop plants including oilseeds, mustards, root crops and vegetables, as well as the model plant *Arabidopsis thaliana* (Jankovska-Bortkevič *et al*., 2022). According to the 'Triangle of U' theory (Nagaharu, 1935), *B. napus* is thought to have originated from natural hybridization between two ancestral diploid progenitors, *Brassica rapa* (turnip, AA genome, n = 10) and *Brassica oleracea* (wild cabbage, kale, CC genome, n = 9). The allotetraploids *Brassica carinata* (Ethiopian mustard, BBCC genome, n = 17) and *Brassica juncea* (Indian mustard, AABB genome, n = 18) are similarly thought to have resulted from crosses between *B. oleracea* and *Brassica nigra* (black mustard, BB genome, n = 8) and *B. rapa* and *B nigra*, respectively (Kimber and McGregor, 1995; Nagaharu, 1935) (Figure 1.5).

Selective breeding of oilseed rape from the 1970s onwards produced the modern double low (00) cultivars with low erucic acid and low glucosinolate levels, starting with cultivar Tower developed in 1974 in Canada (Rapeseed Digest, 1974). In the UK, cultivar Jet Neuf with low erucic acid levels was introduced as a result of similar breeding efforts to improve oilseed genotypes, followed shortly by the first double low cultivar, Darmor. Hybrid cultivars were eventually introduced in the 1990s through improvements in breeding techniques (Booth *et al*., 2005). Over time, rigorous and constant selection for specific traits led to extensive cross-breeding of cultivars worldwide, resulting in considerable genetic bottlenecks, severely reducing the genetic diversity of modern cultivars (Obermeier *et al*., 2022; Snowdon and Iniguez Luy, 2012). This loss of genetic diversity presents an important obstacle for the breeding of new oilseed rape cultivars that remain tolerant to pathogens while also maintaining desirable agronomic traits. Studies have been done to investigate and improve the genetic diversity in oilseed rape (Chen *et al*., 2020; Diers and Osborn, 1994; Elling *et al*., 2009; Lu *et al*., 2019), providing resources to help functionally characterize *B. napus* genotypes and expand breeding efforts. Introgression of compatible ancestral genotypes (secondary gene pools),

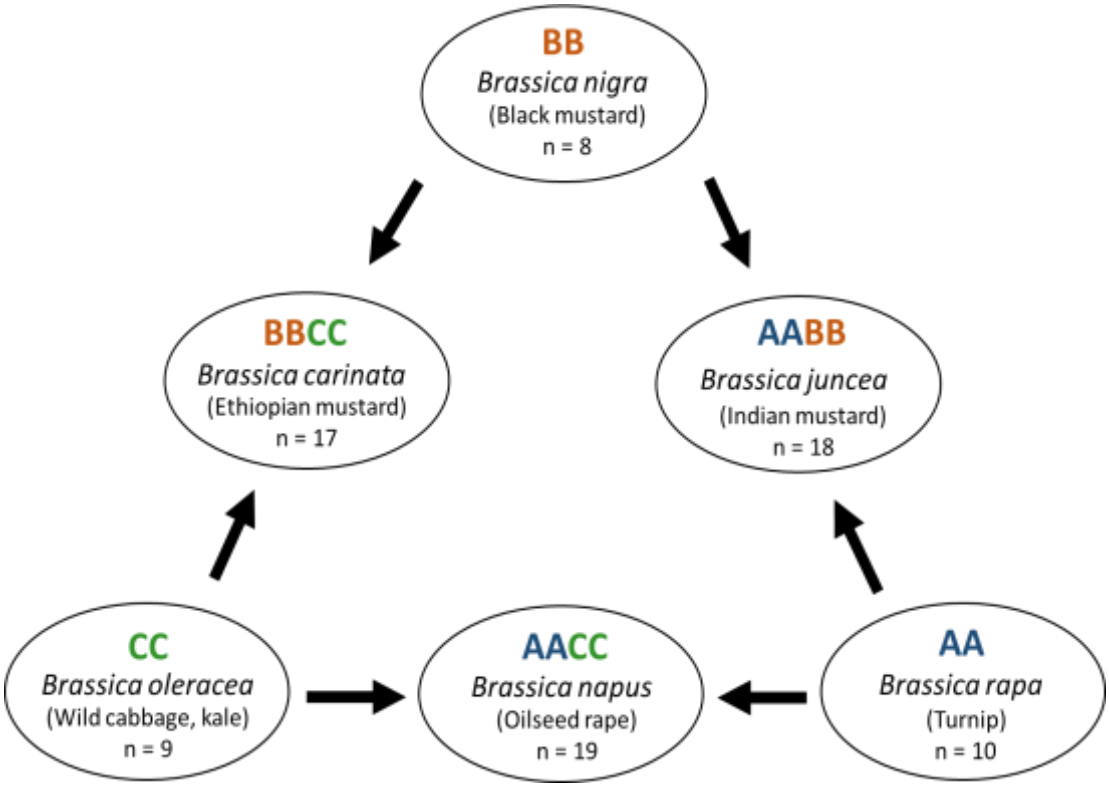

Figure 1.5: 'Triangle of U' explaining the formation of the *Brassica napus* (oilseed rape) genome from *Brassica oleracea* and *Brassica rapa*.

The Triangle of U theory illustrates the evolutionary relationships between six Brassica species. *Brassica napus* (oilseed rape, AACC) originated from natural hybridization between *Brassica rapa* (turnip, AA) and *Brassica oleracea* (wild cabbage, kale, CC). *Brassica carinata* (Ethiopian mustard, BBCC) originated from the fusion between *Brassica nigra* (black mustard, BB) and *B. oleracea*. *Brassica juncea* (Indian mustard, AABB) originated from the fusion between *B. rapa* and *B. nigra* (adapted from Nagaharu, 1935).

namely of *B. rapa* and *B. oleracea*, into *B. napus* may enable introduction of new sources of resistance (Karandeni Dewage *et al*., 2022). Available genome sequences of *B. napus* (Chalhoub *et al*., 2014) and its ancestral species, *B. rapa* (The *Brassica rapa* Genome Sequencing Project Consortium *et al*., 2011) and *B. oleracea* (Liu *et al*., 2014), can also be analysed to identify genes of interest, whether it be agronomic traits or resistance to pathogens. The OREGIN network (oilseed rape genetic improvement network) then provides a number of genetic linkage maps from a reference doubled haploid *B. napus* population that can be used to screen for agronomically important characters for pre-breeding material (https://www.herts.ac.uk/oregin/about-oregin).

1.2 Light leaf spot disease

1.2.1 *Pyrenopeziza brassicae* taxonomy

Pyrenopeziza brassicae Sutton and Rawlinson (anamorph *Cylindrosporium concentricum* Grev.) is a haploid, heterothallic fungus within the class Leotiomycetes and phylum Ascomycota. It is the causative pathogen of light leaf spot in oilseed rape and vegetable brassicas (Karandeni Dewage *et al.*, 2018; Veys *et al*., 2019). The anamorph, *Cylindrosporium concentricum*, was originally described by Greville (1823) based on the concentric ring-like pattern of the asexual sporulation. *P. brassicae* apothecia were first identified in culture by Hickman *et al*. (1955), followed by the first report of disease occurrence under natural conditions (Staunton and Kavanagh, 1966), until *P. brassicae* was formally described as the teleomorph *of C. concentricum* by Rawlinson *et al*. (1978). *P. brassicae* also has a close evolutionary relationship with *Rhynchosporium commune*, causative agent of leaf blotch disease on barley (King *et al*., 2013; 2015). Being heterothallic (sexual reproduction occurring only between strains of opposite mating types), *P. brassicae* has two mating types originally described by Ilott *et al*. (1984) and designated as *MAT 1-1* and *MAT 1-2* by Courtice and Ingram (1987) according to the nomenclature of Yoder *et al*. (1986). They were then later reclassified as *MAT-2* and *MAT-1* according to ascomycete mating-type gene nomenclature (Singh and Ashby, 1998; 1999). Studies by Majer *et al*. (1998) suggested the frequent occurrence of sexual

reproduction and the potential for high genetic diversity among field *P. brassicae* isolates.

As a hemibiotrophic fungus, *P. brassicae* has both endophytic (pathogen requiring living host to survive) and necrotrophic (pathogen kills host cells and feeds on dead matter) phases in its life cycle (Boys *et al*., 2007; Veys *et al*., 2019). As an ascomycete fungus, *P. brassicae* forms discoid ascocarps (fruiting bodies) called apothecia, consisting of asci interspersed with hyaline, filiform, branched and septate paraphyses. Upon maturation, these structures develop an ostiolar opening that further expands, eventually releasing wind-dispersed ascospores (sexual spores) into the environment (Cheah *et al*., 1980; Gilles *et al*., 2001a). In the asexual phase, *P. brassicae* produces white subcuticular acervuli releasing rain-splash-dispersed conidia (asexual spores) (Gilles *et al*., 2001b) (Figure 1.6).

1.2.2 Disease epidemiology

Light leaf spot, caused by *P. brassicae*, is an economically damaging fungal disease of vegetable brassicas, including oilseed rape. In Europe, light leaf spot epidemics have been reported in France since 1978 (Pilet *et al*., 1998b), Germany since the 1980s, although it is considered less damaging there (Karolewski *et al*., 2006; Pilet *et al*., 1998b), Poland since the 1990s following mild, wet winters (Karolewski, 1999; Karolewski *et al*., 2006) and Ireland since 1964, before which the disease was considered to be of minor economic significance (Staunton, 1967). Globally, light leaf spot epidemics first occurred in 1978 following the detection of the teleomorph in New Zealand (Cheah *et al*., 1980) and more recently, light leaf spot occurrence was reported in Oregon, USA, leading to the discovery of a phylogenetically distinct lineage of *P. brassicae* that is currently unique to North America (Carmody *et al*., 2020).

In the UK, the anamorph of *P. brassicae*, *C. concentricum*, was first described in the 1820s (Greville, 1823), but the teleomorph was not documented until 1978 (Rawlinson *et al*., 1978), and the first major UK epidemic was recorded in 1974 (Rawlinson *et al*., 1978; Simons and Skidmore, 1988). Previously, light leaf spot was thought to be a problem primarily in Scotland due to its cold and

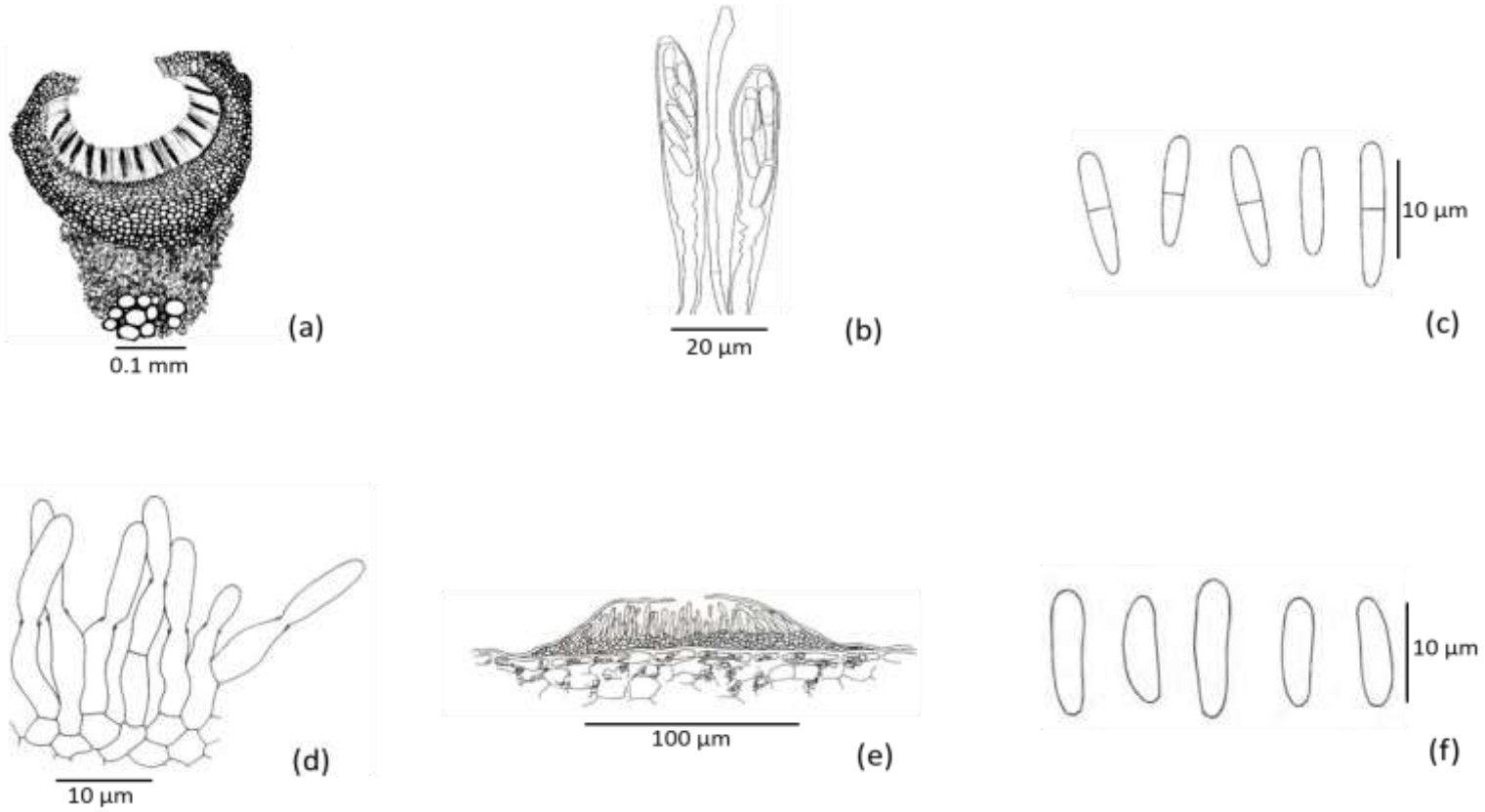

Figure 1.6: Sexual and asexual structures of *Pyrenopeziza brassicae*.

The sexual form of *P. brassicae* consists of (a) discoid apothecia, formed by (b) asci with a conical apex and gradually tapered towards the base, which contain (c) ascospores that are uni- or non-septate, hyaline, straight or slightly curved, with obtuse ends (9.6-14.4 x 2.4-3.1 µm). The asexual form of *P. brassicae* consists of (d) conidiophores that cluster together to form (e) white, subcuticular acervuli, releasing (f) conidia that are hyaline, cylindrical and septate (10-16 x 2-3 µm). Adapted from Karandeni Dewage (2019), Cheah *et al*. (1980) (a), Rawlinson *et al*. (1978) (b-c), Sutton (1977) (e-f).

wet climate favouring disease development, but in recent years, the disease has spread to England as well (Boys *et al*., 2007). Light leaf spot has since surpassed phoma stem canker (caused by two closely related pathogen species, *Leptosphaeria maculans* (Desm.) Ces. & de Not. and *L. biglobosa* Shoemaker & Brun) as the most economically damaging disease of winter oilseed rape in the UK (Karandeni Dewage *et al*., 2018). In England, annual yield losses are estimated to have increased from £18 million to £160 million between 2005 and 2014 (Karandeni Dewage *et al*., 2018) (Figure 1.7). Yield loss data for the last five years has become harder to acquire after the discontinuation of services like CropMonitor and Bayer's Spotcheck initiative to quantify the impact of light leaf spot on the UK farming industry, despite light leaf spot indicence remaining high in many regions of England and Scotland (Figure 1.8).

Disease severity can differ greatly depending on the cropping season, region and crop (Fitt *et al*., 1998a), but severe epidemics are generally favoured by wet weather, which encourages the production and dispersal of conidia in spring (Gilles *et al*., 2000a,b). High genetic diversity due to frequent sexual reproduction of *P. brassicae* has been suggested (Majer *et al*., 1998), promoting the sexual recombination of alleles, followed by newly combined alleles fixed in the population by asexual reproduction (Karandeni Dewage *et al*., 2018). This mixed reproduction system promoting genetic variation in *P. brassicae* populations may contribute to resistance breakdown and increased pathogenicity in the fungus, contributing to increasingly damaging light leaf spot epidemics.

1.2.3 Pathogen life cycle

P. brassicae possesses a complex, polycyclic life cycle involving a long symptomless phase in the winter, which makes pathogen detection in crops challenging (Figure 1.9). Epidemics usually begin in late summer and autumn with the release of airborne ascospores produced during the sexual phase by apothecia on diseased crop debris from the previous cropping season (Calderon *et al*., 2002; Karandeni Dewage *et al*., 2018). Ascospores deposit onto neighbouring plants through wind dispersion and are more infective than

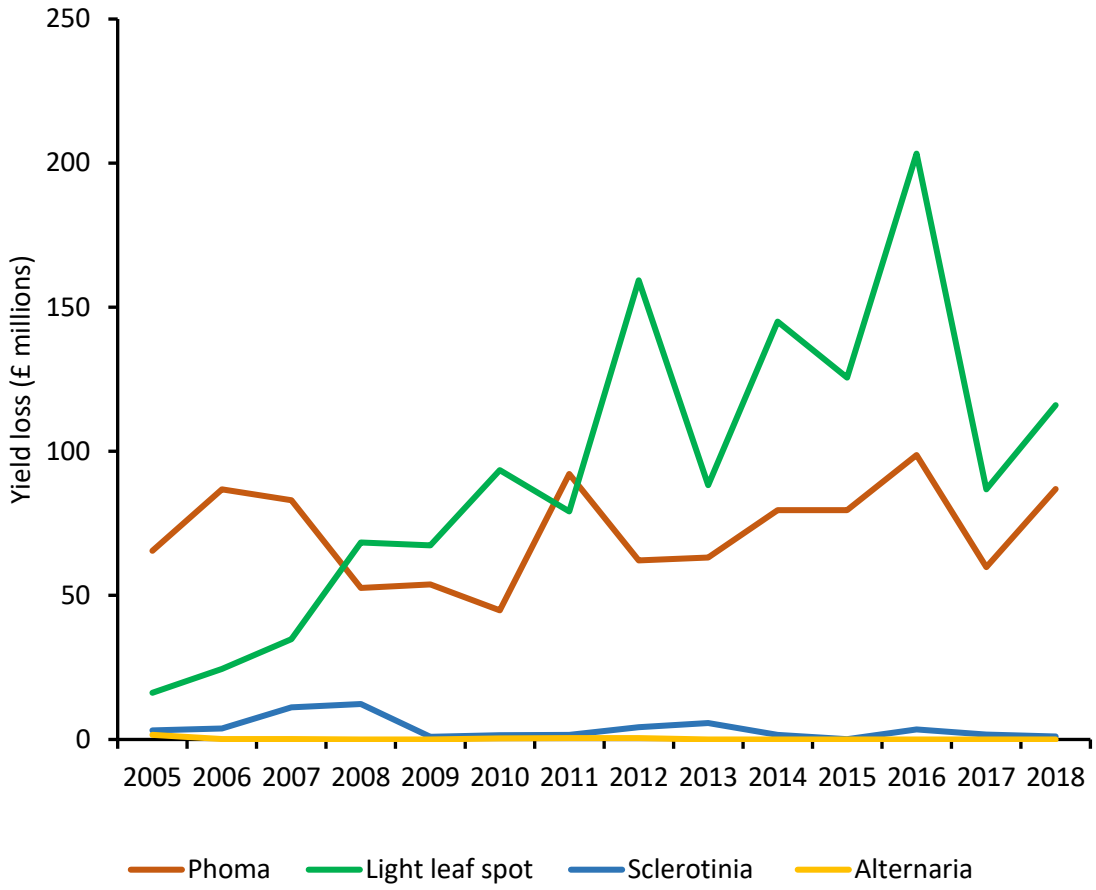

Figure 1.7: Yield losses caused by four major oilseed rape pathogens in England between 2005 and 2018.

Oilseed rape yield losses in England caused by four economically damaging fungal diseases – phoma stem canker, light leaf spot, sclerotinia stem rot and alternaria leaf spot. Since 2008, light leaf spot (*Pyrenopeziza brassicae*) has replaced phoma stem canker (*Lepsosphaeria maculans* and *L. biglobosa*) as the most economically damaging disease of winter oilseed rape in the UK, causing yield losses of up to £200 million per year (CropMonitor, 2018; data provided by Dr James Fortune).

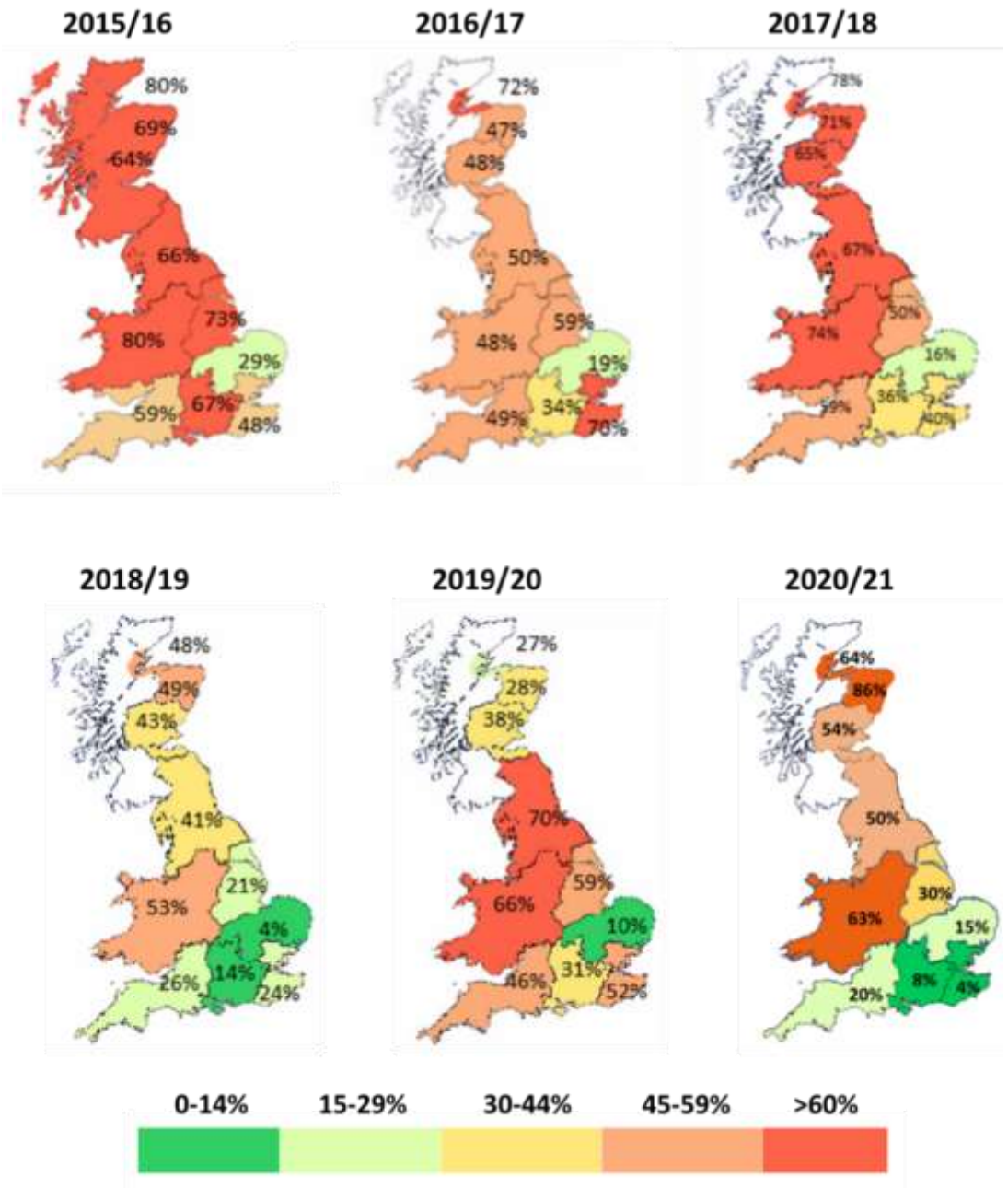

Figure 1.8: Light leaf spot disease incidence in England and Scotland over six cropping seasons between 2015 and 2021.

Regional light leaf spot forecast in England and Scotland over six cropping seasons derived from survey data; % of crops in an area estimated to have more than 25% of plants affected by light leaf spot in spring. Descriptions of each region shown are available on the AHDB website (2024c) (AHDB, 2024c; Accessed 18 April 2024).

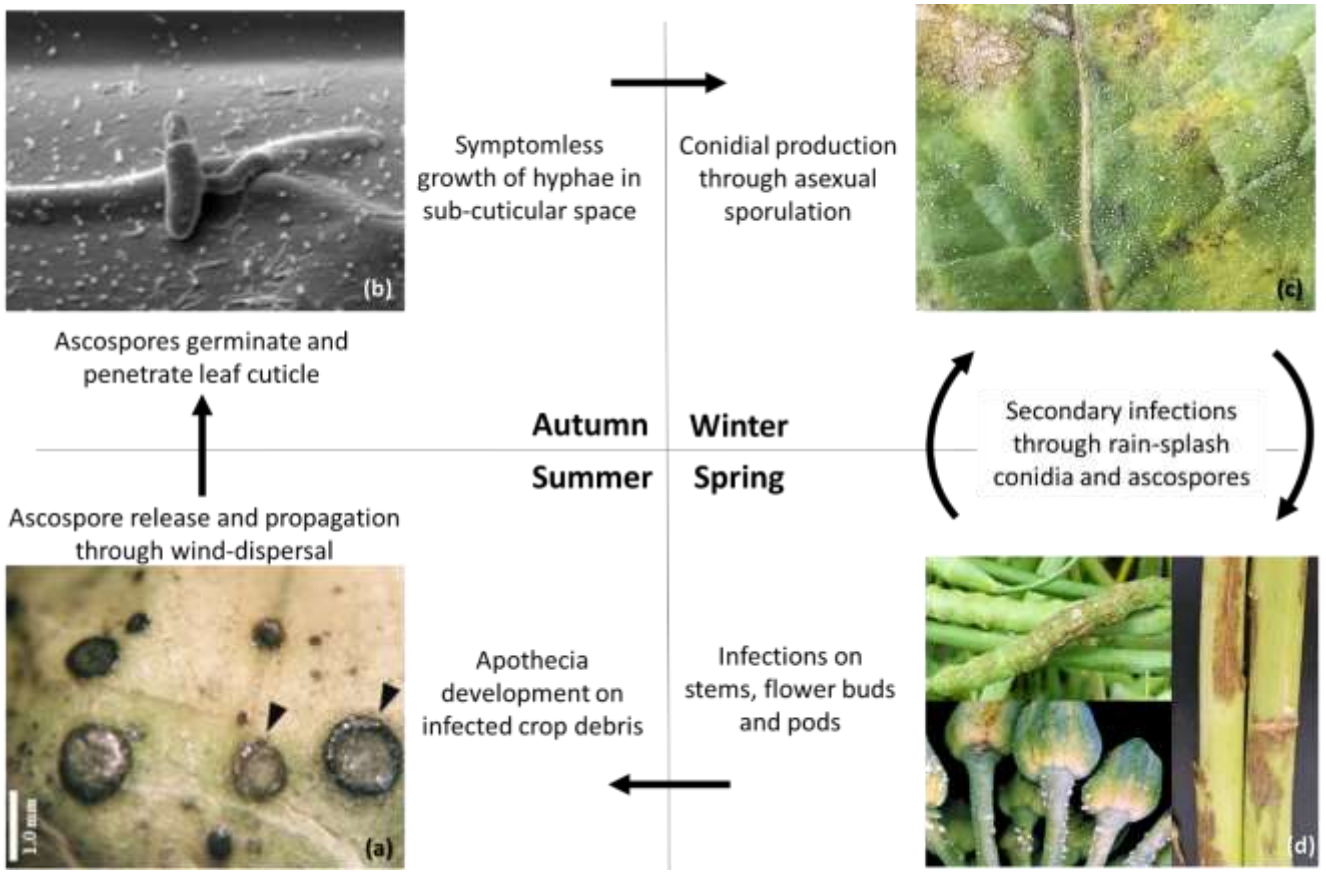

Figure 1.9: Life cycle of *Pyrenopeziza brassicae* (light leaf spot).

Apothecia on infected crop debris release airborne ascospores, which deposit onto nearby plants and penetrate the leaf cuticle, where fungal hyphae grow asymptomatically in the sub-cuticular space. Asexual sporulation in spring then leads to production of splash-dispersed conidia that cause secondary infection on other aerial parts of the plant. Sexual sporulation on senescent leaf debris in summer initiates the next season's epidemic. Photographs show (a) *P. brassicae* apothecia on Brussels sprout leaf (Karandeni Dewage *et al*., 2018), (b) germination and sub-cuticular growth of *P. brassicae* ascospore (Boys, 2009), (c) *P. brassicae* asexual sporulation, (d) Light leaf spot symptoms on pods (photo by Prof Yongju Huang), flower buds (photo by Chris Rawlinson) and stems.

conidia, despite their similar morphology (Gilles *et al*., 2001b). After landing on the leaf surface, ascospores germinate and directly penetrate the leaf cuticle, probably assisted by the use of extracellular cutinases (Li *et al*., 2003), rather than entering through the stomata (Boys *et al*., 2007). Upon penetration, *P. brassicae* forms a hypomycelium and fungal hyphae then continue to slowly grow endophytically between the cuticle and epidermal cells of the leaf. What follows is a long symptomless phase during which the pathogen continues growing through the leaf apoplast, yet remains undetectable to the naked eye (Ashby, 1997; Stotz *et al*., 2014). This long symptomless phase greatly contributes to the diagnostic challenges associated with *P. brassicae* infections, as the inability for early detection of the fungus in crops makes it difficult to assess timings for fungicide spray applications (Fitt *et al*., 1998a; Gilles *et al*., 2000a). Diagnosis of light leaf spot can be improved by incubating plant samples in polyethylene bags (to maintain continuous humidity) at low temperatures for 3 to 5 days to promote sporulation (Fitt *et al*., 1998b).

Upon sufficient accumulation of subcuticular fungal biomass, asexual sporulation then marks the end of the symptomless phase. What follows is the development of visible symptoms, such as white salt-like structures forming circular patterns, resulting from acervuli breaking through the leaf surface, as well as expansive lesions on the leaf lamina (Fitt *et al*., 1998a). Acervuli then release conidia which, though not as infective as ascospores (Gilles *et al*., 2000a), travel short distances by rain-splash dispersal and can cause secondary disease cycles (Boys *et al*., 2007; Evans *et al*., 2003; Gilles *et al*., 2000a). Spatial analyses of light leaf spot showed that autumn infection initiated by wind-dispersed ascospores is randomly distributed; light leaf spot aggregations later in the season, however, suggest localized spread of the disease from initial foci by rain-splashed conidia, creating a ‘patchy’ disease pattern in crops (Evans *et al*., 1999; Gilles *et al*., 2000a). Multiple such infection cycles can occur in the same season (Evans *et al*., 2003). New generations of ascospores produced on affected crop debris can contribute to secondary infections, which affect all parts of the plant, such as nearby leaves, flower buds, stems and seed pods (Gilles *et al*., 2001b; Karandeni Dewage *et al*., 2018). Through pathogen interference with plant hormonal pathways,

symptoms such as leaf distortion or stunting may also present (Ashby, 1997). Lesions that develop on oilseed rape crop stems do not generally affect yield, but infection of the pod in particular greatly contributes to extensive crop damage, as it leads to premature pod shattering and seed release, exacerbating yield losses (Karandeni Dewage *et al.*, 2018). Following harvest in summer, ascospores that survive on diseased crop debris then initiate the next epidemic in autumn.

1.3 Integrated disease management

Integrated disease management, or integrated pest management (IPM), comprises a range of available measures to reduce and control pathogen-induced disease in crops. The main principle of IPM is to encourage the implementation of natural preventative measures that cause the least disruption to existing agro-ecosystems (Kogan, 1998). This may involve reduced reliance on chemical controls like scheduled pesticide applications in favour of introducing modified, more environmentally conscious farming practices such as changes in planting or sowing schedules, crop rotation and usage of resistant cultivars (Radzan *et al*., 2009). Diversification of these control measures, rather than relying on a single management practice, is key in developing a long-term sustainable strategy for disease management (Krupinsky *et al*., 2002). As such, control of light leaf spot and other oilseed rape diseases ideally involves an integrated approach combining cultural, chemical and cultivar resistance control strategies (Bayer Crop Science UK, 2012; Krupinsky *et al*., 2002).

1.3.1 Disease forecasting

The usage of computer programmes to predict disease development is a common practice in agricultural settings. These modelling systems work based on the input of crop, yield, disease, weather and survey data for the evaluation of possible patterns in relation to disease progression. Numerous forecasting tools exist, including airborne inoculum sampling, integrated model-based forecasting frameworks and satellite-based technology (Newlands, 2018). Disease forecasting functions as an essential tool to aid in the correct implementation of IPM strategies.

Disease models as methods for forecasting light leaf spot incidence and severity on winter oilseed rape have been researched. Gilles *et al*. (2001d) developed a model to describe the effects of temperature and leaf wetness duration using controlled-environment experiments. Papastamati *et al*. (2002) monitored the daily progress of light leaf spot epidemics in crops over two cropping seasons. These data, combined with weather parameters, were used to generate an improved model. Disease survey data were also used by Welham *et al*. (2004) to construct statistical models to predict light leaf spot incidence in spring on regional and individual crop scales. Monitoring ascospore release using volumetric air samplers and subsequent spore quantification using microscopy and qPCR-based methods is another signficant forecasting tool for *P. brassicae* incidence over time (Calderon *et al*., 2002; Gilles *et al*., 2001b; McCartney and Lacey, 1990). The concentration of *P. brassicae* ascospore inoculum available in the region has been shown to influence light leaf spot epidemics (Papastamati *et al*., 2002). Generally, forecasting light leaf spot can prove challenging due to the polycyclic nature of the disease, which includes a long asymptomatic phase in autumn, making preliminary forecasts for disease incidence in spring difficult (Gilles *et al*., 2000a). Seasonal variations in weather parameters like temperature and rainfall can also affect the accuracy of predicted disease severity levels (Papastamati *et al*., 2002). Overall, a combination of a varied set of forecasting strategies is necessary to produce the most reliable prediction.

User-oriented, web-based forecasting tools are paramount to guide informed decision-making with regards to IPM strategies (Newlands, 2018). For light leaf spot, an interactive, crop-specific forecast tool was created by Rothamsted Research and AHDB (http://resources.rothamsted.ac.uk/light-leaf-spot-forecast), which used pod incidence data from the previous cropping season, as well as 30 year mean summer temperature data and 30 year mean rainfall data to produce an initial regional risk forecast. This preliminary forecast would be released in autumn and a final forecast would be updated in spring; however, the tool was discontinued in 2023. Parameters such as risk variability, fungicide applications in autumn affecting final light leaf spot levels in crop, and changes in cultivar resistance ratings were not accounted for in the forecast, leading to

inaccuracies in the predictions and causing AHDB to stop supporting the tool. Currently, no forecasting tools for light leaf spot are publicly available, despite increasing disease incidence and economic losses (Karandeni Dewage *et al*., 2018), highlighting the need of these services for farmers and growers to make informed decisions.

Disease modelling systems remain imperfect tools as a result of limited availability of reliable data, both due to the unpredictable nature of the environment, as well as incomplete understanding of pathogens and their diseases (Newlands, 2018). Although there may always be a level of unreliability in every forecast, the future potential of these systems is to provide reliable, cost-effective and accurate information to safeguard crop production. Further research and increased survey data availability will steadily improve the reliability of disease forecasts.

1.3.2 Cultural control

Cultural control strategies are some of the oldest practices used by growers in an effort to contain disease epidemics. They are not as effective as other strategies at suppressing disease outbreaks but rather they act to prevent more severe and longer-lasting epidemics and were mostly used before detailed knowledge of pathogen-crop and environment relationships was available (All, 2004). Cultural control practices are also implemented in IPM programmes against light leaf spot in oilseed rape, mostly to supplement more effective chemical controls. Known practices include destruction of crop debris, sowing date alterations and crop rotation. The primary purpose of these practices is to decrease infection by pathogen inoculum from the previous season.

P. brassicae ascospores on infected crop debris from harvested crops act as the primary inoculum for light leaf spot epidemics in autumn (Gilles *et al*., 2000a; 2001b). Crop residues from fields should therefore ideally be incorporated deep into the soil by ploughing or tilling to prevent sporulation. This greatly reduces the risk of contaminating new crops compared to direct drilling, as buried crop residues are less likely to release spores into the air (FRAG-UK, 2017; SAC, 2009). This includes volunteer oilseed rape, which can

continue to emerge for more than 10 years in subsequent crops (Jursík *et al*., 2023) and can act as a reservoir for *P. brassicae* to survive and infect newly planted crops (Walker *et al*., 2022). Soil cultivation practices are therefore important to remove these volunteer plants.

Changes in the sowing date serve to reduce the development of autumn foliar diseases like phoma leaf spot and light leaf spot, since ascospore release of *L. maculans, L. biglobosa* and *P. brassicae* mainly occurs in autumn (Evans *et al*., 2003; Gilles *et al*., 2001b; Huang *et al*., 2011). The sowing date for oilseed rape is typically in autumn between late August and throughout September, although it varies depending on geographic location; plantings occur earlier in northern areas due to cooler temperatures dictating the need for longer cropping seasons to maximize yield potential (Gilles *et al*., 2000a). Delaying the sowing date by about two weeks can reduce crop exposure to *P. brassicae* ascospores during maximum release periods, decreasing the risk of severe light leaf spot epidemics. This, however, may increase the risk of phoma stem canker (Welham *et al*., 2004). Informed choices need to be made about sowing dates depending on which is the predominant disease in a given area.

Oilseed rape is often used a a break crop in three- to four-year rotations with cereals (Jankovska-Bortkevič *et al*., 2022), although changes in farming practices have led to shorter rotations (Karandeni Dewage *et al*., 2018). Crop rotation is implemented to prevent continuous oilseed rape growth and reduce the amount of debris that produce *P. brassicae* inoculum, to reduce the probability of infection when the next crop is sown (Krupinsky *et a*l., 2002). Separation of crops to reduce the proximity to previous stubble is another recommended practice, as wind-dispersed ascospores can potentially travel long distances and infect new crops if they are planted too close to previous crops (FRAG-UK, 2017).

1.3.3 Chemical control

Chemical control is a fundamental part of oilseed rape disease control programmes. A range of commercially available fungicides with different modes of action exist. Among them, azoles are routinely applied for the treatment of

light leaf spot due to their low cost and broad range of effectiveness (King *et al*., 2021). Azoles work by targeting the sterol 14α-demethylase enzyme CYP51 involved in the biosynthetic pathway of ergosterol, a fundamental component of the fungal cytoplasmic membrane. Binding to this enzyme renders it inactive, preventing the conversion of the precursor lanosterol into ergosterol, which is essential to maintaining fungal membrane structure. This disruption of the pathway depletes ergosterol in the cell and simultaneously causes accumulation of toxic 14α-demethylated sterols, disrupting membrane integrity and ultimately resulting in fungal cell death (Kwok *et al*., 1993; Price *et al*., 2015).

Dosages and number of fungicide applications for light leaf spot vary depending on factors like cropping season, region and disease incidence. Fitt *et al*. (1998a) suggested a three-spray regime involving a first application during the symptomless phase in autumn before symptom appearance, a second application in winter to decrease the secondary pathogen spread and a third application in spring post-flowering to control pod infections (Karandeni Dewage *et al*., 2018). The most important fungicide application remains the first one made in autumn before symptom appearance (Figueroa *et al*., 1994; Fitt *et al*., 1998b; Gilles *et al*., 2000a); however, the inability to predict disease onset can lead to unnecessary fungicide applications that, upon extensive use, may impose selection on the pathogen population, promoting the appearance of fungicide-insensitive strains (Boys *et al*., 2007; Fitt *et al*., 1998b). ‘Resistance’ or insensitivity is defined as reduced disease control in commercial crop protection, resulting from acquired, heritable changes in the genetic constitution of a pathogen population (Brent *et al*., 2007a,b). Insensitivity development is a serious concern, as once a pathogen population has become desensitized to a specific mode of action, insensitivity can persist for many years. One example is the sustained insensitivity of *Sphaerotheca fuliginea*, the causative pathogen of powdery mildew in cucumbers, to benzimidazole fungicides in the Netherlands (Schepers, 1984). Another example is *Zymoseptoria tritici* (previously known as *Mycosphaerella graminicola*), the causative agent of septoria leaf blotch in wheat, with reports of insensitivity against quinone outside inhibitors (also called strobilurins) in the UK and other European

countries (McDonald *et al*., 2015; Torriani *et al*., 2008). Despite insensitivity development, however, a gradual recovery of sensitivity is possible through absence of fungicide selection, as was the case with *Phytophthora infestans*, cause of potato late blight, to phenylamide fungicides in Northern Ireland (Cooke *et al*., 2006). However, insensitivity often means that the fungicide can no longer be used, sometimes for many years, and depending on the choice of fungicides available for control of a specific disease, this can significantly affect disease management strategies.

Given the gravity of insensitivity development and its associated consequences, various anti-insensitivity strategies have been developed in response. The basis of any sound anti-insensitivity strategy involves good crop production, so the usage of resistant cultivars is encouraged, as it reduces the overall amount of fungicide applications needed (Dong *et al*., 2019). Fungicides should also be applied only when justified (favourable conditions for disease development, symptom observations, etc.), as reduced applications prevent unnecessary selection for insensitivity. Additionally, the usage of mixtures or alternations of fungicides with different modes of action is highly recommended, as these reduce selection and therefore reduce the likelihood of emergence of insensitive strains (Staub, 1991). Overall, the best way to minimise insensitivity development is to use less fungicides, through reduced applications or through co-formulations.

Appropriate fungicide management has proven increasingly challenging for the management of light leaf spot, as the selection of available fungicides has gradually decreased over time. The development of fungicides with new modes of action is very costly and time-consuming (Leadbeater, 2015); therefore, it is paramount to preserve currently available treatments, rather than rely on new treatments being released. In 2022, over 350 thousand ha of oilseed rape were grown and 1 million ha of oilseed rape were treated with fungicides. This means that, on average, each crop received 2.5 fungicide sprays, and four out of the five most common formulations were azoles (FERA, 2022). Azoles have been used to manage crop diseases of oilseed rape, including light leaf spot, for more than 40 years after prochloraz, the first azole, was introduced in the 1980s (King

et al., 2021). Since then, reports of azole insensitivity development in UK *P. brassicae* populations have emerged. Reduced sensitivity to methyl benzimidazole carbamate fungicides attributed to amino acid substitutions in the *β*-tubulin gene target has been reported (Carter *et al*., 2013). Reduced sensitivity to azoles attributed to mutations in the *CYP51* gene has been reported as well (Carter *et al*., 2014). More recently, azole insensitivity resulting from substitutions in *CYP51* was also found in *P. brassicae* populations in Ireland (Bucur *et al*., 2024). These reports of azole insensitivity development in selected *P. brassicae* strains, as well as the recent ban of some non-azole alternatives like Refinzar (EC, 2020; HSE, 2018) exacerbate problems due to the already reduced list of available fungicides. The limited availability of fungicides for the control light leaf spot, as well as the likelihood of further insensitivity development in the future, highlight the need for alternative non-chemical disease prevention strategies such as cultivar or host resistance.

1.4 Pathogen populations and host resistance

Cultivar or host resistance is an efficient method of disease control in many agricultural systems that employs resistance genes within the plant host to prevent or slow disease development (Carolan *et al*., 2017; Huang *et al.,* 2018). The usage of resistant cultivars provides a cost-effective and environmentally friendly alternative to control crop diseases, as it minimizes crop damage from machinery and reduces the need for fungicide applications (Gururani *et al*., 2012). There are two types of host resistance: major (*R*) gene-mediated qualitative resistance, which is governed by a single gene and provides complete resistance, and quantitative resistance, which is controlled by multiple genes working together to give partial resistance (Brun *et al*., 2010; Huang *et al*., 2009; Mitrousia *et al*., 2018). The Agricultural and Horticultural Development Board (AHDB) in the UK compiles yearly AHDB Recommended Lists for cereals and oilseeds (https://ahdb.org.uk/rlarchive) comprising independent information on quality and yield performance, as well as agronomic features and market criteria, to assist UK growers with cultivar selection. Among the criteria displayed, cultivars are also assigned a resistance rating for pathogens that cause major diseases, including phoma stem canker and light leaf spot.

These disease resistance ratings are given on a scale from 1 to 9, with higher numbers correlating to better resistance, that are determined through field trials (AHDB, 2024a).

Major-resistance genes (*R* genes) within the host plant function through the recognition of a corresponding effector (*Avr*) gene carried by a pathogen, often triggering a resistance phenotype, such as a hyper-sensitive response manifested as rapid cell death, although that may not always be the case. A major class of *R* proteins include nucleotide-binding (NB), leucine rich repeat (LRR) proteins with a putative coiled coil (CC) domain or TIR region (homologous to the mammalian toll-interleukin-1-receptor) at the N-terminus (Gururani *et al*., 2012). In oilseed rape, host resistance has been studied for the management of *L. maculans* (phoma stem canker) (Haddadi *et al*., 2019; Huang *et al*., 2006; 2018; Mitrousia *et al*., 2018). The *Rlm* genes are widely used in oilseed rape cultivars across the UK and work at the leaf infection stage to prevent leaf lesion development through a host defence response, causing cell death at the infection site and thus preventing further *L. maculans* spread (Brun *et al*., 2010; Huang *et al*., 2006). *R* gene-mediated resistance is race-specific (Gururani *et al.*, 2012; Huang *et al.*, 2018), meaning resistance relies on the predominance of the avirulent (*Avr*) allele of the corresponding effector gene within the pathogen population (Table 1.1). Therefore, *R* gene-mediated resistance can become ineffective if pathogens develop mutations in their effector genes to avoid the recognition by the receptor of the host resistance gene. Resistance breakdown has led to phoma stem canker epidemics in oilseed rape crops before, such as breakdown of *Rlm1* or *LepR3* mediated resistance (Rouxel *et al*., 2003; Sprague *et al*., 2006); however, resistance breakdown can be managed. Crop rotation facilitating the routine deployment in time and space of cultivars with different *R* genes, combined with pathogen population monitoring schemes (Stachowiak *et al*., 2006; Marcroft *et al*., 2012) has been shown to help reduce selection for mutations that render the pathogen virulent.

Table 1.1: Gene-for-gene interactions within *R* gene-mediated resistance in plants.

Receptor (*R*) genes in the host plant are either resistant (*R*) dominant or susceptible (*r*) recessive. Similarly, effector (*Avr*) pathogen genes can either be avirulent (*Avr*) dominant or virulent (*avr*) recessive. The interaction of host receptors coded by dominant *R* genes and pathogen effectors coded by dominant *Avr* genes gives an incompatible interaction, triggering a resistance response in the host. This incompatible reaction between an *R* protein and an *Avr* protein triggers a resistance phenotype, often a hyper-sensitive response (HR), particularly against biotrophic pathogens. Conversely, all other combinations are considered compatible between host and pathogen, causing disease symptoms to develop (taken from Kistner *et al*., 2022).

		Genotypes of the pathogen	
		AA /Aa (*Avr*)	aa (*avr*)
Genotypes of the host	RR /Rr (*R*)	**RESISTANCE** Incompatible reaction	**SUSCEPTIBILITY** Compatible reaction
	rr (*r*)	**SUSCEPTIBILITY** Compatible reaction	**SUSCEPTIBILITY** Compatible reaction

Quantitative resistance, also called incomplete or minor-gene mediated resistance, is associated with reduction but not absence of disease and is controlled by the additive action of multiple genes that interact with each other and the environment. Unlike *R* gene-mediated resistance, quantitative resistance does not fit Mendelian segregation ratios, making it harder to incorporate in plant breeding programmes, and many of its underlying mechanisms remain undetermined (French *et al*., 2016). However, because multiple loci are involved in resistance responses, polygenic quantitative resistance is considered race-non-specific (i.e. acting against all isolates of a pathogen species) and therefore more durable than qualitative resistance (Brun *et al*., 2010; Huang *et al*., 2018). In the management of phoma stem canker in oilseed rape, quantitative resistance decreases canker development later in the season, so while it is unable to stop the pathogen completely, it effectively slows pathogen colonization, reducing overall disease severity and improving yields (Delourme *et al*., 2006; Travadon *et al*., 2009). There is evidence suggesting that combining both types of host resistance can provide the most stable control of phoma stem canker, as quantitative resistance acts as a 'safety net' in the case of *R* gene resistance breakdown (Brun *et al*., 2010; Huang *et al*., 2018). Currently, not much is known about operation of quantitative resistance against *L. maculans* in oilseed rape, but quantitative trait loci (QTLs) for resistance have been identified (Huang *et al*., 2016; Pilet *et al*., 1998a,b). This makes screening for cultivars with quantitative resistance challenging compared to selecting for *R* gene resistance, which can be screened at the seedling stage in cotyledon tests (Balesdent *et al*., 2000; Huang *et al*., 2009).

For the best implementation of host resistance, it is paramount to have information about both the plant host genotype and regional pathogen races, in order to match resistance types with pathogen populations that are avirulent against them. While there have been advances made to understand *L. maculans* populations for the management of phoma stem canker, the operation of host resistance against light leaf spot, as well as the variations in pathogenic *P. brassicae* races, remain understudied.

1.4.1 Current evidence of variations in pathogenicity of *P. brassicae* populations/isolates

Determining the genetic structures of *P. brassicae* populations is an important step for the deployment of resistance to improve disease management. Majer *et al*. (1998) studied the genetic diversity and population structures among field isolates of *P. brassicae* using amplified fragment length polymorphism (AFLP) markers. Results suggested a high level of genetic diversity, particularly within regions, implying frequent sexual reproduction, although infrequently through long-distance dispersal (Majer *et al*., 1998). *P. brassicae* is heterothallic with a mixed reproduction system (Karandeni Dewage *et al*., 2018), and with evidence suggesting frequent allele exchange between its two mating types, *MAT-1* and *MAT-2*, newly combined alleles can be fixed in the population during the asexual reproduction phase in spring, leading to genetically diverse populations. The study by Majer *et al*. (1998), however, was done using neutral DNA markers, which may not correlate with differences in pathogenicity determinants. Evidence pointing towards differences in pathogenic races of *P. brassicae* specifically may be seen in the progressive breakdown of resistance in previously less sensitive cultivars. Boys *et al*. (2007) suggested that cultivar Bristol, which was previously considered resistant, may have carried a major resistance gene that was rendered ineffective, as cultivar Bristol appeared to become increasingly susceptible to light leaf spot. Similarly, cultivar Apex, which retained moderate resistance until the late 1990s, showed a more gradual increase in disease severity, which may have been due to the progressive erosion of quantitative resistance (Boys *et al*., 2007). In both cases, increased susceptibility to *P. brassicae* occurred after the cultivars had been widely grown across the UK (Boys *et al*., 2007), possibly applying selection and promoting pathogen diversification towards virulent alleles. More recently, studies also suggested the breakdown of resistance of resistant cultivar Cuillin in Scotland but not in England (Evans *et al*., 2017). These instances show the potential of *P. brassicae* populations to change from avirulent to virulent, implying both differences in pathogenic *P. brassicae* races and highlighting the importance of studying their underlying mechanisms.

Investigating differences in *P. brassicae* pathogenic races can be done at different levels, from assessing light leaf spot severity in crops, to comparing individual *P. brassicae* genotypes. Evans *et al*. (2017) compared light leaf spot disease development by PCR-based spore analysis over three years at different sites, showing differences between cultivars, locations and cropping seasons, implying variations between *P. brassicae* populations. Single-spore *P. brassicae* isolate studies done to investigate the specific genetic components of resistance (Bradburne *et al*., 1999; Karandeni Dewage *et al*., 2021; Karolewski, 1999) typically showed differences in disease phenotypes between isolates or cultivars and their interactions, further implying differences in pathogen genotypes. These may be followed by a range of molecular techniques to investigate differences in pathogen genotypes, including random amplified polymorphic DNA (RAPD) markers (Boys, 2009), AFLP markers (Majer *et al*., 1998), microsatellites (Mahfooz *et al*., 2017), up to *de novo* genome sequencing and assembly of isolates of interest (Carmody *et al*., 2020).

1.4.2 Current evidence of host resistance against *P. brassicae*

Compared to the advances made in understanding both major- and minor-gene mediated resistance against *L. maculans* in oilseed rape, there is currently a limited number of studies investigating host resistance against *P. brassicae* (Table 1.2). Bradburne *et al*. (1999) investigated qualitative resistance on breeding lines of oilseed rape with introgressed segments of the genomes from wild *Brassica* species. Seedling resistance was attributed two major-gene loci, *PBR1* on linkage group N1 (chromosome A1) associated with a symptomless disease phenotype after infection, and *PBR2* on linkage group N16 (chr C6) associated with black necrotic flecking and limited asexual sporulation. Later, Boys *et al*. (2012) confirmed through field and controlled-environment experiments on cultivar Imola (produced from breeding material studied by Bradburne *et al*. (1999)) that black necrotic flecking (collapsed epidermal cells associated with *P. brassicae* infection, Figure 2.5) and limited asexual (but not

Table 1.2: Summary of work done to study host resistance against *Pyrenopeziza brassicae* in oilseed rape.

Research has been done on the identification and mapping of both major- and minor-gene mediated resistance against *P. brassicae*. Two phenotypes of resistance, limited asexual sporulation and black necrotic flecking (collapsed epidermal cells) have been identified on cultivars and breeding lines with either resistance type.

Study	Experiment description	Resistance type	Host genotype	Resistance identified
Pilet *et al*. 1998b	Field experiments with 11-point scale assessment (1 is most resistant)	Quantitative resistance	DH lines cross between OSR cvs Darmor-*bzh* and Yudal	Ten (six environmentally stable) resistance QTL
Bradburne *et al*. 1999	Detached cotyledon tests scored for presence/ absence of *P. brassicae* asexual sporulation and for presence/absence of black flecking	Major gene-mediated	OSR commercial lines with introgressed resistance from wild *Brassicas*	*PBR1* locus on chr A1 *PBR2* locus on chr C6
Boys *et al*. 2012	Controlled-environment and field experiments with 9-point scale assessment (1 is most resistant)	Major gene-mediated	OSR cultivar Imola	Chr A1
Karandeni Dewage *et al*. 2022	Controlled-environment and glasshouse experiments with visual estimation of % foliar *P. brassicae* asexual sporulation and presence/absence of black flecking	Quantitative resistance	DH lines with introgressed resistance from secondary gene pools	Four resistance QTL
Fell *et al*. 2023	Glasshouse experiments with 6-point scale assessment (1 is most resistant)	Quantitative resistance	Non-commercial OSR diversity set	Eight resistance QTL and one susceptibility QTL

sexual) sporulation resulted from the action of a single *R* gene located at the bottom of chr A1. Quantitative resistance against *P. brassicae* was first investigated by Pilet *et al*. (1998b). Several resistance QTL, two of which were considered 'multiple disease resistance' QTL that matched QTL previously identified for phoma stem canker resistance, were identified in field trials using a doubled haploid mapping population (Pilet *et al*., 1998a,b). Similarly, Karandeni Dewage *et al*. (2022) mapped four QTL in controlled-environment and glasshouse experiments using a doubled haploid mapping population with resistance introgressed from a secondary gene pool. Most recently, Fell *et al.* (2023) identified four novel gene loci associated with quantitative disease resistance to *P. brassicae* from 195 *B. napus* lines using genome-wide association analyses. Findings included a heterozygous locus for a cytochrome P450 gene co-localising with a previously described QTL for seed glucosinolate content, and a a HXXXD-type acyl-transferase as a potential susceptibility gene. Results reported by Fell *et al*. (2023) were then included in a separate study that used associative transcriptomics to identify novel gene loci involved in quantitative disease resistance against four separate *B. napus* fungal pathogens, including *P. brassicae*. The study found broad-spectrum loci associated with either resistance or susceptibility dependent on the pathogen lifestyle, highlighting potential new targets for crop breeding for multi-pathogen resistance (Jacott *et al*., 2024). There are few to no overlaps in the QTL identified by the studies discussed, probably due to differences in host genotypes and pathogen inoculum used. Overall, two phenotypes of resistance have currently been identified: absence of asexual sporulation and black necrotic flecking. However, both phenotypes were observed on cultivars or breeding lines with either *R* gene-mediated resistance or quantitative resistance (Boys *et al*., 2012; Karandeni Dewage *et al*., 2018; 2022).

Major gene-mediated resistance against biotrophic pathogens often involves a hyper-sensitive response, but that may not be the case with *P. brassicae*. Boys *et al*. (2012) observed that, within the first 10 days of infection with *P. brassicae*, the change in amount of *P. brassicae* DNA measured in *B. napus* leaf samples did not significantly differ between susceptible and resistant genotypes. Host recognition happened only after 10 days post-inoculation, once *P. brassicae*

produced acervuli following asexual sporulation. This suggests that a host defence response is not triggered during the early stages of invasion, when *P. brassicae* grows endophytically inside the host, but only once sporulation occurs. Additionally, *R* gene-mediated resistance against apoplastic fungal pathogens like *P. brassicae* may involve effector recognition by cell-surface receptor-like proteins (RLP) (Stotz *et al*., 2014). In oilseed rape, the resistance genes *Rlm2* and *LepR3* encode leucine-rich repeat RLPs (LRR-RLPs) that operate against *L. maculans* for the management of phoma stem canker (Larkan *et al*., 2013; 2015). Receptor-like kinases (RLKs) are another newly discovered class of race-specific *R* genes in oilseed rape. The resistance genes *Rlm9*, *Rlm4* and *Rlm7* encode wall-associated kinase-like 10 (WAKL10) genes providing race-specific resistance against isolates of *L. maculans* (Larkan *et al*., 2020; Noel *et al.,* 2024). Although these genes are involved with resistance against *L. maculans*, further screening of host genotype regions of interest specifically for *R* genes that encode RLPs or RLKs may also present a novel avenue for selective breeding of resistance against *P. brassicae*.

Although both major-gene loci and resistance QTL have been identified, many of these areas of interest have yet to be cloned and characterised. There is therefore great incentive to further investigate the findings from these studies for the development of molecular markers for resistance breeding in commercial cultivars. Novel genomic approaches to characterize sequences of interest have been discussed (Karandeni Dewage *et al*., 2018). The genomes of five brassica species, *B. napus* (Chalhoub *et al*., 2014) and its progenitors *B. rapa* (A genome) (The *Brassica rapa* Genome Sequencing Project Consortium *et al*., 2011) and *B. oleracea* (C genome) (Liu *et al*., 2014), as well as *B. juncea* (Yang *et al*., 2016) and *B. nigra* (Paritosh *et al*., 2020), have been sequenced. These available genome sequences, combined with high-density *B. napus* single-nucleotide polymorphism (SNP) arrays and transcriptome sequencing for genome-wide association studies (GWAS), as well as next generation sequencing technologies, among others, offer unprecedented molecular tools to assist in breeding for resistance to *P. brassicae* (Karandeni Dewage *et al*., 2018; Snowdon and Iniguez Luy, 2012). Identification of these ancestral genomes has also been used for the introgression of secondary gene pools into

B. napus lines, to counteract the genetic bottleneck formed through selective breeding of agronomic traits and improve resistance breeding (Karandeni Dewage *et al*., 2022). Maintenance of potential resistance genes then becomes of great importance to extend their usage and prevent resistance breakdown. Combining resistance QTL with multiple modes of action, combining both major and minor-gene mediated resistance and pyramiding several *R* genes in commercial cultivars are all strategies that offer more durable resistance, since pathogens require multiple mutations to overcome several resistances simultaneously (Pilet-Nayel *et al*., 2017).

1.4.3 Exploitation of potential resistance mechanisms

The potential areas where host resistance could be used in relation to the *P. brassicae* life cycle have been discussed (Boys *et al*., 2007). *P. brassicae* infection on oilseed rape begins with adhesion and germination of ascospores on the leaf cuticle (Figure 1.9b). *P. brassicae* directly penetrates the leaf cuticle (Ashby, 1997; Davies *et al*., 2000) and it was shown that the thickness and topology of cuticular waxes greatly affect germination and leaf penetration by *P. brassicae*. The plant cuticle itself is a hydrophobic, three-dimensional network of cutin and integrated and superimposed lipids, or ‘waxes’, acting as a protective skin above the leaf epidermis (Koch *et al*., 2008). The properties of the cuticular wax greatly depend on the environmental conditions (Koch *et al*., 2005); for example, certain herbicides like dalapon (2,2-dichloropropionic acid) favour the pathogen by altering plant wax structures (Rawlinson *et al*., 1978), as more resistant cultivars typically presented greater wax coverage than susceptible cultivars (Davies, 1997, as cited in Boys *et al*., 2007). Cuticular penetration by *P. brassicae* is believed to involve enzymatic degradation by an extracellular cutinase, Pbc1 (Davies *et al*., 2000; Li *et al*., 2003), as cutinase-deficient *P. brassicae* mutants failed to penetrate the cuticular layer and were unable to induce disease symptoms (Li *et al*., 2003). At this stage of *P. brassicae* colonization, improvements in host cuticular wax structures, as well as inhibition of fungal cutinases, may act as potential mechanisms for resistance.

Next, *P. brassicae* undergoes endophytic growth in the sub-cuticular space between the cuticle and epidermal cells of the leaf (Ashby, 1997; Stotz *et al*., 2014), which is thought to involve both Pbc1 (Li *et al*., 2003) and an extracellular serine protease, Psp1 (Ball *et al*., 1991; Batish *et al*., 2003). Psp1 is thought to be a determinant of pathogenicity, since transformed *P. brassicae* mutants that were deficient in the extracellular protease were also deficient in pathogenicity (Ball *et al*., 1991), and presence of the protease was detected *in planta* up to 13 days post inoculation with *P. brassicae* (Batish *et al*., 2003). Psp1 may be involved in the breakdown of host intercellular matrices to generate space for subcuticular growth and nutrient acquisition (Boys *et al*., 2007). Fungal cytokinins are also believed to be involved in this stage of *P. brassicae* colonization. Cytokinins are a class of plant growth regulators whose roles include metabolism and transportation of nutrients, as well as regulating plant cell division (Ashby, 1997). *P. brassicae* is capable of producing cytokinins *in vitro* under growth-limiting conditions (Murphy *et al*., 1997), suggesting that fungal cytokinin production by *P. brassicae in planta* may function as a key pathogenicity determinant by increasing host metabolism and translocating nutrients towards the site of infection (Ashby, 1997; Boys *et al*., 2007; Murphy *et al*., 1997). Altogether, the deployment of host lines expressing cutinase or protease inhibitors, spraying of cutinase inhibitors such as ebelactones, or the modification of the protease substrate to prevent proteolytic cleavage, present possible areas for exploitation of host resistance during endophytic apoplastic growth of *P. brassicae* (Ashby, 1997; Boys *et al*., 2007).

The symptomless phase of *P. brassicae* colonization ends upon onset of asexual sporulation (Evans *et al*., 2003; Fitt *et al*., 1998a) (Figure 1.9c). These splash-borne conidia initiate and perpetuate secondary disease cycles which greatly contribute to yield loss (Karandeni Dewage *et al*., 2018) (Figure 1.9d). Resistance preventing asexual sporulation would therefore be of considerable importance in limiting subsequent disease cycles, reducing the overall disease incidence in crops. Major gene-mediated resistance identified by Pilet *et al*. (1998b) and Boys *et al*. (2012) was suggested to limit asexual sporulation. While exploiting this resistance would not completely prevent conidial production, meaning that infected crops could still act as potential reservoirs for

subsequent epidemics, it would greatly decrease disease severity and improve yields. Similarly, limiting sexual sporulation on infected crop debris at the start of the cropping season (Figure 1.9a), as well as on senescent infected leaves in spring (Gilles *et al*., 2001b), would greatly limit light leaf spot infection, since ascospores are the primary inoculum initiating epidemics in new crops in autumn (Boys *et al*., 2007; Gilles *et al*., 2001b,c). Potential resistance mechanisms to prevent ascospore release include delayed foliar senescence in spring and inhibition of apothecial development by mimicking fungal sex factors (Boys *et al*., 2007).

Exploitation of resistance identified in genetically related fungal species may also be of great value. Sequencing of the internal transcribed spacer region revealed that *Rhyncosporium commune*, a hemibiotrophic fungal pathogen causing leaf blotch in barley, rye and other graminaceous species, is closely related to *P. brassicae* (Goodwin, 2002; King *et al*., 2015). The genome of *R. commune* has been sequenced (Penselin *et al*., 2016), and the potential for exploiting the phylogenetic relationship between *R. commune* and *P. brassicae* for the identification of candidate *P. brassicae* effector genes has been discussed (Fell *et al*., 2023; Karandeni Dewage *et al*., 2018).

1.5 Aims and objectives

Chemical control has proven insufficient for disease management strategies due to fungicide insensitivity development in *P. brassicae* populations and the loss of important fungicides. Host resistance is an economical and environmentally conscious alternative disease control method already employed for the management of phoma stem canker, another important oilseed rape disease. Control of light leaf spot through host resistance is becoming increasingly relevant; however, there is currently limited information available on the operation of resistance against *P. brassicae* in oilseed rape, as well as limited knowledge of pathogenic *P. brassicace* races that determine resistance deployment. Therefore, the overall aim of this PhD project is to provide further understanding of *P. brassicae* pathogen populations and host resistance against the light leaf spot pathogen in winter oilseed rape. To meet the aims, there are three objectives presented in three experimental chapters.

1. To investigate the host, pathogen and environmental factors that contribute to light leaf spot disease in oilseed rape (Chapter 3)
2. To improve the understanding of pathogenic *P. brassicae* populations using controlled-environment, glasshouse and field experiments (Chapter 4)
3. To characterise the resistance phenotype and map the genetic locus/loci of resistance against *P. brassicae* using a DH population of *B. napus* (Chapter 5)

Chapter 2 General materials and methods

2.1 Preparation of *Pyrenopeziza brassicae* isolates

2.1.1 Media preparation

Malt extract agar (MEA) was used to grow *P. brassicae* isolates. Media stocks were prepared by adding 30 g of malt extract (Oxoid) and 15 g of Agar (Oxoid) to 1 l of distilled water. Media was portioned in 400 ml aliquots in Duran bottles and autoclaved at 121°C for 2 hr. Once cooled to 50°C, bottles were amended with 50 µg/ml of penicillin and 50 µg/ml of streptomycin to prevent bacterial growth. The media were then poured into 9 cm diameter Petri dishes in approx. 20 ml aliquots.

2.1.2 Single-spore isolation of *P. brassicae*

Diseased oilseed rape leaves were sampled from crops, wrapped in dampened tissue paper, placed in sealed polyethylene bags to retain humidity and incubated at 4°C for 7-9 days. After incubation, leaves were examined for the presence of *P .brassicae* asexual sporulation (acervuli visible as white salt-like structures on the leaf lamina, Figure 2.1a). Single acervuli were then picked from leaves using a sterile needle and placed in a 0.5 ml microfuge tube containing 50 µl of sterile distilled water. Tubes were briefly vortexed to liberate the conidia (asexual spores) and the spore suspension was subsequently transferred onto a MEA plate and spread using a sterile L-shaped spreader. The plates were then sealed with Parafilm and incubated at 15°C for 7 days, or until individual colonies were visible (Figure 2.1b).

2.1.3 Culturing *P. brassicae* for spore and mycelium production

Individual *P. brassicae* colonies grown on MEA plates (section 2.1.2) were selected for sub-culturing. Mycelium from a single colony was scraped using a sterile scalpel, and placed in a 1.5 ml Eppendorf tube containing 300 µl of sterile distilled water. 100 µl of the suspended mycelium was then transferred onto one of three MEA plates. Two plates were standard MEA plates for spore production (section 2.2.1) and one plate was amended with a cellulose disc to

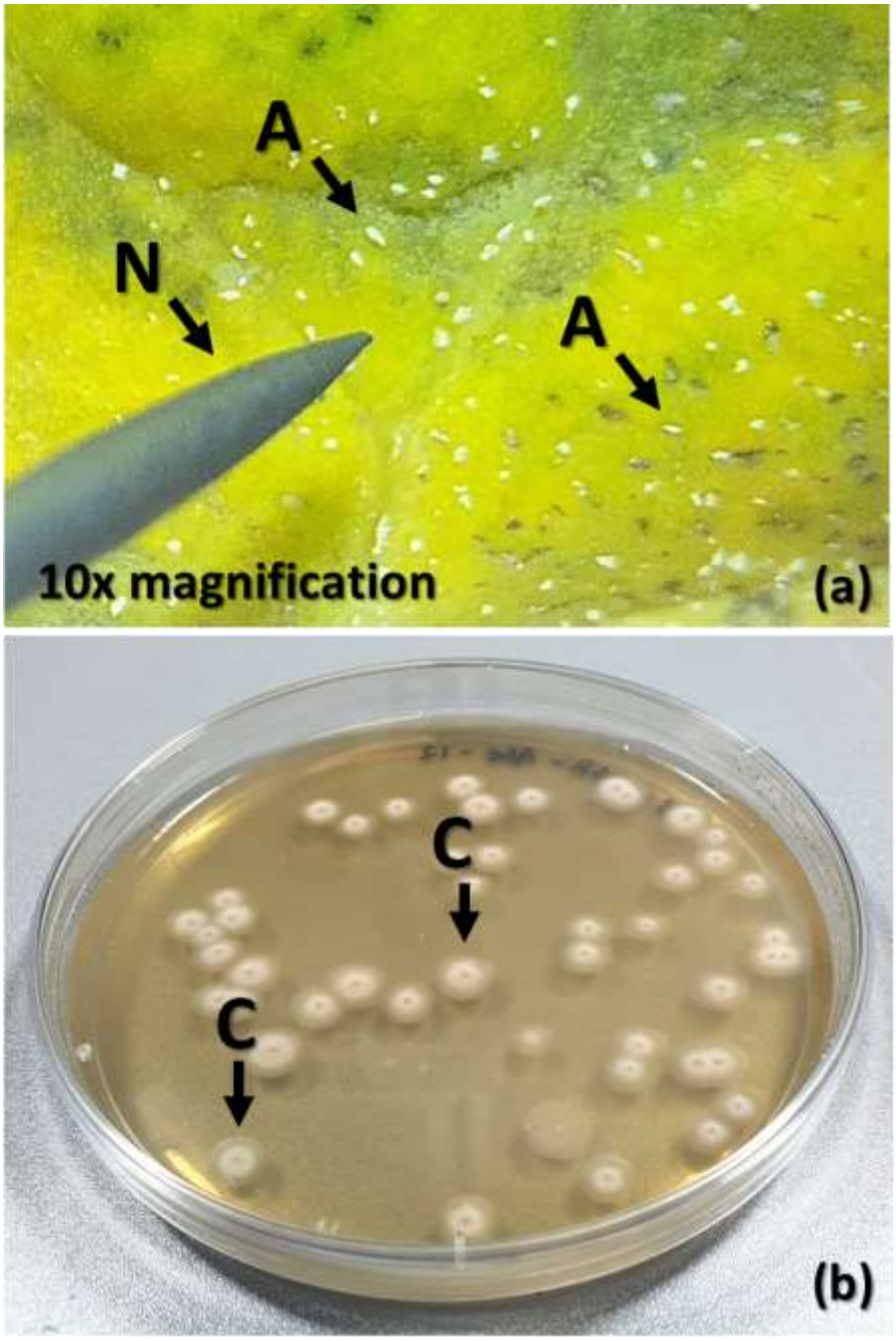

Figure 2.1: Preparation of individual *Pyrenopeziza brassicae* conidial isolates.

Leaves with light leaf spot symptoms were harvested from crops to obtain single conidial colonies of *P. brassicae*. (a) Single acervuli (A) were picked from leaves using a sterile needle (N) and placed in a 0.5 ml microfuge tube containing 50 µl of sterile distilled water. Tubes were briefly vortexed to liberate the conidia and the spore suspension was subsequently transferred onto a malt extract agar (MEA) plate and spread using a sterile L-shaped spreader. The plates were then sealed with Parafilm and incubated at 15°C for about 7 days, or (b) until individual colonies (C) were visible. Single colonies were further sub-cultured onto MEA plates for spore or mycelium production.

promote mycelium production. All plates were sealed with Parafilm and incubated at 15°C in the dark for 3-5 weeks. Mycelium was scraped from the cellulose disc MEA plates using sterile forceps and stored in 2 ml screw-cap tubes at -20°C until needed for DNA extraction.

2.2 *P. brassicae* inoculum preparation

2.2.1 *P. brassicae* inoculum production *in vitro*

MEA plates containing *P. brassicae* cultures prepared for spore production (section 2.1.2) were flooded with 10 ml of sterile distilled water and mycelium was macerated using a sterile L-shaped spreader. The mycelium was left for 5 min to liberate spores and the suspension was then filtered through a glass funnel layered with sterile Miracloth (Cambiochem, USA) into 15 ml Falcon tubes. The spore concentration was determined by counting spore numbers using a Bright-Line haemocytometer (Sigma Aldrich, UK) and spore suspensions were diluted to a concentration of 10^5 spores/ml. Spore suspensions were then stored in 2 ml Eppendorf tubes at -20°C and 20% glycerol stocks were also prepared for long-term storage at -80°C.

2.2.2 *P. brassice* inoculum production from diseased plant material

Diseased oilseed rape leaves with light leaf spot symptoms were sampled from crops and incubated at 4°C for 7-9 days to induce sporulation. Visible conidia on leaves were washed into a 500 ml beaker containing 100-150 ml (adjusted depending on the amount of sporulation) of sterile distilled water. Spore suspensions were filtered through a glass funnel layered with sterile Miracloth (Cambiochem, USA) into 50 ml Falcon tubes. The spore suspension concentration was determined by counting spore numbers using a Bright-Line haemocytometer (Sigma Aldrich, UK) and adjusted to 10^5 spores/ml. Spore suspensions were stored in 40 ml and 15 ml aliquots at -20°C until needed. Three *P. brassicae* populations and a single-spore *P. brassicae* isolate were used (Table 2.1). One of the *P. brassicae* populations was derived from *Brassica napus* in England, one was derived from *B. napus* in Scotland, and one was derived from *B. oleracea* in England.

Table 2.1: Origin of *Pyrenopeziza brassicae* inoculum used in controlled environment and glasshouse experiments

P. brassicae conidial suspensions were used to spray inoculate oilseed rape plants in controlled environment or glasshouse experiments. Four main inoculum sources were used, which were produced using the method described in section 2.2.2.

Inoculum name	Type	Crop*	Cultivar	Location	Year
15WOSR64-SS1	Single-spore isolate	*B. napus*	Bristol	Herefordshire, England	2015
England-Pb	Population	*B. napus*	Multiple cultivars	Norfolk, England	2016
Scotland-Pb	Population	*B. napus*	Multiple cultivars	Aberdeen, Scotland	2015
Kale-Pb	Population	*B. oleracea*	Nero di Toscana	Hertfordshire, England	2021

* *B. napus* – oilseed rape; *B. oleracea* - kale

2.3 Preparation of plant material and inoculation of plants

2.3.1 Plant growth in controlled environment/glasshouse conditions

Oilseed rape seeds were pre-germinated in a Petri dish containing a dampened filter paper for 48 hr in the dark at 20°C (Figure 2.2a). Pre-germinated seeds were sown into 40-cell (single cell: 4 cm x 4 cm x 5 cm) seed trays containing Miracle-Gro all-purpose compost (The Scotts Company) and John Innes No. 3 compost (LBS Horticulture) mixed in a 1:1 ratio. Seed trays were then placed in a plastic outer tray (22 cm x 35 cm) containing a capillary mat. The compost surface was covered with a thin layer of vermiculite to retain moisture and trays were watered regularly from below. Seedlings were maintained in a controlled environment cabinet (FITOCLIMA D1200, ARALAB, Rio de Mouro, Portugal) with a 12 hr photoperiod at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 20°C day/18°C night temperatures and 60% relative humidity. After seedlings reached growth stage 1,0-1,1 (Sylvester-Bradley, 1985; Appendix D), they were transplanted into pots (5 cm x 5 cm x 9 cm) containing the same compost mixture and, depending on experiment, either kept in the controlled environment or transferred to a temperature-controlled glasshouse with natural daylight supplemented by a 12 hr photoperiod and 20°C day/18°C night temperatures (Figure 2.2b). Plants were maintained until they reached growth stage 1,4-1,5 for inoculation (Figure 2.2c).

2.3.2 Plant inoculation in controlled environment/glasshouse conditions

The concentrations of *P. brassicae* spore suspensions were measured using a Bright-Line haemocytometer and adjusted to a suitable concentration, usually 10^5 spores/ml. Before inoculation, 0.005% Tween 80 was added to the spore suspensions as a wetting agent. Spore suspensions were sprayed onto the plants using a hand-held sprayer until the leaves were evenly covered with fine droplets. After inoculation, plants were covered with polyethylene covers for 48 hr to retain humidity and promote *P. brassicae* spore infection (Figure 2.3). After inoculation, controlled environment or glasshouse temperatures were decreased to 16°C day/14°C night, respectively.

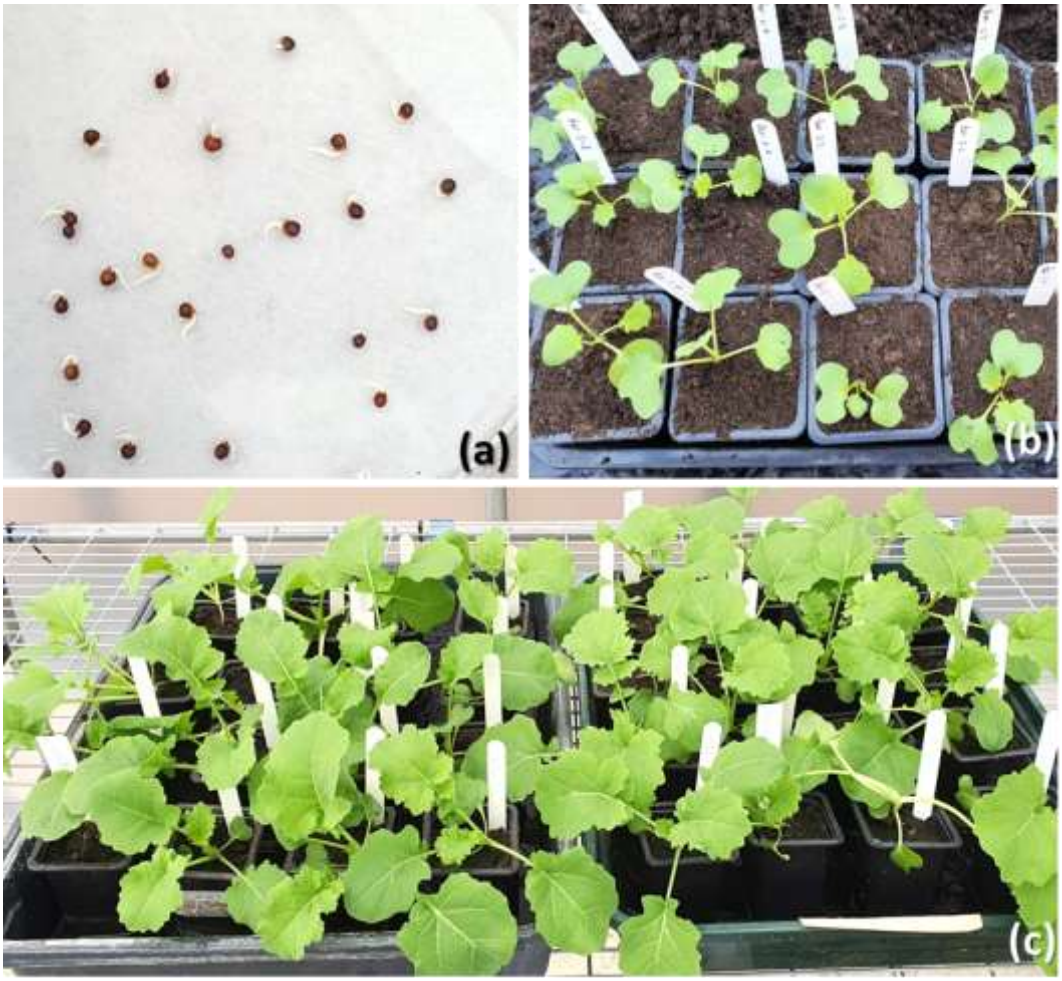

Figure 2.2: Growth stages of oilseed rape in controlled environment/glasshouse experiments.

(a) Oilseed rape seeds were pre-germinated in a Petri dish containing a dampened filter paper for 48 hr in the dark at 20°C. (b) Seedlings that reached growth stage 1,0-1,1 (one true leaf, Sylvester-Bradley, 1985, Appendix D) were transplanted into individual pots. (c) Plants were ready for inoculation upon reaching growth stage 1,4-1,5 (four or five true leaves).

Figure 2.3: Oilseed rape plants covered in polyethylene bags after spray inoculation with *Pyrenopeziza brassicae*.

Oilseed rape plants were grown and spray inoculated with *P. brassicae* conidial suspensions. Plants were subsequently covered with polyethylene covers for 48 hr to retain humidity and promote *P. brassicae* spore infection. (a) Each tray (22 cm x 35 cm) containing oilseed rape plants was covered using a polyethylene bag in a controlled-environment cabinet. (b) Polyethylene cover using a scaffolding was erected on a glasshouse bench to cover many inoculated oilseed rape plants simultaneously.

2.4 Light leaf spot disease assessment

2.4.1 Light leaf spot foliar severity score on a 1-8 scale

Light leaf spot disease severity was assessed in controlled environment and glasshouse experiments using a 1-8 scale adapted from Fortune (2022) (Table 2.2 and Figure 2.4), with 1 being no disease.

2.4.2 Percentage leaf area covered with *P. brassicae* acervuli (asexual sporulation)

The most characteristic symptom of light leaf spot infection in a controlled environment was the appearance of sporulation on the leaf surface as white *P. brassicae* acervuli. These were usually present as concentric rings, but may also be scattered pustules. To promote sporulation, infected leaves were wrapped in dampened tissue paper, placed in sealed polyethylene bags to retain humidity, and incubated at 4°C for 7-9 days. Disease severity was then assessed by visually estimating the percentage leaf area covered in *P. brassicae* sporulation.

2.4.3 Assessment of other symptoms

Although disease score and percentage leaf area covered in *P. brassicae* asexual sporulation are the most significant symptoms used to assess light leaf spot in controlled environments, other symptoms that appeared were also recorded. These included leaf deformations such as curling or distortion, petiole elongation, leaf discoloration to yellow, red or purple, or premature leaf senescence (Figure 2.5). These symptoms can be disease non-specific and caused by general nutrient deficiencies; therefore, when doing disease assessments, they were not considered of the same significance as the disease score, or percentage leaf area with sporulation.

A necrotic response (collapsed epidermal cells creating a “black flecking” phenotype), which is considered as a phenotype of resistance to light leaf spot (Boys *et al*., 2012), was also assessed. Presence or absence of necrotic

Table 2.2: Light leaf spot disease severity score. See Figure 2.3 for illustrations of selected scores.

A 1-8 point disease severity score (with 1 being no disease) was used to assess *Pyrenopeziza brassicae* (light leaf spot) symptoms. The disease severity score used was adapted from Fortune (2022).

Score	Description
1*	No disease
2	Trace of disease
3	Few scattered lesions and/or less than 20% leaf area affected
4*	Several scattered lesions and/or 20-40% leaf area affected
5	Many small lesions/ scattered large lesions and/or 40-60% leaf area affected
6*	Many small lesions/ scattered large lesions and/or 60-80% leaf area affected
7*	Severely diseased plants > 80% leaf area affected
8	Plants heavily stunted/ dead

*See Figure 2.4 for illustrations of the scores.

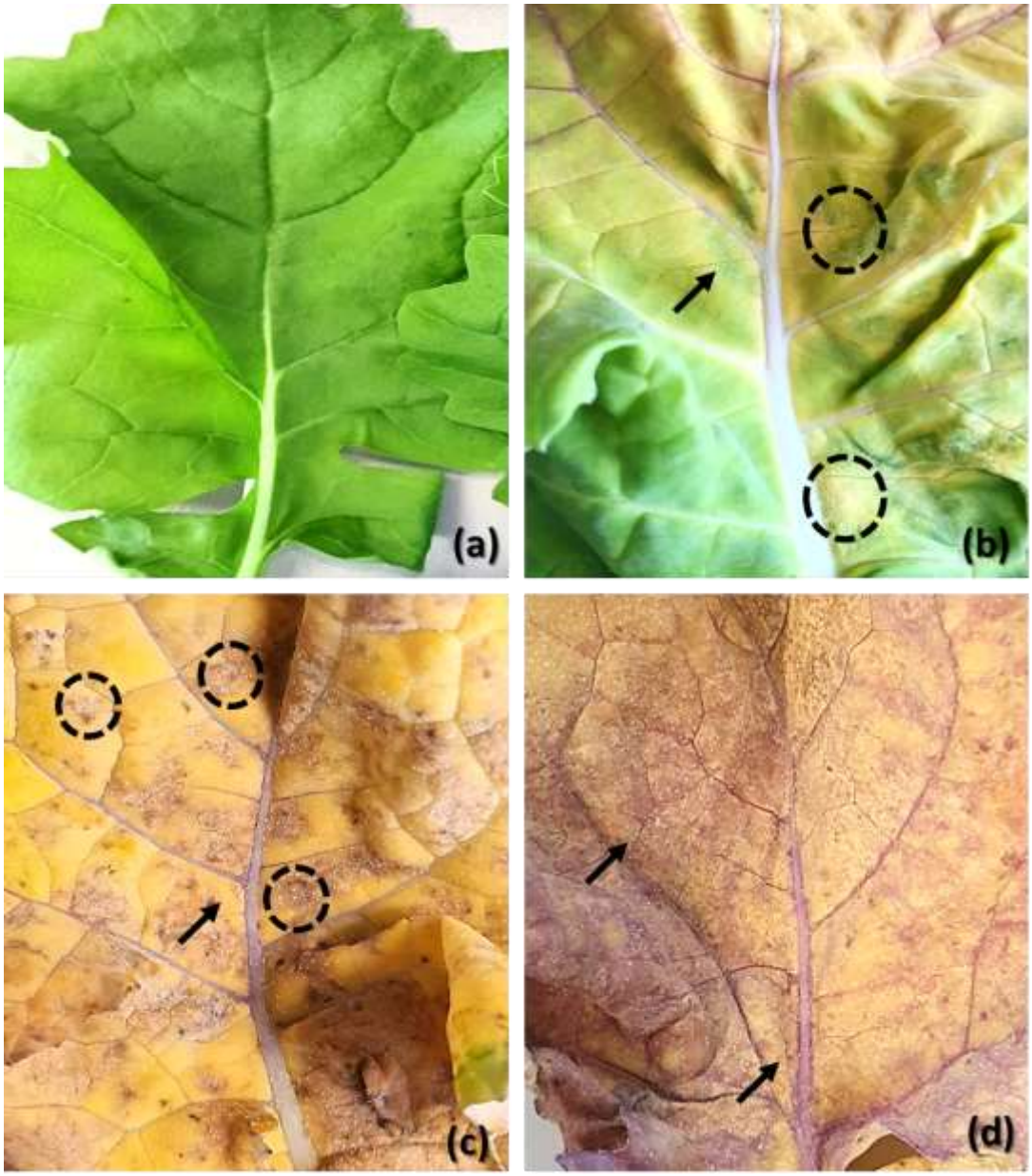

Figure 2.4: Light leaf spot disease severity score key. See Table 2.2 for descriptions of these scores.

A 1-8 point disease severity score (with 1 being no disease) was used to assess *Pyrenopeziza brassicae* (light leaf spot) infection. (a) Asymptomatic oilseed rape leaf (score 1). (b) Leaf showing few scattered lesions (encircled areas) with 20-40% leaf area covered in *Pyrenopeziza brassicae* sporulation (arrowhead) (score 4). (c) Leaf showing many lesions with 60-80% leaf area covered in sporulation (score 6). (d) Severely diseased leaf with >80% leaf area covered in sporulation (score 7).

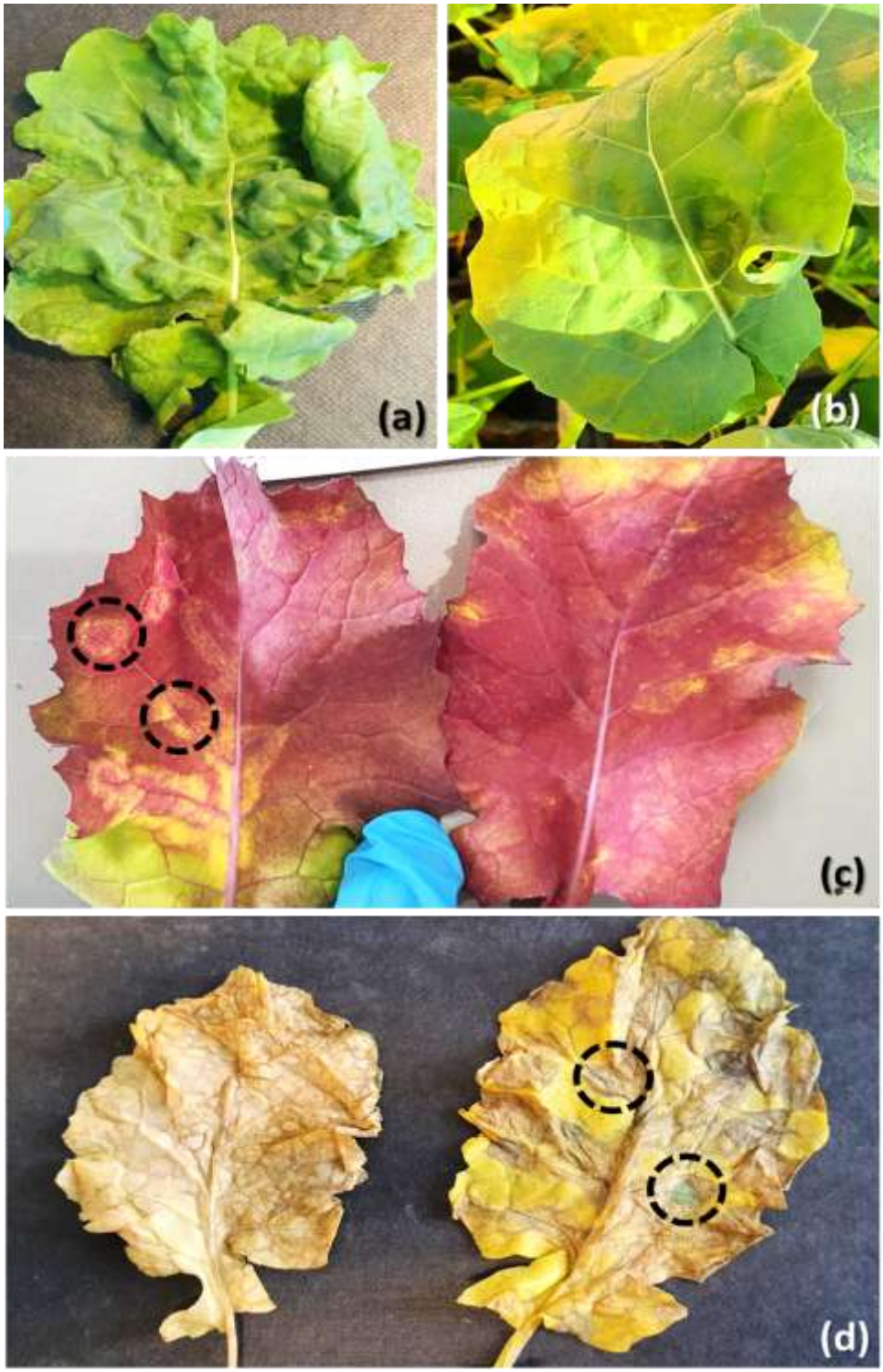

Figure 2.5: Changes in oilseed rape leaf morphology caused by light leaf spot disease.

Oilseed rape plants inoculated with *Pyrenopeziza brassicae* (light leaf spot) may undergo changes in leaf morphology in response to pathogen colonization interfering with key plant signaling pathways. These changes include (a) (b) leaf curling, (c) leaf discolouration, with visible green islands (areas of high photosynthetic activity associated with *P. brassicae* infection, encircled areas), (d) premature leaf senescence.

flecking on different areas of the leaf (petiole, midrib and lamina) was recorded as a qualitative measure (Figure 2.6).

2.5 Experimental design and statistical analysis

Three main experimental designs were used: alpha design (generated using Microsoft Excel (Office 365)), split-plot design (generated using Experiment Design Generator and Randomiser (EDGAR) (Brown, 2005)) and complete randomised block design (generated using EDGAR). Statistical analysis was done with R (version 4.2.2) and Genstat (Payne *et al*. 2011). Specific statistical tests are explained in the corresponding experimental chapters. Graphs were plotted using Microsoft Excel. Transformations were applied to normalize the data. Foliar morphology data were expressed as percentages by dividing the numbers of symptomatic leaves (deformed, dead, discoloured, etc. leaves) by the total number of leaves on the plant. The percentage of symptomatic leaves calculated, as well as *P. brassicae* sporulation data (the % leaf area covered in *P. brassicae* sporulation), were arc-sine-transformed by taking the arc-sine of the square root of the data expressed on a 0-1 scale (10% as 0.1, 25% as 0.25, etc.) using the following formula in Microsoft Excel:

$$X = ASIN(SQRT(data))$$

The inverse of the function was used to back-transform the data:

$$Data = (SIN(X))^2$$

This was done to present the data on the back-transformed scale where applicable.

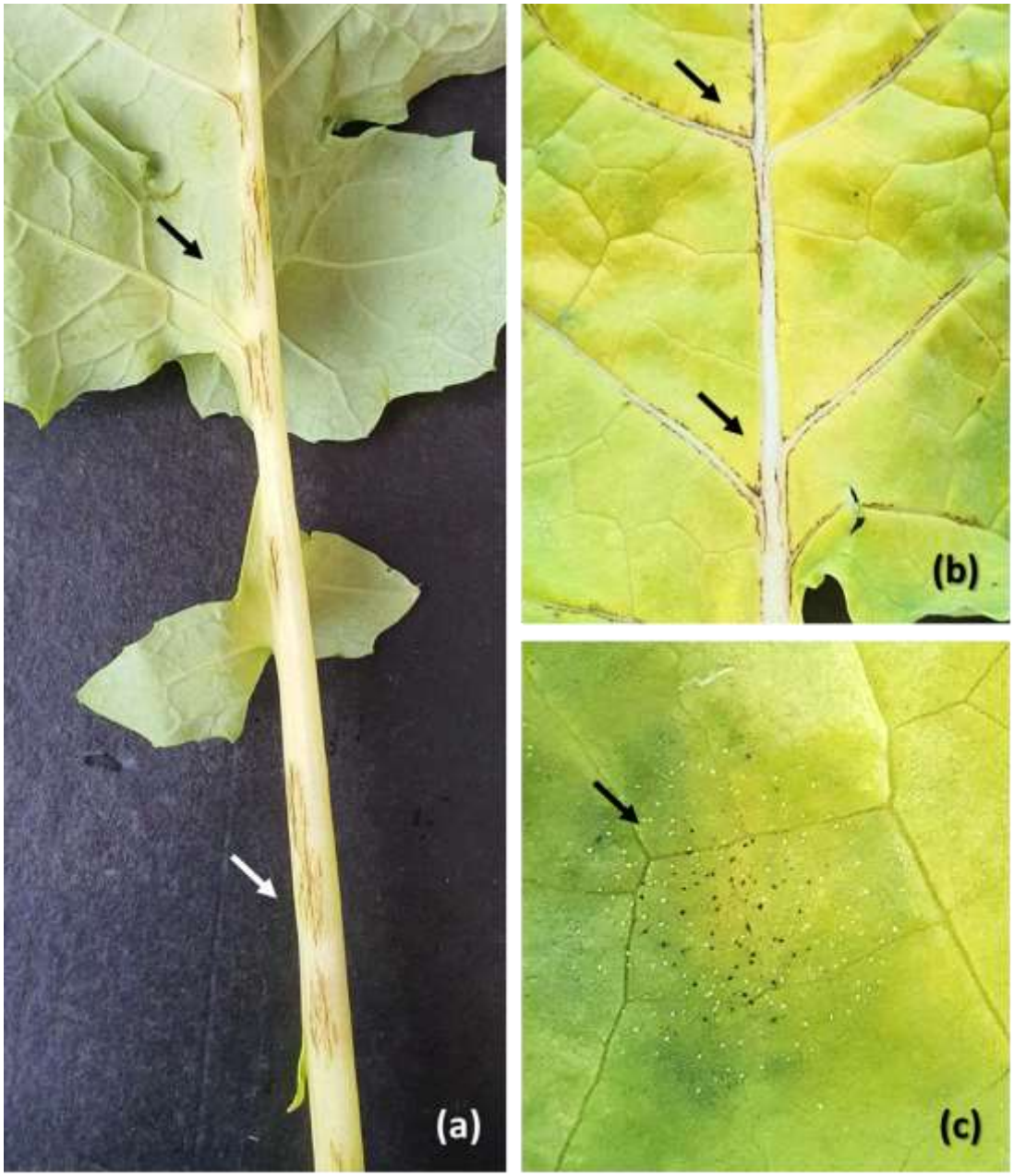

Figure 2.6: Necrotic flecking on oilseed rape leaf as a response to *Pyrenopeziza brassicae*.

Oilseed rape plants inoculated with *Pyrenopeziza brassicae* (light leaf spot) may develop a necrotic response (collapsed epidermal cells creating a “black flecking” phenotype), which is considered a phenotype of resistance (Boys *et al*., 2012). Necrotic flecking (arrowheads) may appear on the (a) petiole, (b) midrib and (c) lamina of the leaf.

Chapter 3 Factors affecting light leaf spot disease development

3.1 Introduction

The development of plant diseases is determined by the interactions between the host, the pathogen and the environment, forming a three-way relationship referred to as the disease triangle. The growth stage of the host and its susceptibility to the pathogen can determine the severity of the disease. The pathogen virulence influences colonization of the host and the pathogen can use the environment to spread its inoculum (e.g. through spore dispersal). Finally, the environment can affect both the host susceptibility by predisposing it for infection and the pathogen effectiveness by providing favourable conditions (e.g. humidity) for infection (Keane and Kerr, 1997). Understanding these factors and their interactions within a particular pathosystem is critical to control epidemics and improve disease prevention strategies.

Certain structural components of host plants (e.g. cuticle, epidermis, wax composition, etc.) affect levels of resistance to the pathogen. Plant metabolites and other chemical barriers may also be produced in response to infection (Doughari, 2015). Sometimes, these innate plant host defence mechanisms are insufficient and characterized resistance genes can be introduced into the host gene pool through selective breeding (Stuthman *et al*., 2007). Another contributing factor to disease development is host maturity. Studies have shown that plant age at the time of infection can greatly affect the severity of disease development in various host-pathogen systems (Gongora-Canul and Leandro, 2011; Sharabani *et al*., 2012; Zhu *et al.*, 2022). Defining the period of susceptibility to infection within a given host could be paramount to understanding the etiology of disease and improving its management. For light leaf spot, few studies have tried to correlate host maturity with disease severity, such as work done by Hartill and Cheah (1984) studying the effects of temperature, humidity and plant growth stage on light leaf spot development on cauliflower. For oilseed rape, plants grown in controlled-environment experiments are typically inoculated at the 1,4-1,5 growth stage (Sylvester-

Bradley, 1985), although resistance assessments on cotyledons have also been done (Bradburne *et al*., 1999; Thomas *et al*., 2019). Work done by Karolewski *et al*. (2006) compared disease development between plants at either the 1,4 or 1,6-1,7 growth stages. However, there are currently no studies that compare the light leaf spot disease phenotype at different earlier growth stages, providing limited information on how plant age influences disease development in oilseed rape.

Next, the most important factor that determines a pathogen's ability to infect its host is pathogenicity, which includes pathogen virulence (the ability to infect) and aggressiveness (the vigour of the infection) (Van der Plank, 1963). Disease will occur in the presence of a pathogenic strain of the pathogen; however, the severity of disease caused is closely determined by the number of infective propagules (e.g. spores), where a greater number of viable propagules correlates with greater disease incidence (Keane and Kerr, 1997). This means that even pathogenic strains may not cause significant disease at small inoculum concentrations. Similarly, the genetic variation within a pathogen population will influence its potential to cause disease. Populations carrying greater genetic diversity are thought to have greater evolutionary potential than populations with less diversity (McDonald *et al*., 2022) and have therefore a greater potential to overcome host resistance. *P. brassicae* populations are thought to have high evolutionary potential (Majer *et al.*, 1998) but information about their genomics remains largely unknown. Understanding the threshold levels of *P. brassicae* inoculum necessary for infection, as well as comparing the severity of disease between different pathogen populations, would provide valuable information to help further identify the pathogen's underlying mechanisms of pathogenicity.

Finally, the environment, including weather conditions and soil properties, may have the greatest influence on disease development (Keane and Kerr, 1997). A susceptible host may not be infected by a virulent pathogen if the environmental conditions are unfavourable for disease development (Velásquez *et al*., 2018). Conversely, a suitable environment can promote disease, even in conventionally resistant hosts (Wang *et al.*, 2009; West *et al*., 2012). The effects of weather parameters such as temperature, atmospheric

humidity, sunlight and air movement have long been studied and are frequently included in plant disease forecasting models (Nath *et al*., 2020; Peries, 1971). For light leaf spot, epidemiological studies (Gilles *et al*., 2000a; Papastamati *et al.*, 2002) have shown that epidemics are initiated by ascospores produced on infected oilseed rape stubble in late summer and early autumn. Low temperatures and high humidity (leaf wetness) greatly influence *P. brassicae* asexual sporulation in winter and spring (Gilles *et al*., 2000b). Since weather patterns are closely related to light leaf spot disease outbreaks, regional disease forecasting systems are effective to help predict epidemics and time fungicide applications (Gilles *et al*., 2000a). A common detection method used is to monitor and quantify *P. brassicae* airborne ascospore release over time using an air sampler (Calderon *et al*., 2002; Karolewski *et al*., 2012). This method employs visual (microscopy) or PCR-based assays to study the spatio-temporal distribution of airborne inoculum. When done over several cropping seasons, this practice can help predict the onset of seasonal release of *P. brassicae* ascospores and anticipate light leaf spot disease outbreaks in a given area.

Altogether, plant diseases develop because of complex interactions between host, pathogen and environment. It is paramount to understand each host-pathogen system in its given environment to best deploy suitable management strategies. Some factors that influence light leaf spot development in oilseed rape have been documented, while others are still largely understudied. The hope is that a better understanding of certain key factors can help elucidate light leaf spot disease mechanisms.

3.1.1 Aims and objectives

Experimental work in this chapter aimed to improve the understanding of host, pathogen and environmental factors that contribute to light leaf spot disease development on oilseed rape. Four separate objectives were investigated:

- To monitor the timing and abundance of *P. brassicae* air-borne ascospore release and related seasonal weather conditions

- To study the effects of host plant age on light leaf spot disease development
- To investigate the influence of type of inoculum and inoculum concentration on light leaf spot disease development
- To investigate the effects of light intensity and exposure time on light leaf spot development

3.2 Materials and methods

3.2.1 Effects of weather conditions on *Pyrenopeziza brassicae* air-borne ascospore release

3.2.1.1 Burkard spore tape preparation

Burkard drums were prepared following the method described by Lacey and West (2006). Each drum was first cleaned with 1% anigene and Melinex tape was then tightly wound around the drum and affixed using double-sided tape. Under a fume hood, the drum and Melinex tape were covered with a thin layer of coating solution (10 g petroleum jelly/20 ml hexane mixture) using a Burkard drum coating apparatus (Figure 3.1). The drums were then left to dry for 1-2 hr and placed into individual labelled metal containers.

3.2.1.2 Burkard sampler set up

A Burkard 7-day recording air sampler was set up as described by Lacey and West (2006) at the experimental site at Bayfordbury, Hertfordshire (Figure 3.2). The sampler was operated all year round for three cropping seasons (2020/21, 2021/22, 2022/23). For each season, in August, oilseed rape stubble from the previous cropping season was collected from an untreated field site at Rothamsted Research, Harpenden, and placed around the sampler. Prepared drums were secured onto the Burkard spore sampler according to manufacturers' instructions. The sampler was then operated for periods of seven continuous days before the drum was replaced with a new one. Used drums were stored at 4°C until processing.

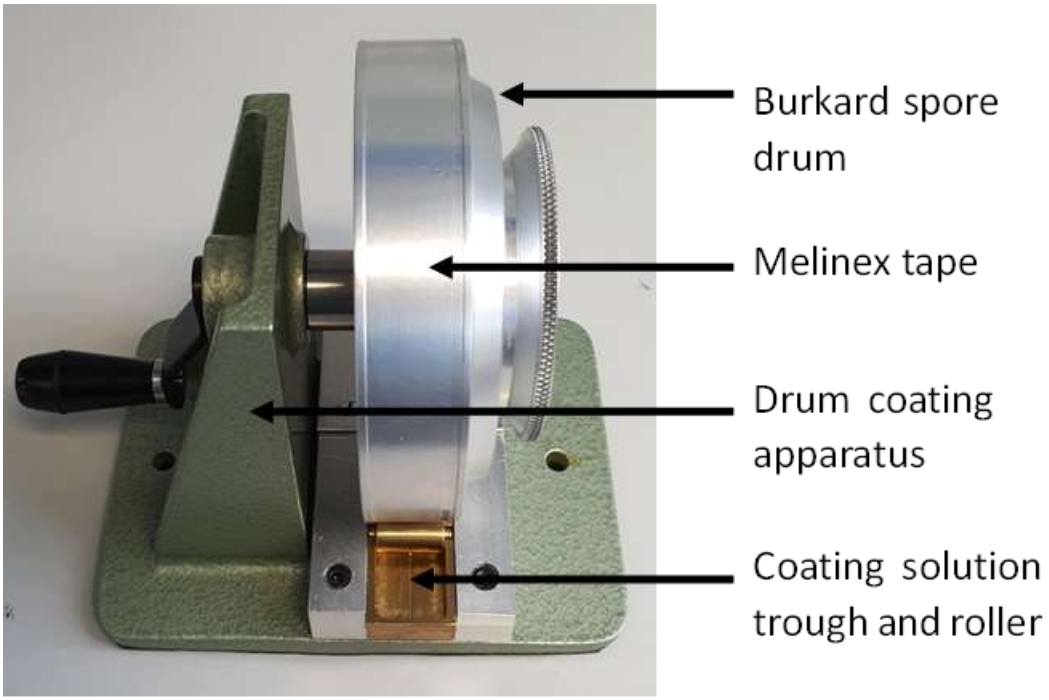

Figure 3.1: Burkard spore drum coating apparatus.

Burkard drums were tightly wound with Melinex tape and subsequently placed onto the drum coating apparatus. A coating solution consisting of a 10 g petroleum jelly/20 ml hexane mixture was added to the coating solution trough. By rotating the drum against the roller within the trough, the coating mixture was transferred from the trough onto the drum, coating the Melinex tape (Lacey and West, 2006).

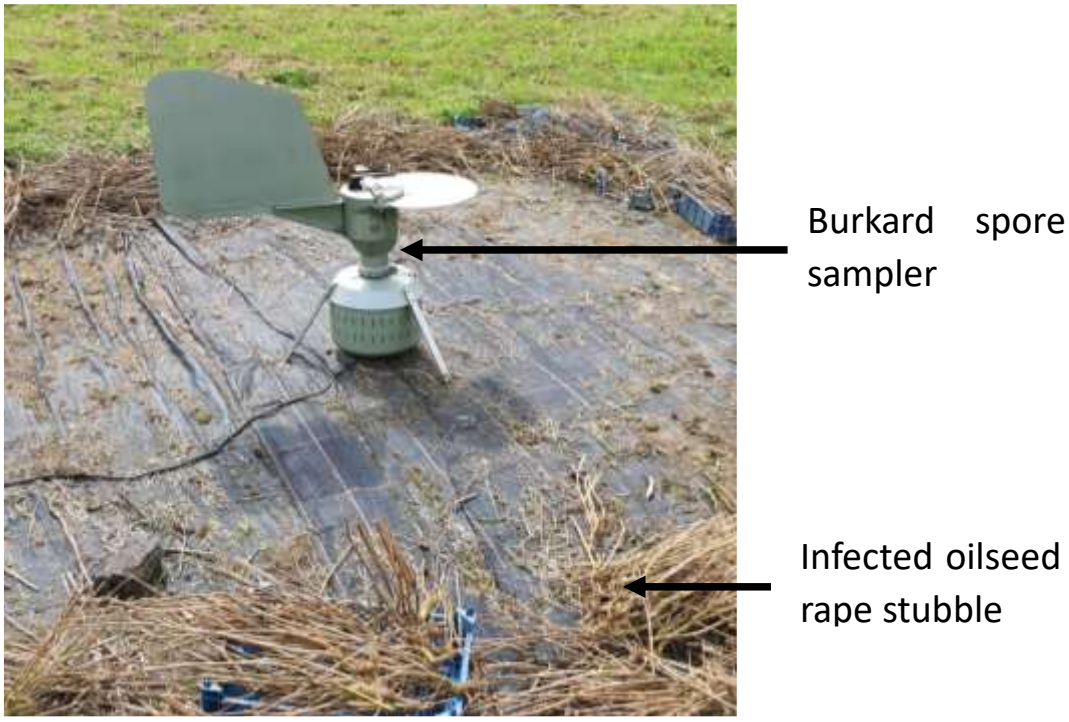

Figure 3.2: Burkard 7-day recording air sampler surrounded by oilseed rape stubble.

A Burkard 7-day recording air sampler containing a prepared Burkard drum lined with Melinex tape was set up at Bayfordbury, Hertfordshire. The sampler was surrounded with infected oilseed rape stubble from the previous cropping season, which released fungal ascospores into the air. *Pyrenopeziza brassicae* airborne ascospores were sampled through an orifice outside of the sampler and deposited onto the tape-coated drum. The drum rotated within the sampler at a fixed speed and over time ascospores were deposited onto the entire tape surface.

3.2.1.3 Burkard spore tape sample processing

Burkard drums were processed following the method described by Lacey and West (2006). The used spore sampler tape was removed from the drum and transferred with the exposed side facing up onto a pre-marked cutting template. The tape was placed starting at the left end of the cutting template and cut into seven equal 48 mm long pieces, each piece corresponding to a 24-hour period of the tape. Each piece was then further cut horizontally, producing two pieces of equal size. One half of the tape was mounted on a slide for other use (e.g. counting numbers of *Leptosphaeria* ascospores), while the other half was placed in a 2 ml screw cap tube and stored at -20°C for DNA extraction.

3.2.1.4 DNA extraction from Burkard spore tape samples

DNA was extracted from samples at alternating dates using a modified version of the CTAB protocol (Graham *et al*., 1994; Kaczmarek *et al.,* 2009; 2024). First, 0.5 g of acid-washed glass beads were added to each sample, followed by 440 µl of DNA extraction buffer (500 mM NaCl, 400 mM Tris-HCl, 50 mM EDTA at pH 8 added to a 1:1 ratio of SDW, 2% PVP and 5mM phenanthroline, followed by 0.1% β-mercaptoethanol at the time of use). Samples were then lysed using a Fast-prep machine at 6.0 m/sec for 40 sec three times, with 2-5 min cooling on ice in between cycles. Next, 400 µl of 2% SDS was added to each sample, then vortexed and incubated for 30 min in a 65°C water bath. After incubation, 800 µl of the bottom phase of cold phenol:chloroform:isoamyl alcohol mixture (25:24:1) was added to each sample. Next, samples were vortexed and centrifuged at 13000 rpm for 10 min at 4°C. The supernatant was then pipetted into a new 1.5 ml Eppendorf tube containing 30 µl ammonium acetate (7.5 M), 480 µl isopropanol and 1 µl glycogen (20 µg μL^{-1}) and samples were inverted and stored at -20°C for 16 hr to precipitate the DNA. The samples were then centrifuged at 13000 rpm for 30 min at 4°C before removing the supernatant. The DNA pellet was then washed with 300 µl of cold 70% ethanol and further centrifuged at 13000 rpm for 10 min at 4°C. The remaining ethanol was then removed, first with a pipette, then by letting excess ethanol evaporate. Once dry, the pellet was resuspended in 30 µl SDW and stored at -20°C for species-specific quantitative PCR.

3.2.1.5 Quantitative PCR

Quantitative PCR (qPCR) reactions were done using a Mx3005 qPCR instrument (Agilent Technologies, UK). Each reaction was a 20 µl standard volume containing 10 µl Brilliant III Ultra-Fast SYBR® Green qPCR master mix with low ROX (Agilent Technologies, UK), 0.6 µl of 10 µM forward primer, 0.6 µl of 10 µM reverse primer, 6.3 µl nuclease-free water and 2.5 µl DNA sample. No-template controls were included by replacing sample DNA with nuclease-free water. A set of six standards was prepared as a 10x dilution series ranging from 1000 pg to 0.01 pg of a pure culture of *P. brassicae* DNA. The *P. brassicae*-specific primers used were PbITSF (5′-TTGAACCTCTCGAAGAAGTTCAGTCT-3′) and PbITSR (5′-AGATTTGGGGGTTGTTGGCTAA-3′) (Karolewski *et al*., 2006).

Each run consisted of a 96-well plate containing DNA standards, sample DNA and no-template controls, all in duplicate. The thermal cycling parameters were: initial denaturation at 95°C for 2 min, followed by 50 cycles at 95°C for 15 sec, 58°C for 45 sec, 72°C for 45 sec and 84°C for 15 sec. After the final amplification, temperature was raised to 95°C for 1 min, then 58°C for 30 sec and 95°C for 30 sec, with a melting curve reading done between the two final steps (Figure 3.3).

Results were visualised and analysed using MxPro qPCR software (Agilent) according to Karandeni Dewage (2019). An example amplification plot of a qPCR run is shown in Figure 3.4. For each run, a standard curve was plotted with the six standards and used to calculate the amount of *P. brassicae* DNA in the unknown samples. The performance of the qPCR reactions was evaluated using the reaction efficiency (with a threshold value of 70%) and the R^2 value of the standard curve (Figure 3.5). The production of the correct amplicon was confirmed using the dissociation curve (Figure 3.6).

3.2.1.6 Weather data collection and formatting

Weather data were collected from a weather station set up at Bayfordbury, Hertfordshire. The main weather parameters used were temperature (°C) and daily rainfall (mm). Data were collected for the period between July 2020 and

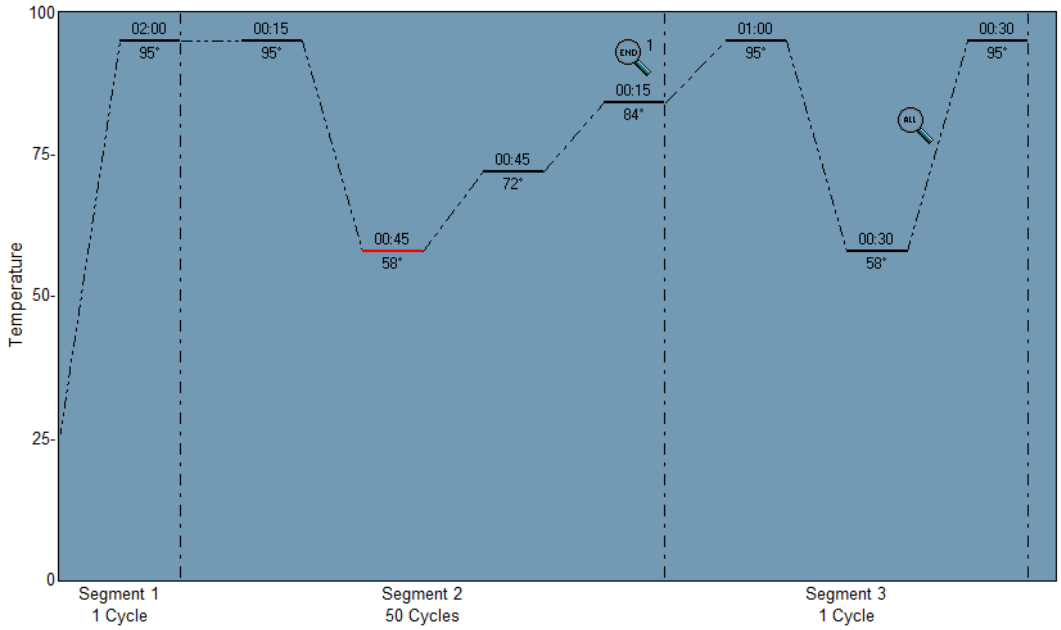

Figure 3.3: Quantitative PCR thermal profile for *Pyrenopeziza brassicae.*

A thermal profile consisting of three segments was used to quantify the amount of *P. brassicae* DNA in an unknown sample. The thermal cycling parameters were: initial denaturation at 95°C for 2 min, followed by 50 cycles at 95°C for 15 sec, 58°C for 45 sec, 72°C for 45 sec and 84°C for 15 sec. After the final amplification, temperature was raised to 95°C for 1 min, then 58°C for 30 sec and 95°C for 30 sec, with a melting curve reading done between the two final steps.

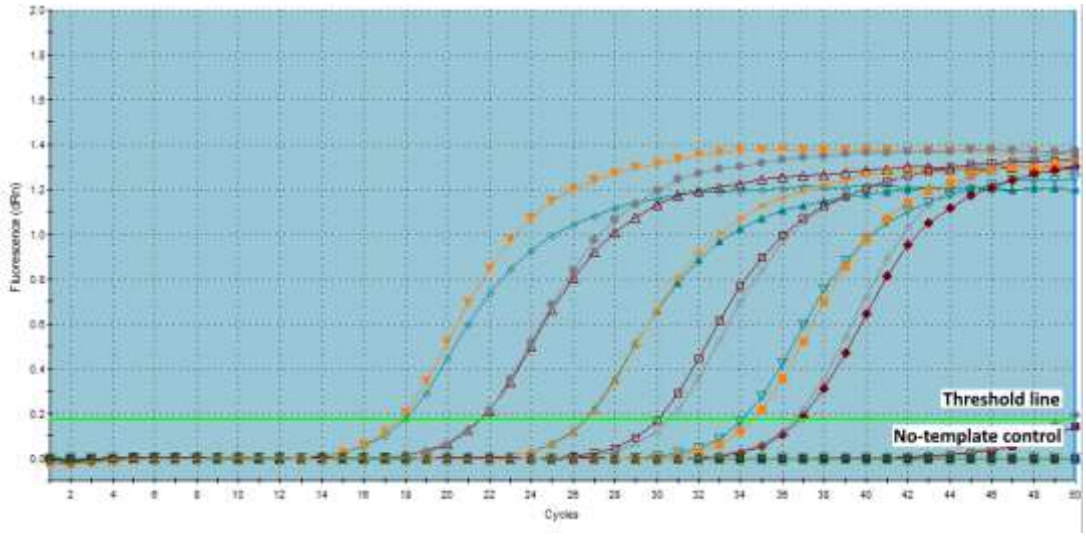

Figure 3.4: Quantitative PCR amplification curve for *Pyrenopeziza brassicae.*

An example amplification curve of the six *P. brassicae* DNA standards in duplicate used in qPCR (prepared as a 10x dilution series ranging from 1000 pg to 0.01 pg of *P. brassicae* DNA) plotted as an increase in fluorescence signal against the cycle number. The intersection between the fluorescence threshold (background fluorescence) and an amplification curve was used to measure the threshold cycle (C_t) for each sample.

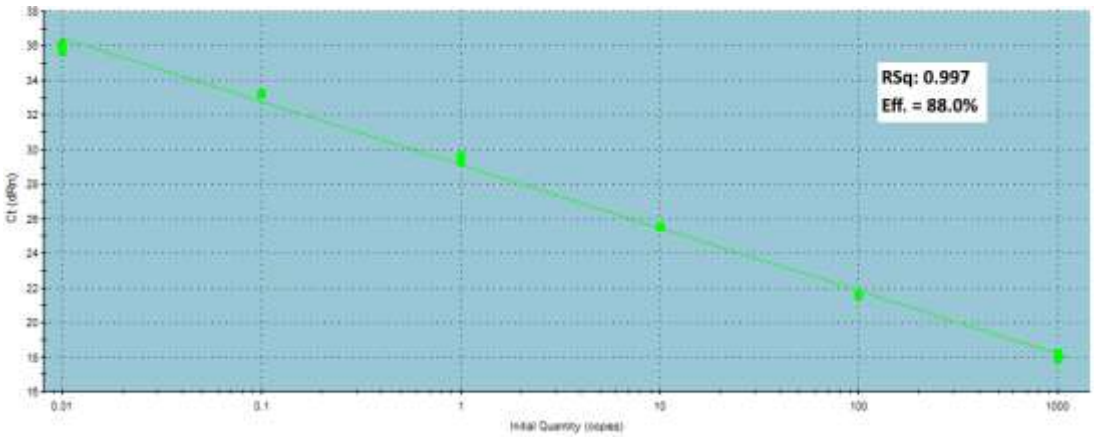

Figure 3.5: Quantitative PCR standard curve for *Pyrenopeziza brassicae.*

Six standards prepared in duplicate as a 10x dilution series ranging from 1000 pg to 0.01 pg of *P. brassicae* DNA. For each qPCR run, the standard curve generated was used to calculate the amount of *P. brassicae* DNA in the unknown samples. The performance of the qPCR reactions was evaluated using the reaction efficiency (with a threshold value of 70%) and the R^2 value of the standard curve.

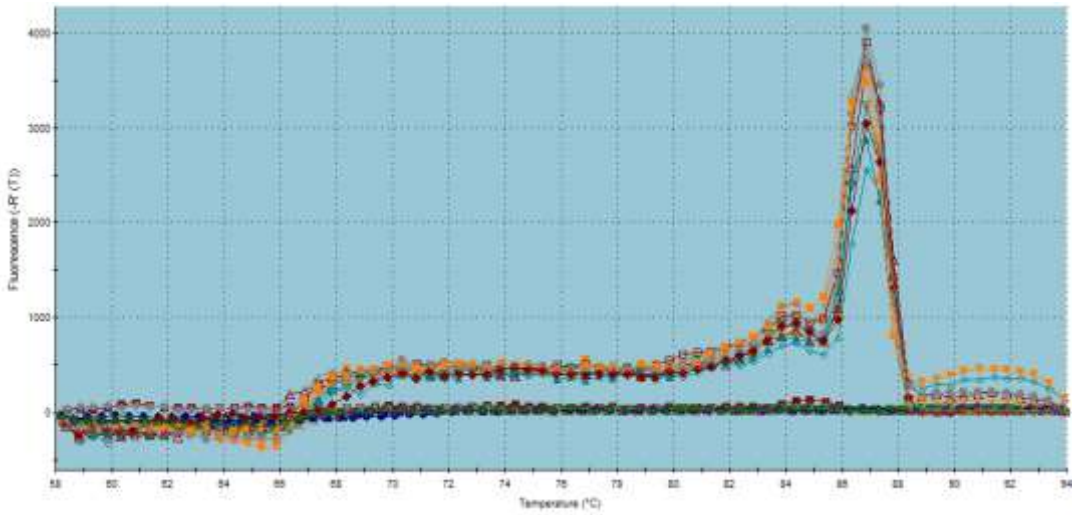

Figure 3.6: Quantitative PCR dissociation curve for *Pyrenopeziza brassicae.*

An example dissociation curve of the six *P. brassicae* DNA standards in duplicate used in qPCR (prepared as a 10x dilution series ranging from 1000 pg to 0.01 pg of *P. brassicae* DNA). The presence of a unique dissociation peak as the melting temperature (T_m) of the amplicon confirmed the specificity of the qPCR reaction.

October 2023. Mean daily averages were used to calculate mean monthly averages.

3.2.2 Effects of plant age on light leaf spot symptom development

Experimental work in this section consisted of two experiments. One experiment investigated light leaf spot disease phenotype in plants aged four-, three-, two- or one-week-old in controlled environment and glasshouse conditions (experiment 1). The other experiment investigated light leaf spot disease phenotype in plants aged six- or four-weeks-old in glasshouse conditions (experiment 2). The individual experiments are described below and summarized in Table 3.1.

3.2.2.1 Preparation of plant material

Susceptible oilseed rape cultivar Charger (LLS resistance rating 4 on a 1-9 scale where 9 is most resistant, AHDB Recommended List 2016/2017) was used for this study. Plants were grown in controlled environment conditions according to the method described in section 2.3.1 until they reached the designated growth stages (e.g. one-, two-, three-, four- or six-weeks-old). For experiment 1, plants were arranged in a complete randomized design, with four inoculated replicates for each treatment. For experiment 2, plants were arranged in a split-plot design generated using Experiment Design Generator and Randomiser (EDGAR) (Brown, 2005), where replicates were blocks, plant age was the main plot and inoculum treatments were the sub-plots. For each treatment, twelve replicate plants were used.

3.2.2.2 Preparation of *P. brassicae* inoculum

P. brassicae inoculum 15WOSR64-SS1 (Table 2.1), which is a single-spore *P. brassicae* isolate, was used in both experiment 1 and experiment 2. For experiment 2, a second inoculum, Pb-Scotland (Table 2.1), which is a *P. brassicae* population collected from a field experiment in Scotland, was also included. The inoculum was bulked up from diseased leaves from oilseed rape cultivar Charger collected from controlled environment-grown plants and incubated at 4°C for 7-9 days. *P. brassicae* inoculum was prepared from the

Table 3.1: Summary of controlled environment and glasshouse experiments.

Two experiments were done to investigate the effects of plant age on light leaf spot symptom development. Location, plant age in weeks at inoculation and *Pyrenopeziza brassicae* inoculum concentration for each experiment are shown.

Experiment	Location	Plant age (weeks) at inoculation	*P. brassicae* inoculum type	*P. brassicae* inoculum concentration
1	Controlled environment or Glasshouse	1, 2, 3 or 4	Single-spore isolate	10^4 or 10^5 spores/ml
2	Glasshouse	4 or 6	Single-spore isolate, Population	10^5 or 10^6 spores/ml

incubated leaves following the method described in section 2.2.2. Plants were spray-inoculated with conidial suspensions amended with 0.005% Tween 80 as described in section 2.3.2. For experiment 1, two concentrations, 10^4 and 10^5 spores/ml, were chosen. For experiment 2, two concentrations, 10^5 and 10^6 spores/ml, were chosen.

3.2.2.3 Assessment of light leaf spot symptoms

Plants were harvested at 23 or 24 days post inoculation (dpi), placed in polyethylene bags and incubated at 4°C for 7-9 days to induce *P. brassicae* sporulation. Individual plants were then scored for light leaf spot disease using a 1-8 scale (with 1 being no disease, Table 2.2) and for percentage leaf area covered with *P. brassicae* asexual sporulation (acervuli). Presence of other symptoms, such as total number of leaves, numbers of deformed leaves, dead leaves and yellow/senescent leaves, were also recorded. Plant height (cm) and wet weight (g) were also measured, after which plants were desiccated for 48 hr at 60°C, before measuring dry weight (g).

3.2.2.4 Statistical analysis

Analysis of variance was done using R (version 4.2.2). Data transformations were applied to all parameters, except for the light leaf spot disease score, height, wet weight and dry weight. The numbers of deformed leaves, dead leaves and yellow/senescent leaves were expressed as percentages by dividing the numbers of affected leaves by the total number of leaves on each plant. These values, along with the percentage leaf area covered by *P. brassicae* sporulation, were transformed using an arc-sine transformation.

Correlations between light leaf spot disease score, % leaf area with sporulation, % deformed leaves, % dead leaves and % yellow/senescent leaves were analysed using Microsoft Excel. Plots were generated in Microsoft Excel using calculated means for the different treatments.

Analyses of position and parallelism (Fitt *et al*., 1998b; Payne *et al*., 1993) were done to investigate the relationship between light leaf spot disease score and plant age in days at inoculation between the two different inoculum

concentrations (10^4 and 10^5 spores/ml). Regression analyses were based on all the data-points. Plots were generated using Genstat (Payne *et al*., 2011).

3.2.3 Effects of type of *P. brassicae* inoculum and inoculum concentration on light leaf spot symptom development

The experiment described in this section is the same as experiment 2 from section 3.2.2, but only plants aged four-weeks-old were included in this analysis, as that is the standard inoculation age and growth stage used for light leaf spot studies.

3.2.3.1 Preparation of plant material

Susceptible oilseed rape cultivar Charger was used for this experiment. Plants were grown in controlled environment conditions according to the method described in section 2.3.1 until they reached growth stage 1,4-1,5 (Sylvester-Bradley, 1985). Plants were arranged in a split-plot design generated using Experiment Design Generator and Randomiser (EDGAR) (Brown, 2005), where replicates were blocks, plant age was the main plot, and inoculum treatments were the sub-plots. For each treatment, 12 replicate plants were used. Each age group included an uninoculated control group.

3.2.3.2 Preparation of *P. brassicae* inoculum

P. brassicae inoculum Scotland-Pb (population) and 15WOSR64-SS1 (single-spore *P. brassicae* isolate) (Table 2.1) were used. The two types of inoculum were bulked up from diseased leaves from oilseed rape cultivar Charger collected from controlled environment-grown plants and incubated at 4°C for 7-9 days. *P. brassicae* inoculum was prepared from the incubated leaves following the method described in section 2.2.2. For both types of *P. brassicae* inoculum, spore concentration was adjusted to either 10^5 spores/ml or 10^6 spores/ml, amended with 0.005% Tween 80. Plants were spray-inoculated with conidial suspensions as described in section 2.3.2.

3.2.3.3 Assessment of light leaf spot symptoms

Plants were harvested and assessed at 24 dpi, placed in polyethylene bags and incubated at 4°C for 7-9 days to induce *P. brassicae* sporulation. Individual plants were scored for light leaf spot disease using a 1-8 scale (with 1 being no disease) and for percentage leaf area covered with *P. brassicae* asexual sporulation (acervuli). Presence of other symptoms such as total number of leaves, numbers of deformed leaves, dead leaves, discoloured leaves (red, purple, etc.) and yellow/senescent leaves were also recorded. Presence of a necrotic response in different areas of the leaf (petiole, midrib and lamina) was also assessed.

3.2.3.4 Statistical analysis

Analysis of variance was done using R (version 4.2.2). Since one of the age groups, which comprised the main plots in the split-plot design, was disregarded for this analysis, the plants were considered as arranged in a randomized block design instead. Data transformations were applied to all parameters, except for the light leaf spot disease score. The numbers of deformed leaves, dead leaves, discoloured leaves and yellow/senescent leaves were expressed as percentages by dividing the number of symptomatic leaves by the total number of leaves on each plant. These values, along with the percentage leaf area covered in *P. brassicae* sporulation, were transformed using an arc-sine transformation. The presence or absence of a necrotic response in different areas of the leaf was taken as a qualitative measure.

Correlations between light leaf spot disease score, % leaf area with sporulation, % deformed leaves, % dead leaves, % discoloured leaves and % yellow/senescent leaves were analysed using Microsoft Excel. Plots were generated in Microsoft Excel using calculated means for the different treatments.

3.2.4 Effects of light intensity on light leaf spot symptom development

This experiment was conducted following observations from a different glasshouse experiment. A detailed rationale is outlined in section 3.3.4.1.

3.2.4.1 Preparation of plant material and lighting times conditions

Susceptible oilseed rape cultivar Charger was used for this experiment. Plants were grown in controlled environment conditions according to the method described in section 2.3.1 until they reached growth stage 1,4-1,5. Plants were arranged in a complete randomized design. For each treatment, 15 inoculated replicates and five uninoculated controls were used. The design was repeated on two shelves of the controlled-environment cabinet with different lighting

conditions; one shelf operated on a 12 hr light/12 hr dark period at a light intensity of 249 µmol m^{-2} s^{-1}, and the other shelf operated on a 8 hr light/16 hr dark period at a higher light intensity of 326 µmol m^{-2} s^{-1}.

3.2.4.2 Preparation of *P. brassicae* inoculum

P. brassicae inoculum Scotland-Pb (Table 2.1) was used. The inoculum was bulked up from diseased leaves from oilseed rape cultivar Charger collected from controlled environment-grown plants and incubated at 4°C for 7-9 days. *P. brassicae* inoculum was prepared from the incubated leaves following the method described in section 2.2.2. Plants were spray-inoculated with conidial suspensions (10^5 spores/ml + 0.005% Tween 80) as described in section 2.3.2.

3.2.4.3 Assessment of light leaf spot symptoms

Plants were originally planned to be harvested and assessed at 24 dpi; however, due to the early onset of symptoms, plants were instead harvested and assessed at 18 dpi. Individual plants were scored for light leaf spot disease using a 1-8 scale (with 1 being no disease) and for percentage leaf area covered with *P. brassicae* asexual sporulation (acervuli). Presence of other symptoms such as total numbers of leaves, deformed leaves, dead leaves, discoloured leaves (red, purple, etc.) and yellow/senescent leaves, were recorded at 3, 8, 14, 16 and 18 dpi. The presence of a necrotic response in

different areas of the leaf (petiole, midrib and lamina) was also recorded. Plants were then incubated at 4°C for 7-9 days to induce *P. brassicae* sporulation, and both disease score and % leaf area with sporulation were recorded. After assessment, *P. brassicae* sporulation was removed from the plants through leaf washing (section 2.2.2). Plants were further incubated at 4°C for 7-9 days and % leaf area with sporulation was recorded. Leaf washing, incubation and % leaf area with sporulation assessment were then done one more time. Overall, % leaf area with sporulation was assessed at 18, 27, 34 and 41 dpi, with leaf washing done between each assessment. Plant height (cm) and wet weight (g) were also measured, after which plants were desiccated for 48 hr at 60°C, before measuring dry weight (g).

3.2.4.4 Statistical analysis

Analysis of variance was done using R (version 4.2.2). Data transformations were applied to all parameters, except for the light leaf spot disease score, height, wet weight and dry weight. The numbers of dead leaves, deformed leaves, discoloured leaves and yellow/senescent leaves were expressed as percentages by dividing the number of affected leaves by the total number of leaves on each plant. These values, along with the percentage leaf area covered by *P. brassicae* sporulation, were transformed using an arc-sine transformation. The presence or absence of a necrotic response in different areas of the leaf was taken as a qualitative measure.

3.3 Results

3.3.1 Effects of weather conditions on *Pyrenopeziza brassicae* air-borne ascospore release

P. brassicae DNA quantified from spore tapes using qPCR over three cropping seasons (2020/21, 2021/22, 2022/23) showed that there were differences in timing and abundance of ascospore release. Amount of DNA was then analysed in relation to temperature and rainfall data over the three seasons. In the 2020/2021 season, airborne ascospore release was monitored from 24 September, meaning that data for August and September 2020 were unavailable. The average daily temperature ranged between a minimum of -

1.5°C in February to a maximum of 20.7°C in July. Average monthly temperature was at its lowest in January (3.6°C) and it was at its highest in July (17.67°C) (Table 3.2). The average daily rainfall ranged between a minimum of 0 mm at various times throughout the cropping season, to a maximum of 30 mm in July. Monthly rainfall was at its lowest in April (0.1 mm) and at its highest in October (4.1 mm) (Table 3.3). There were two major periods of ascospore release. The first period was throughout October, with a maximum of 256.92 pg of DNA detected on 5 October. The second period was between mid-June and July, with a maximum of 664.68 pg of DNA, the highest in the season, detected on 28 July. Temperature differed between the two maximum ascospore release periods. Mean temperature during the first maximum on 5 October was 12°C, while the temperature during the second maximum on 28 July was 16°C. Mean temperature throughout the first ascospore release period in October was 11°C, while mean temperature during the second period from mid-June to July was 16.5°C. Rainfall was recorded before and during maximum ascospore release periods. The first maximum on 5 October was preceded by 22.2 mm of rainfall on 3 October, the highest value in that month, and the second maximum on 28 July was preceded by 30 mm of rainfall on 25 July, the greatest value in the cropping season. Overall, ascospore release was maximum when rainfall was >10 mm and at temperatures between 10-16°C (Figure 3.7a).

In the 2021/2022 season, the average daily temperature ranged between a minimum of -0.25°C in December and a maximum of 28.3°C in July. Monthly temperature was at its lowest in January (4.7°C) and it was at its highest in July (19.5°C) (Table 3.2). The average daily rainfall ranged between a minimum of 0 mm at various times throughout the cropping season and a maximum of 22.8 mm in September. Monthly rainfall was at its lowest in April (0.62 mm) and it was at its highest in October (2.87 mm) (Table 3.3). There were two major periods of ascospore release. The first period was from August until mid-September, with a maximum of 766.98 pg of DNA detected on 18 August and another maximum of 885.3 pg on 10 September. There was a long period of ascospore release from mid-April until mid-July, with a maximum of 474.24 pg

Table 3.2: Mean monthly temperatures over three cropping seasons at Bayfordbury.

Weather data were collected from a weather station set up at Bayfordbury, Hertfordshire, and used to calculate mean monthly temperatures (°C). Data were collected over three cropping seasons (2020/2021, 2021/2022, 2022/2023), where each new season began in August after new oilseed rape stubble was scattered around the Burkard spore sampler to monitor *Pyrenopeziza brassicae* ascospore release.

Temperature (°C)

Month	Season		
	2020/2021	2021/2022	2022/2023
August	18.47	16.10	19.40
September	14.57	15.88	14.56
October	10.88	12.08	13.07
November	8.68	7.41	9.64
December	5.54	7.14	4.01
January	3.68	4.78	5.04
February	5.55	7.39	5.99
March	7.15	7.77	7.39
April	6.33	9.42	8.68
May	10.49	13.47	12.30
June	16.41	15.83	17.45
July	17.68	19.51	16.92
Season mean	10.45	11.40	11.20

Table 3.3: Mean monthly rainfall over three cropping seasons at Bayfordbury.

Weather data were collected from a weather station set up at Bayfordbury, Hertfordshire, and used to calculate mean daily rainfall per month (calculated using mean daily rainfall (mm)). Data were collected over three cropping seasons (2020/2021, 2021/2022, 2022/2023), where each new season began in August after new oilseed rape stubble was scattered around the Burkard spore sampler to monitor *Pyrenopeziza brassicae* ascospore release.

Rainfall (mm) (mean daily rainfall per month)

Month	Season		
	2020/2021	2021/2022	2022/2023
August	3.86	1.24	1.32
September	0.98	1.89	1.96
October	4.12	2.88	2.88
November	1.49	0.37	3.71
December	2.52	1.96	1.72
January	2.76	0.66	2.95
February	1.23	2.65	0.24
March	0.96	1.10	3.40
April	0.11	0.62	2.03
May	3.39	1.23	1.25
June	1.68	0.92	0.94
July	3.62	0.21	2.21
Season mean	2.23	1.31	2.05

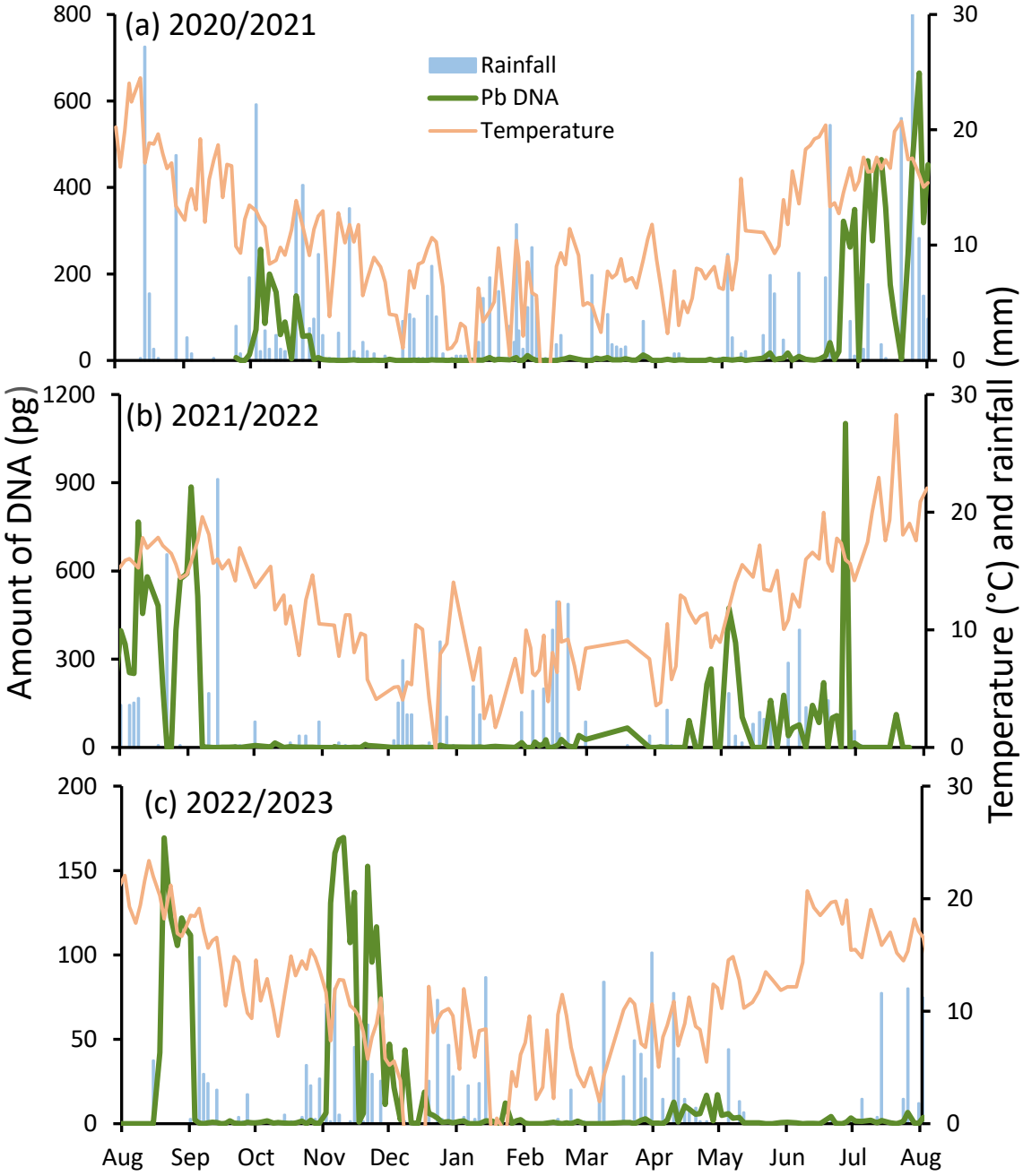

Figure 3.7: Amount (pg) of *Pyrenopeziza brassicae* DNA in relation to mean daily temperature (°C) and rainfall (mm) from (a) 2020/2021, (b) 2021/2022, (c) 2022/2023 cropping seasons at Bayfordbury.

A Burkard spore sampler surrounded by oilseed rape crop stubble from the previous cropping season was set up at Bayfordbury, Hertfordshire, to monitor *P. brassicae* ascospore release. Amount (pg) of *P. brassicae* DNA was quantified from spore tapes in 30 µl of final volume using qPCR and plotted in relation to mean daily temperature (°C) and rainfall (mm) from (a) 2020/2021, (b) 2021/2022 and (c) 2022/2023 cropping seasons. The primary y axes (showing the amount of *P. brassicae* DNA in pg) are different scales for each cropping season.

of DNA detected on 15 May and another maximum of 1101.54 pg on 3 July, which was also the greatest maximum in the season. Temperature ranged between 15-17°C during both ascospore release periods. Mean temperature during the first ascospore release period in August-September was 16.4°C, with mean daily temperatures of 17.8°C and 18.09°C during the two maxima on 18 August and 10 September, respectively. Mean temperature during the second period in April-July was 14.42°C, with mean daily temperatures of 14.48°C and 15.83°C during the two maxima on 15 May and 3 July, respectively. Mean rainfall was overall less than in the previous season. During both maxima in the first ascospore release period, daily rainfall reached only 4.6 mm and was even less during both maxima in the second ascospore release period, reaching only 2 mm. Overall, ascospore release this time happened with less rain than in 2020/21 and at temperatures between 14-17°C (Figure 3.7b).

In the 2022/2023 season, the average daily temperature ranged between a minimum of -3.02°C in December, the coldest period across all three cropping seasons, and a maximum of 23.37°C in August. Monthly temperature was at its lowest in December (4°C), and it was at its highest in August (19.39°C) (Table 3.2). The average daily rainfall ranged between a minimum of 0 mm at various times throughout the cropping season and a maximum of 15.2 mm in March. Monthly rainfall was at its lowest in February (0.2 mm) and it was at its highest in November (3.7 mm) (Table 3.3). There were again two major periods of ascospore release, but this time, the overall amount of DNA was significantly less than in the previous two seasons, and a second maximum occurred in winter, rather than summer. The first period was in the second half of August, with a maximum of 161.26 pg of DNA detected on 20 August. The second period was from November until mid-December, with two maxima at 169.56 pg of DNA detected on 10 November (the highest in the season) and 152.58 pg of DNA detected on 21 November. Temperatures were different between the two ascospore release periods. Mean temperature during the first period in August was 18.46°C, while mean temperature during the second period in November-December was 6.35°C, which included an unusual frost period between 8-17 December 2022. Mean temperature during the first maximum on 20 August was

18.21°C, while temperatures during the two later maxima were 12.76°C on 10 November and 5.73°C on 21 November, respectively. Rainfall was almost entirely absent in August; however, major ascospore release in this period began shortly after the only recorded rainfall (5.6 mm on August 15). During the second period, high rainfall on 2 November (10.6 mm) and again on 6 November (12 mm) preceded a period of substantial ascospore release between 2-15 November. The second maximum of that period on 21 November was also associated with high rainfall (8.8 mm). Overall, ascospore release in this season was much less than in the previous two seasons and one major release occurred in winter at lower temperatures than previously observed. Ascospore release, however, once again was maximal when there was moderate rainfall (> 5 mm) (Figure 3.7c).

Overall, most *P. brassicae* DNA was detected in the summer months (May – July), with smaller maxima in early-to-late autumn (August -November). One notable exception was the 2022/2023 season, where minimal DNA detection occurred during the summer, and spore release instead occurred in late autumn-winter. Spore release was most abundant in the 2021/2022 season and was at its lowest in the 2022/2023 season. Temperatures during periods of maximum ascospore release ranged between 10-17°C, with a notable exception in the 2022/2023 season, where ascospore release happened in November, with average temperature of 6°C. Rainfall seemed to precede or accompany major ascospore releases, although rainfall was significantly less in the 2021/2022 season, despite this also being the season with the highest ascospore release maximum across all three seasons (1101.54 pg of DNA on 3 July). Most ascospore release did not occur between January and April, except for some ascospore release in March during the 2021/2022 season, which was then followed by a long second ascospore release period between May and July.

3.3.2 Effects of plant age on light leaf spot symptom development

Results of two separate experiments are presented. The first experiment investigated light leaf spot disease phenotypes on plants aged one-, two-, three- or four-weeks-old in controlled environment and glasshouse conditions

(experiment 1) and the second experiment investigated disease phenotypes on plants aged four- or six-weeks-old in glasshouse conditions (experiment 2). The results from light leaf spot disease score (1-8 scale with 1 being no disease) and percentage leaf area with *P. brassicae* sporulation in experiment 1 showed significant differences between treatments, although both disease score and % area with sporulation were small across all treatments (mean score min: 2, max: 5; mean % area with sporulation min: 0, max: 12.5) (Figure 3.8). There were no significant differences between controlled environment and glasshouse conditions for disease score ($P > 0.59$) and % area with sporulation ($P > 0.07$). There were significant correlations between plant age and disease score (sample correlation coefficient = 0.81, $P < 0.001$, $n = 16$) and plant age and % sporulation (sample correlation coefficient = 0.73, $P < 0.001$, $n = 16$) (Figure 3.9). Both disease score ($P < 0.001$) and % area with sporulation ($P < 0.001$) significantly increased with plant age and there was a positive relationship between light leaf spot disease score and plant age for both inoculum concentrations, with 72.2% of the variance accounted for in linear regressions (Figure 3.10). The greater inoculum concentration also tended to cause greater disease scores ($P < 0.002$) across all age groups but showed no significant differences for % area with sporulation ($P > 0.15$). There was a good correlation between the two assessment techniques (sample correlation coefficient = 0.88, $P > 0.001$, $n = 16$) (Figure 3.11).

Results from experiment 2 in the glasshouse showed significant differences between treatments (Figure 3.12). There were no significant differences between age groups for disease score ($P > 0.74$), but four-week-old plants showed significantly greater % area with sporulation ($P > 0.02$) than six-week-old plants. Plants inoculated with the *P. brassicae* population, compared to the single *P. brassicae* isolate, also had greater disease scores ($P < 0.001$) and % area with sporulation ($P < 0.001$). Overall, plants inoculated with the *P. brassicae* population at 10^6 spores/ml had the greatest disease score and % area with sporulation for both age groups. There was a significant correlation between the two assessment techniques (sample correlation coefficient = 0.99, $P < 0.001$, $n = 10$) (Figure 3.13). Altogether, light leaf spot disease severity increased with plant age and inoculum concentration.

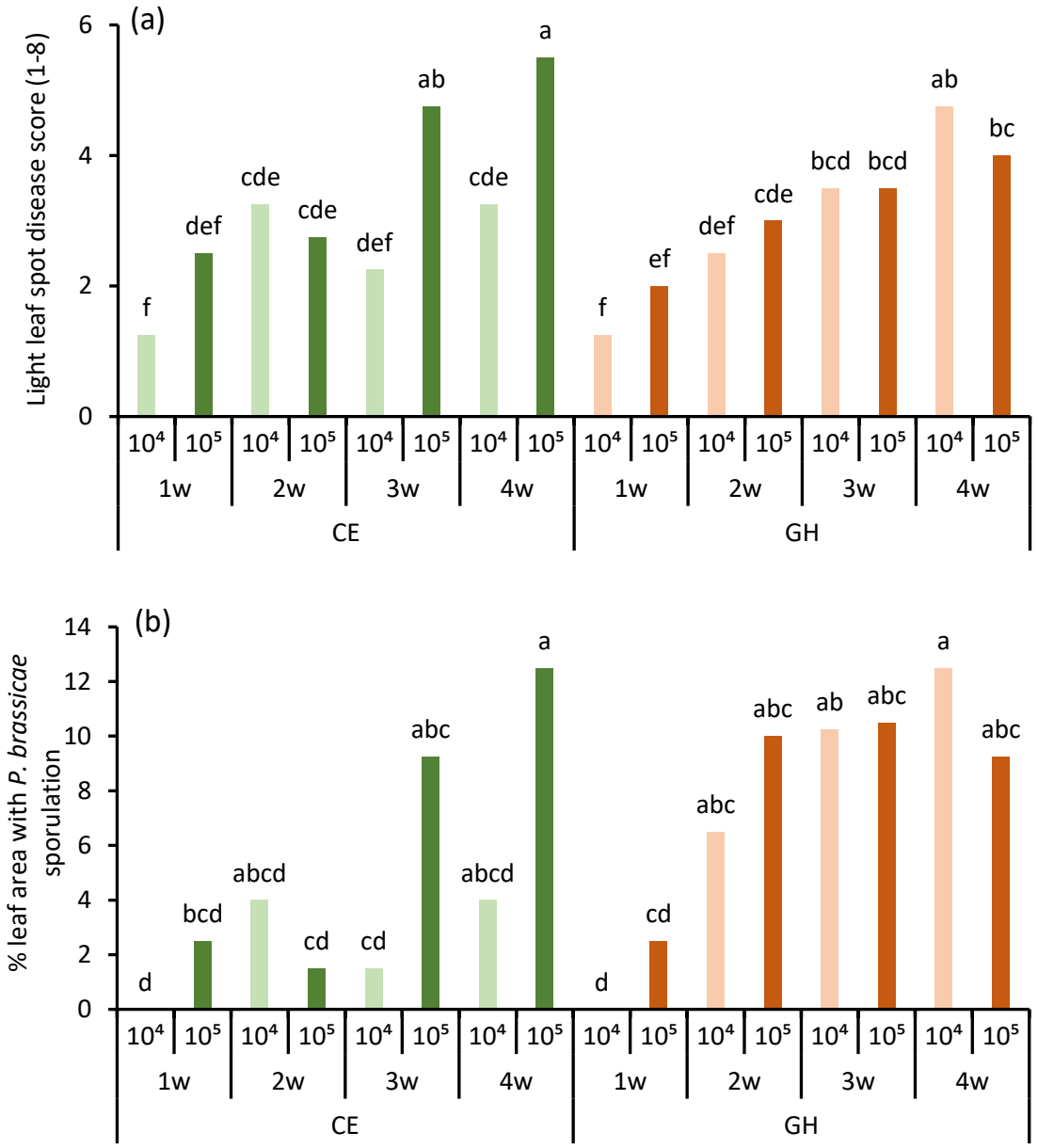

Figure 3.8: (a) Light leaf spot disease score and (b) percentage leaf area covered with *Pyrenopeziza brassicae* sporulation on oilseed rape plants at different locations, plant ages and inoculum concentrations.

Experiment investigating light leaf spot disease phenotype on plants grown in two different conditions (controlled-environment cabinet (CE, green) or glasshouse (GH, red)), aged four-, three-, two- or one-week-old, and inoculated with *P. brassicae* conidial suspensions at either 10^4 spores/ml (light columns) or 10^5 spores/ml (dark columns). Disease severity was measured using (a) disease score on a 1-8 scale (with 1 being no disease) and (b) percentage leaf area covered by *P. brassicae* sporulation. Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, d.f. = 48, (a) LSD = 1.31, (b) LSD = 0.209).

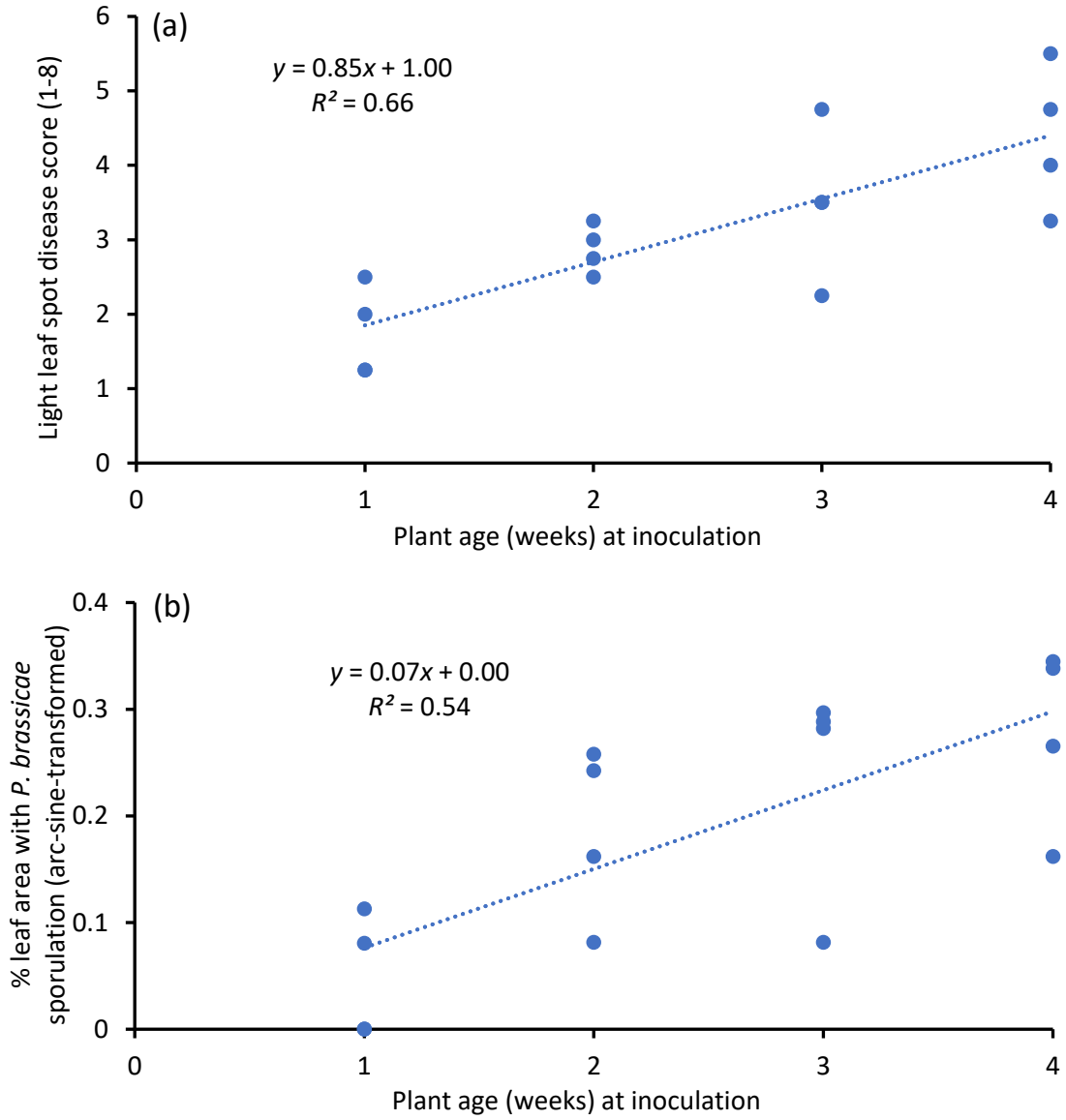

Figure 3.9: Correlation between plant age (weeks) at inoculation and (a) light leaf spot disease score or (b) percentage leaf area covered with *Pyrenopeziza brassicae* sporulation.

Experiment investigating light leaf spot disease phenotype in plants grown in two different conditions (controlled-environment cabinet or glasshouse), aged four-, three-, two- or one-week-old, and inoculated with *P. brassicae* conidial suspensions at either 10^4 spores/ml or 10^5 spores/ml. Plants were assessed for light leaf spot severity using a disease score on a 1-8 scale (with 1 being no disease) and by visual estimation of the % leaf area covered in *P. brassicae* sporulation after samples were incubated at 4°C for 7-9 days. Regression analysis was done for plant age against disease score (sample correlation coefficient = 0.81, $P < 0.001$, $n = 16$) and plant age against % leaf area with sporulation (sample correlation coefficient = 0.73, $P < 0.001$, $n = 16$). Percentage leaf area with sporulation values are presented on an arc-sine-transformed scale.

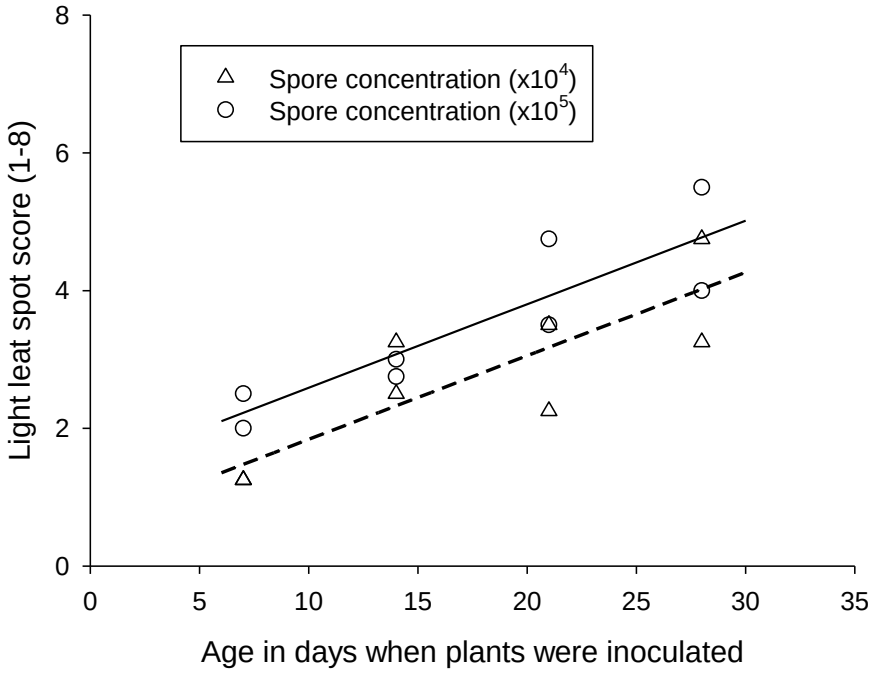

Figure 3.10: Relationship between light leaf spot disease score and plant age (days) at inoculation.

Experiment investigating light leaf spot disease phenotype in plants grown in two different conditions (controlled-environment cabinet or glasshouse), aged four-, three-, two- or one-week-old, and inoculated with *Pyrenopeziza brassicae* conidial suspensions at either 10^4 spores/ml or 10^5 spores/ml. Analysis of position and parallelism showed that the two spore concentrations differed significantly in the line position but shared the same slope. Equations of the fitted lines are: $y = 0.63 + 0.13x$ for inoculum concentration of 10^4 spores/ml (dotted line) and $y = 1.38 + 0.13x$ for 10^5 spores/ml (solid line). The fitted lines accounted for 72.2% of the variation in observed light leaf spot severity scores by plant age (days) at inoculation.

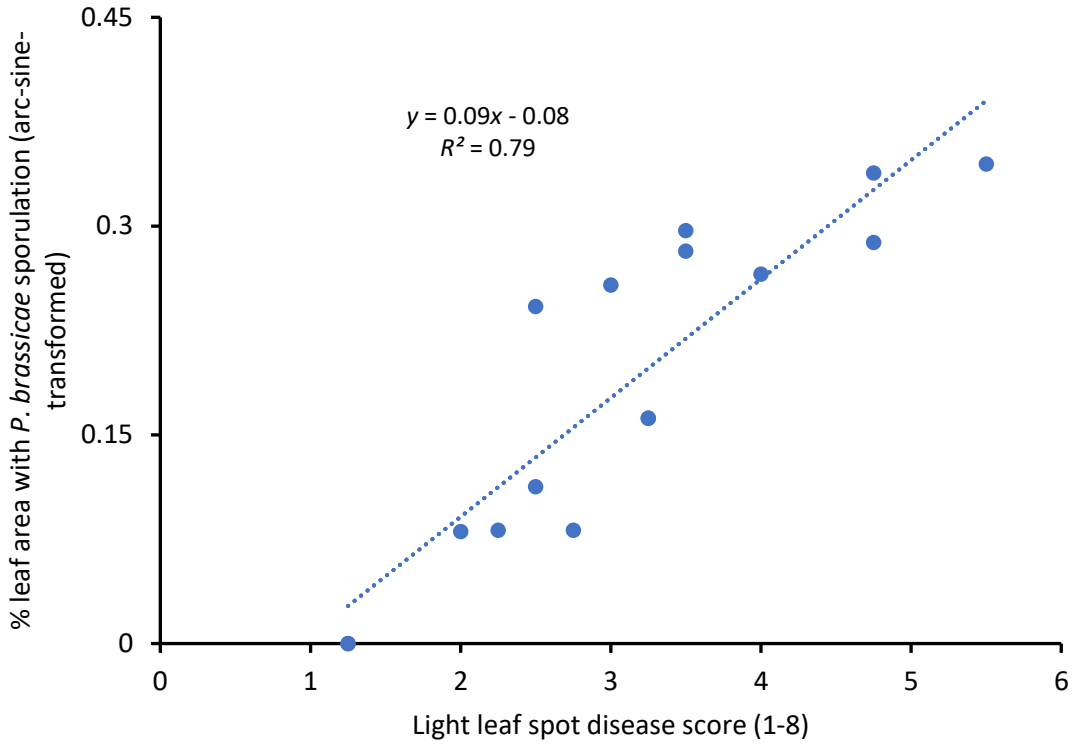

Figure 3.11: Correlation between light leaf spot disease score and percentage leaf area covered with *Pyrenopeziza brassicae* sporulation.

Experiment investigating light leaf spot disease phenotype in plants grown in two different conditions (controlled-environment cabinet or glasshouse), aged four-, three-, two- or one-week-old, and inoculated with *P. brassicae* conidial suspensions at either 10^4 spores/ml or 10^5 spores/ml. Plants were assessed for light leaf spot severity using a disease score on a 1-8 scale (with 1 being no disease) and by visual estimation of the % leaf area covered in *P. brassicae* sporulation after samples were incubated at 4°C for 7-9 days (sample correlation coefficient = 0.88, $P > 0.001$, $n = 16$). Percentage leaf area with sporulation values are presented on an arc-sine-transformed scale.

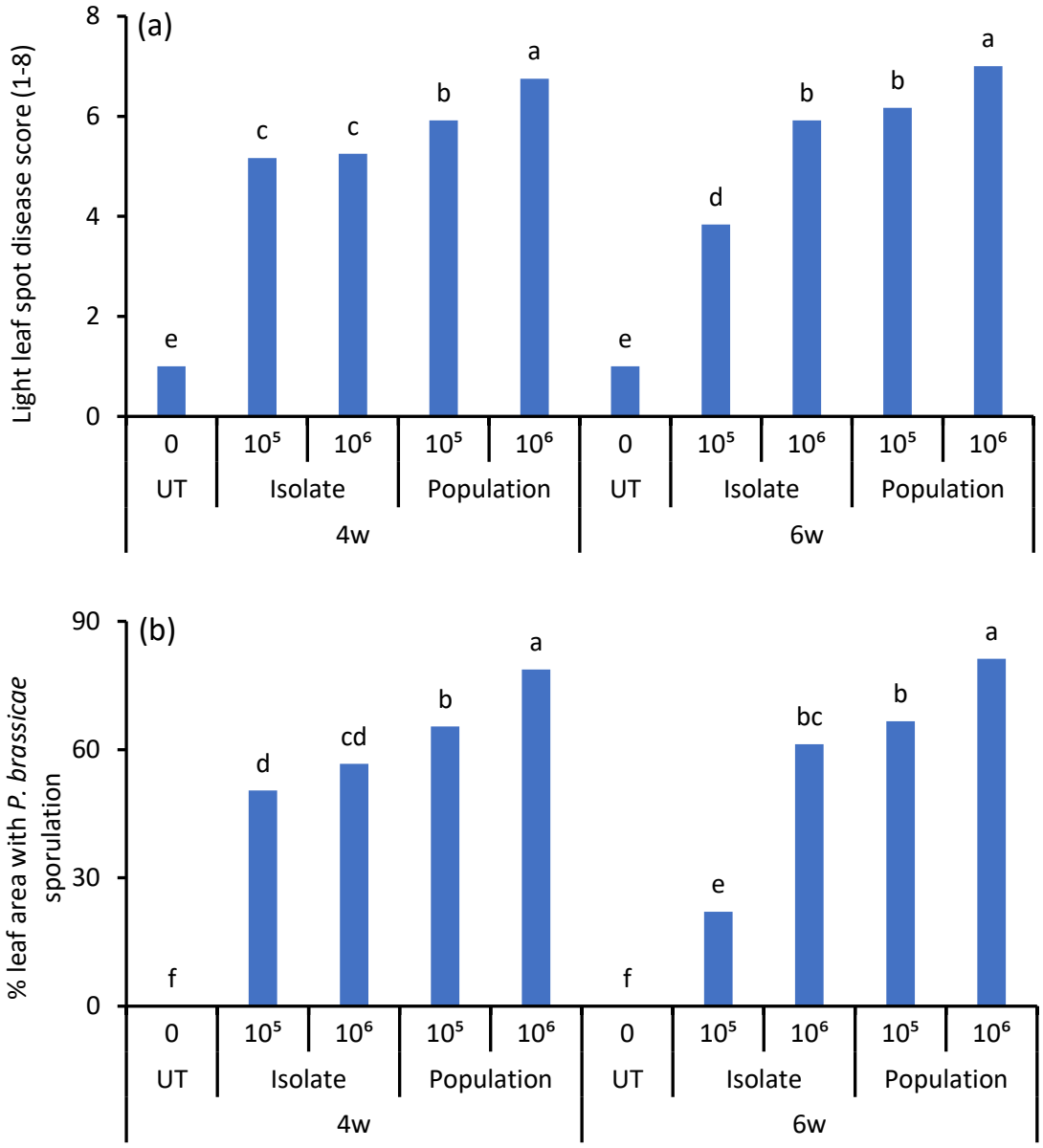

Figure 3.12: (a) Light leaf spot disease score and (b) percentage leaf area covered with *Pyrenopeziza brassicae* sporulation on oilseed rape plants at different plant ages, with different inoculum types and inoculum concentrations.

Glasshouse experiment investigating light leaf spot disease phenotype in plants aged six- or four-weeks-old, inoculated with either a single *P. brassicae* isolate or a *P. brassicae* population at either 10^5 spores/ml or 10^6 spores/ml. Disease severity was measured using (a) disease score on a 1-8 scale (with 1 being no disease) or (b) % leaf area covered with *P. brassicae* sporulation. Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, d.f. = 88, (a) LSD = 0.47, (b) LSD = 0.08).

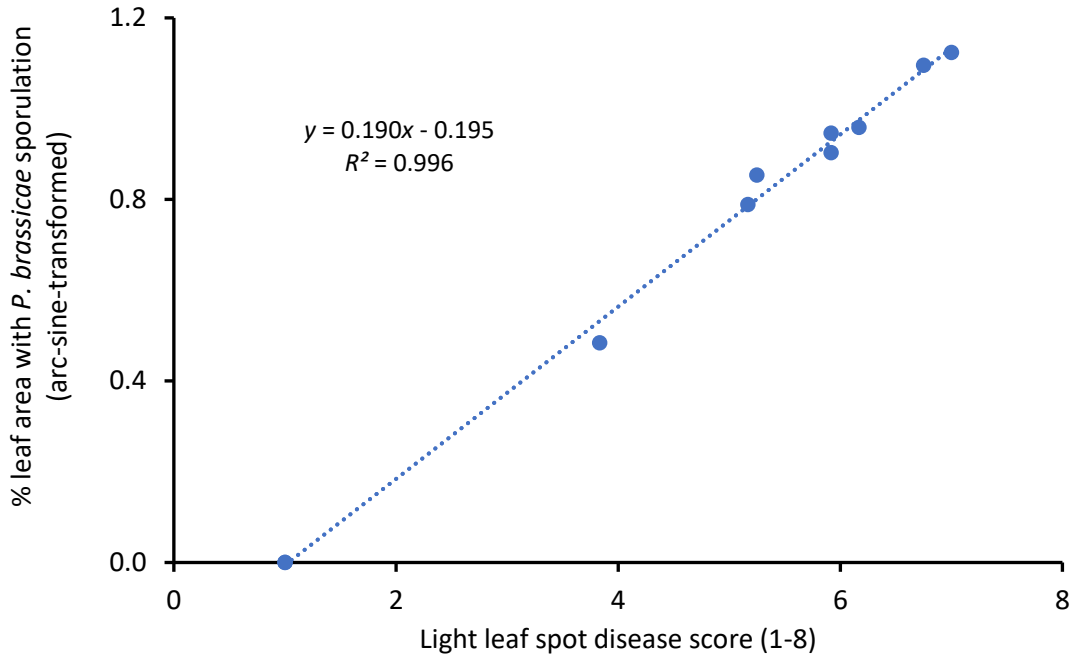

Figure 3.13: Correlation between light leaf spot disease score and percentage leaf area covered with *Pyrenopeziza brassicae* sporulation.

Experiment investigating light leaf spot disease phenotype in plants aged six or four weeks, inoculated with either a single *P. brassicae* isolate or a *P. brassicae* population at either 10^5 spores/ml or 10^6 spores/ml. Plants were assessed for light leaf spot severity using a disease score on a 1-8 scale (with 1 being no disease) and by visual estimation of the % leaf area covered in *P. brassicae* sporulation after samples were incubated at 4°C for 7-9 days (sample correlation coefficient = 0.99, $P < 0.001$, $n = 10$). Percentage leaf area with sporulation values are presented on an arc-sine-transformed scale.

The percentages of deformed leaves, dead leaves, and yellow/senescent leaves were calculated. For experiment 1, glasshouse-grown plants had significantly more dead leaves compared to plants in the controlled-environment ($P < 0.02$), whereas there were no differences for % deformed ($P > 0.1$) or % yellow ($P > 0.19$) leaves. There were significant differences in % deformed ($P < 0.001$), % dead ($P < 0.001$) and % yellow ($P < 0.001$) leaves between age groups. The % of symptomatic leaves generally increased with plant age, although plants aged one- or two-weeks-old did not present any symptomatic leaves, except for % deformed leaves in the glasshouse. Finally, the inoculum concentration did not significantly affect the % deformed ($P > 0.68$), % dead ($P > 0.16$) or % yellow leaves ($P > 0.08$) (Table 3.4). Correlation between disease score and % deformed leaves was only just significant (sample correlation coefficient = 0.48, $P < 0.05$, $n = 16$), but correlation was more significant between disease score and % dead leaves (sample correlation coefficient = 0.78, $P < 0.001$, $n = 16$) and % yellow leaves (sample correlation coefficient = 0.71, $P < 0.001$, $n = 16$). For experiment 2, there was a significant difference in % deformed leaves ($P < 0.02$) between replicates. Plants aged six-weeks-old had significantly greater % dead leaves ($P < 0.001$) compared to plants aged four-weeks-old, while there were no significant differences for % deformed ($P > 0.18$) or % yellow ($P > 0.19$) leaves. Plants inoculated with the *P. brassicae* population had significantly greater % deformed ($P < 0.001$) and % dead ($P < 0.001$) leaves than plants inoculated with the isolate, with no differences for % yellow ($P > 0.6$) leaves (Table 3.5). Correlation between disease score and % deformed leaves was again just significant (sample correlation coefficient = 0.62, $P < 0.05$, $n = 10$), but was not significant for % dead leaves (sample correlation coefficient = 0.44, $P > 0.19$, $n = 10$) or for % yellow leaves (sample correlation coefficient = 0.14, $P < 0.68$, $n = 10$). Correlation was significant for % discoloured leaves (sample correlation coefficient = 0.68, $P < 0.02$, $n = 10$).

For each plant, height (cm), wet weight (g) and dry weight (g) (after desiccating the plants for 48 hr at 60°C) were measured. For experiment 1, glasshouse-grown plants did not show significant differences in height ($P > 0.16$). For

Table 3.4: Light leaf spot foliar symptoms on oilseed rape plants inoculated with *Pyrenopeziza brassicae* at different locations, plant ages and with different inoculum concentrations.

Experiment investigating light leaf spot disease phenotype on plants grown at two different locations (controlled-environment cabinet (CE) and glasshouse (GH)), aged four-, three-, two- or one-week-old, and inoculated with *P. brassicae* conidial suspensions at either 10^4 spores/ml or 10^5 spores/ml. Foliar symptoms were assessed as % deformed leaves, % dead leaves and % yellow/senescent leaves. Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (alpha = 0.05, d.f. = 48; % deformed leaves LSD = 0.264; % dead leaves LSD = 0.194; % yellow leaves LSD = 0.172).

	Proportion of leaves with symptoms		
Treatment*	% deformed leaves	% dead leaves	% yellow leaves
CE-1w-10^4	0.0 d	0.0 d	0.0 e
CE-1w-10^5	0.0 d	0.0 d	0.0 e
CE-2w-10^4	4.2 cd	0.0 d	0.0 e
CE-2w-10^5	4.2 cd	0.0 d	3.6 de
CE-3w-10^4	16.9 ab	3.1 cd	6.3 cde
CE-3w-10^5	3.6 cd	10.7 bc	21.4 a
CE-4w-10^4	25.3 a	11.3 bc	21.1 a
CE-4w-10^5	22.0 a	29.8 a	11.3 bc
GH-1w-10^4	6.3 cd	0.0 d	0.0 e
GH-1w-10^5	12.5 bcd	0.0 d	0.0 e
GH-2w-10^4	15.0 abc	0.0 d	0.0 e
GH-2w-10^5	5.0 cd	0.0 d	0.0 e
GH-3w-10^4	3.6 cd	0.0 d	0.0 e
GH-3w-10^5	19.6 ab	0.0 d	7.7 cd
GH-4w-10^4	22.6 a	11.9 b	23.2 a
GH-4w-10^5	24.4 a	8.3 bc	19.6 ab

*Treatment name structure: condition (CE or GH) – plant age (4, 3, 2, 1 week (w)) – inoculum concentration (10^4 or 10^5 spores/ml)

Table 3.5: Light leaf spot foliar symptoms on oilseed rape plants inoculated with *Pyrenopeziza brassicae* at different plant ages and with different inoculum types and inoculum concentrations.

Glasshouse experiment investigating light leaf spot disease phenotype in plants aged six- or four-weeks-old, inoculated with either a single *P. brassicae* isolate or a *P. brassicae* population at either 10^5 spores/ml or 10^6 spores/ml. Foliar symptoms were assessed as % deformed leaves, % dead leaves and % yellow/senescent leaves. Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (alpha = 0.05, d.f. = 88; % deformed leaves LSD = 0.08; % dead leaves LSD = 0.109; % yellow leaves LSD = 0.179).

	Proportion of leaves with symptoms		
Treatment*	% deformed leaves	% dead leaves	% yellow leaves
4w-UT	9.9 d	0.0 c	6.2 bcd
4w-Is-10^5	41.8 bc	8.1 b	10.0 abcd
4w-Is-10^6	52.9 a	6.4 b	11.5 abc
4w-Pop-10^5	52.8 a	25.4 a	19.3 a
4w-Pop-10^6	52.3 a	33.5 a	12.3 abc
6w-UT	41.5 bc	30.1 a	14.8 ab
6w-Is-10^5	47.8 ab	26.4 a	3.6 cd
6w-Is-10^6	37.8 c	28.3 a	8.8 abcd
6w-Pop-10^5	42.3 bc	31.9 a	2.4 d
6w-Pop-10^6	42.9 bc	34.6 a	2.4 d

*Treatment name structure: plant age (6, 4 week (w)) – inoculum type (untreated (UT), single *P. brassicae* isolate (Is) or *P. brassicae* population (Pop)) – inoculum concentration (10^5 or 10^6 spores/ml)

cabinet-grown plants, there were significant height differences between plant ages ($P < 0.008$). Cabinet-grown plants inoculated with the lower inoculum concentration were significantly taller compared to those inoculated with the greater inoculum concentration ($P < 0.001$), suggesting the greater inoculum concentration negatively impacted plant growth. There were no differences in wet weight between locations ($P > 0.05$) and inoculum concentrations ($P > 0.06$), but there were significant differences between plant ages ($P < 0.001$). For dry weight measurements, glasshouse-grown plants were significantly lighter than controlled-environment-grown plants ($P < 0.01$), as were plants inoculated with the greater inoculum concentration ($P < 0.005$) than those inoculated with the smaller inoculum concentration and dry weight was significantly affected by plant age ($P < 0.001$) (Table 3.6). There was a modest correlation between height and wet weight (sample correlation coefficient = 0.52, $P < 0.001$, $n = 64$) and a significant correlation between wet weight and dry weight (sample correlation coefficient = 0.8, $P < 0.001$, $n = 64$), but no correlation between height and dry weight (sample correlation coefficient = 0.11, $P > 0.36$, $n = 64$). For experiment 2, plant age did not affect height ($P > 0.67$) and, although inoculum type significantly affected plant height ($P < 0.004$), mean treatment heights ranged only between 20-21 cm. Plants aged six-weeks-old had significantly greater wet weight ($P < 0.002$) and dry weight ($P < 0.001$) compared to plants aged four-weeks-old. Plants inoculated with the *P. brassicae* population at 10^6 spores/ml had significantly lower wet weight ($P < 0.001$) and dry weight ($P < 0.04$) compared to the other treatments (Table 3.7). There was modest correlation between height and wet weight (sample correlation coefficient = 0.3, $P < 0.008$, $n = 120$) and between wet weight and dry weight (sample correlation coefficient = 0.63, $P < 0.001$, $n = 120$), but no correlation between height and dry weight (sample correlation coefficient = 0.14, $P > 0.1$, $n = 120$).

3.3.3 Effects of *P. brassicae* inoculum type on light leaf spot symptom development

Results presented in this section overlap with results from section 3.3.2, as the same glasshouse experiment (experiment 2) is discussed. In this section, the

Table 3.6: Height, wet weight and dry weight data of oilseed rape plants inoculated with *Pyrenopeziza brassicae* at different locations, plant ages and with different inoculum concentrations.

Experiment investigating light leaf spot disease phenotype on plants grown at two different locations (controlled-environment cabinet (CE) or glasshouse (GH)), aged four-, three-, two- or one-week-old, and inoculated with *P. brassicae* conidial suspensions at either 10^4 spores/ml or 10^5 spores/ml. For each plant, height (cm), wet weight (g) and dry weight (g) (after desiccating the plants for 48 hr at 60°C) were assessed. Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (alpha = 0.05, d.f. = 48; height LSD = 2.8; wet weight LSD = 2.96; dry weight LSD = 0.49).

Plant height and weight data

Treatment*	Height (cm)	Wet weight (g)	Dry weight (g)
CE-1w-10^4	18.9 bcde	8.7 f	0.5 h
CE-1w-10^5	15.1 g	9.0 f	0.9 h
CE-2w-10^4	21.2 ab	17.2 cd	1.4 g
CE-2w-10^5	16.2 efg	16.3 de	2.2 def
CE-3w-10^4	23.4 a	25.3 a	2.3 cdef
CE-3w-10^5	17.7 cdefg	19.9 bc	3.0 ab
CE-4w-10^4	20.3 bc	21.0 b	2.4 cd
CE-4w-10^5	15.0 g	13.8 e	2.4 cde
GH-1w-10^4	16.3 efg	7.9 f	0.6 h
GH-1w-10^5	17.1 efg	9.8 f	0.7 h
GH-2w-10^4	18.1 cdef	13.9 e	1.9 efg
GH-2w-10^5	16.9 efg	14.9 de	1.8 fg
GH-3w-10^4	20.0 bcd	20.4 b	3.0 ab
GH-3w-10^5	20.0 bcd	22.2 b	3.2 a
GH-4w-10^4	15.6 fg	16.7 de	2.8 abc
GH-4w-10^5	17.4 defg	17.1 cd	2.6 bcd

*Treatment name structure: condition (CE or GH) – plant age (4, 3, 2, 1 week (w)) – inoculum concentration (10^4 or 10^5 spores/ml)

Table 3.7: Height, wet weight and dry weight data of oilseed rape plants inoculated with *Pyrenopeziza brassicae* at different plant ages and with different inoculum types and inoculum concentrations.

Glasshouse experiment investigating light leaf spot disease phenotype in plants aged six- or four-weeks-old, inoculated with either a single *P. brassicae* isolate or a *P. brassicae* population at either 10^5 spores/ml or 10^6 spores/ml. For each plant, height (cm), wet weight (g) and dry weight (g) (after desiccating the plants for 48 hr at 60°C) were assessed. Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (alpha = 0.05, d.f. = 88; height LSD = 1.11; wet weight LSD = 1.41; dry weight LSD = 0.33).

Plant height and weight data

Treatment*	Height (cm)	Wet weight (g)	Dry weight (g)
4w-UT	21.9 ab	16.6 abc	2.3 c
4w-Is-10^5	20.7 c	15.8 c	2.3 c
4w-Is-10^6	21.2 abc	15.9 c	2.2 c
4w-Pop-10^5	22.2 a	16.5 abc	2.4 c
4w-Pop-10^6	20.5 c	13.9 d	2.2 c
6w-UT	21.5 abc	17.2 abc	3.3 a
6w-Is-10^5	20.5 c	17.6 a	3.4 a
6w-Is-10^6	21.3 abc	17.7 a	3.5 a
6w-Pop-10^5	21.4 abc	17.5 ab	3.1 ab
6w-Pop-10^6	20.9 bc	16.2 bc	2.9 b

*Treatment name structure: plant age (6, 4 week (w)) – inoculum type (untreated (UT), single *P. brassicae* isolate (Is) or *P. brassicae* population (Pop)) – inoculum concentration (10^5 or 10^6 spores/ml)

results regarding the effects of inoculum type and inoculum concentration are further analysed. Only plants aged four-weeks-old were included in this analysis, as that is the standard inoculation age and growth stage for light leaf spot studies.

The results for light leaf spot disease score and % leaf area with sporulation are shown in Figure 3.12. Overall, plants inoculated with the *P. brassicae* population showed greater disease score ($P < 0.001$) and % area with sporulation ($P < 0.001$) than those inoculated with the single *P. brassicae* isolate. The concentration of the *P. brassicae* inoculum also affected both disease score ($P < 0.009$) and % area with sporulation ($P < 0.001$). The *P. brassicae* population at the greater concentration (10^6 spores/ml) caused greater disease compared to the lower concentration (10^5 spores/ml), whereas the inoculum concentration of the single *P. brassicae* isolate did not affect disease development.

Presence of a necrotic response was assessed on the leaf petiole, lamina and midrib. None of the plants across any treatments developed flecking on the petiole, and only plants inoculated with a single *P. brassicae* isolate at 10^6 spores/ml developed flecking on the midrib. All affected plants, except those inoculated with a single *P. brassicae* isolate at 10^5 spores/ml, developed necrotic flecking on the lamina. Untreated plants showed no necrotic response in any area of the leaf.

The percentages of deformed, dead and yellow/senescent leaves were calculated, and the results are shown in Table 3.5. Plants inoculated with the *P. brassicae* population had significantly greater % deformed leaves ($P < 0.001$) and % dead leaves ($P < 0.001$), compared to those inoculated with the single *P. brassicae* isolate, and all inoculated plants had significantly greater % deformed and % dead leaves compared to the untreated controls. The inoculum concentration did not affect any of the foliar symptoms. The % yellow leaves was the same across all treatments ($P > 0.41$).

3.3.4 Effects of light intensity on light leaf spot symptom development

3.3.4.1 Rationale

This study resulted from observations made during a different glasshouse experiment, where oilseed rape plants inoculated with *P. brassicae* conidial suspensions were exposed to unequal amounts of light, causing differences in light leaf spot symptom development. The experiment was done in December 2022, a period that had an average of eight hours of daily sunlight. The glasshouse that the experiment was done was fitted with supplementary lighting operating at a 12 hr photoperiod; however, the lights were unevenly distributed across the glasshouse space. This meant that some plants, which were not underneath the supplementary lighting, received about four hours less light each day, compared to the plants exposed to the supplementary lighting. Over the course of the three weeks of disease development period following *P. brassicae* inoculation, plants that were not exposed to the supplementary lights did not develop symptoms at the same rate as those under the lights, and instead appeared to develop symptoms more slowly (Figure 3.14). Upon moving the unexposed plants under the supplementary lights for a few additional days, they developed better visible symptoms (data not shown).

These observations led to the hypothesis that the duration of exposure to light or light intensity affects the rate and severity of light leaf spot disease development. The objective of the following study was to test whether plants exposed to light for longer time periods develop light leaf spot disease symptoms more quickly, compared to plants that are exposed to light for shorter time periods.

3.3.4.2 Experimental results

The results for light leaf spot disease score (1-8 scale with 1 being no disease) and percentage leaf area with *P. brassicae* sporulation showed that there were significant differences between treatments (Figure 3.15). Between the two lighting conditions, plants exposed to 8 hr of more intense light developed greater disease score ($P < 0.002$) and % area with sporulation ($P < 0.001$) compared to plants exposed to 12 hr of less intense light. The percentage leaf

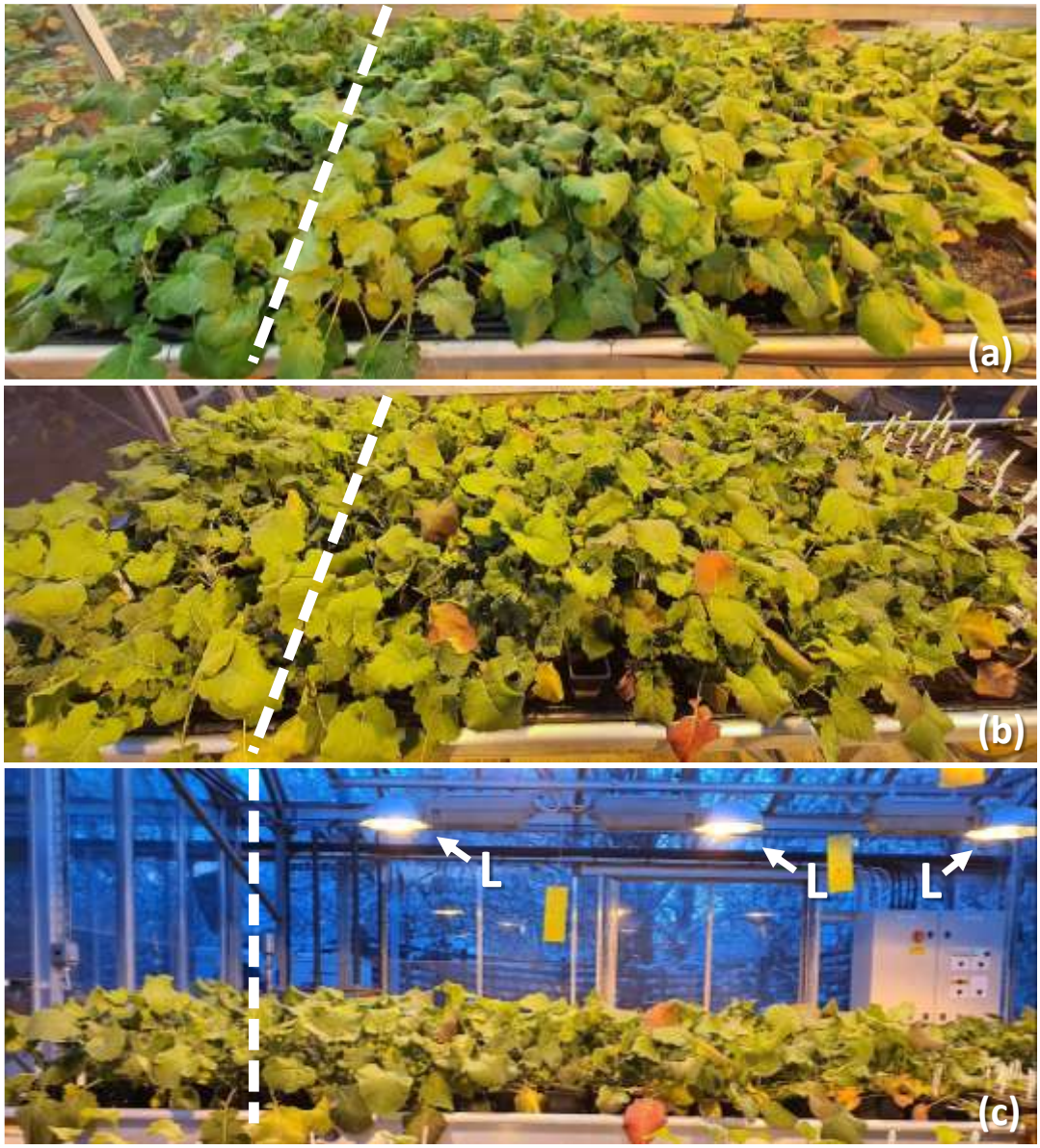

Figure 3.14: Influence of light treatment on light leaf spot symptom development on oilseed rape plants in glasshouse.

Glasshouse experiment where oilseed rape plants inoculated with *Pyrenopeziza brassicae* conidial suspensions were exposed to unequal amounts of light, developing light leaf spot symptoms at different rates. The same bench is shown, where plants on the left of the white line were not underneath a supplementary light, and therefore received only natural light (8 hr daily average, compared to supplementary lighting operating at a 12 hr photoperiod). These plants appear asymptomatic compared to others, whose leaves appear visibly more distorted and discoloured at (a) 15 days post inoculation (dpi) and (b & c) 24 dpi. (c) Shows positions of the supplementary lights (L) above the bench.

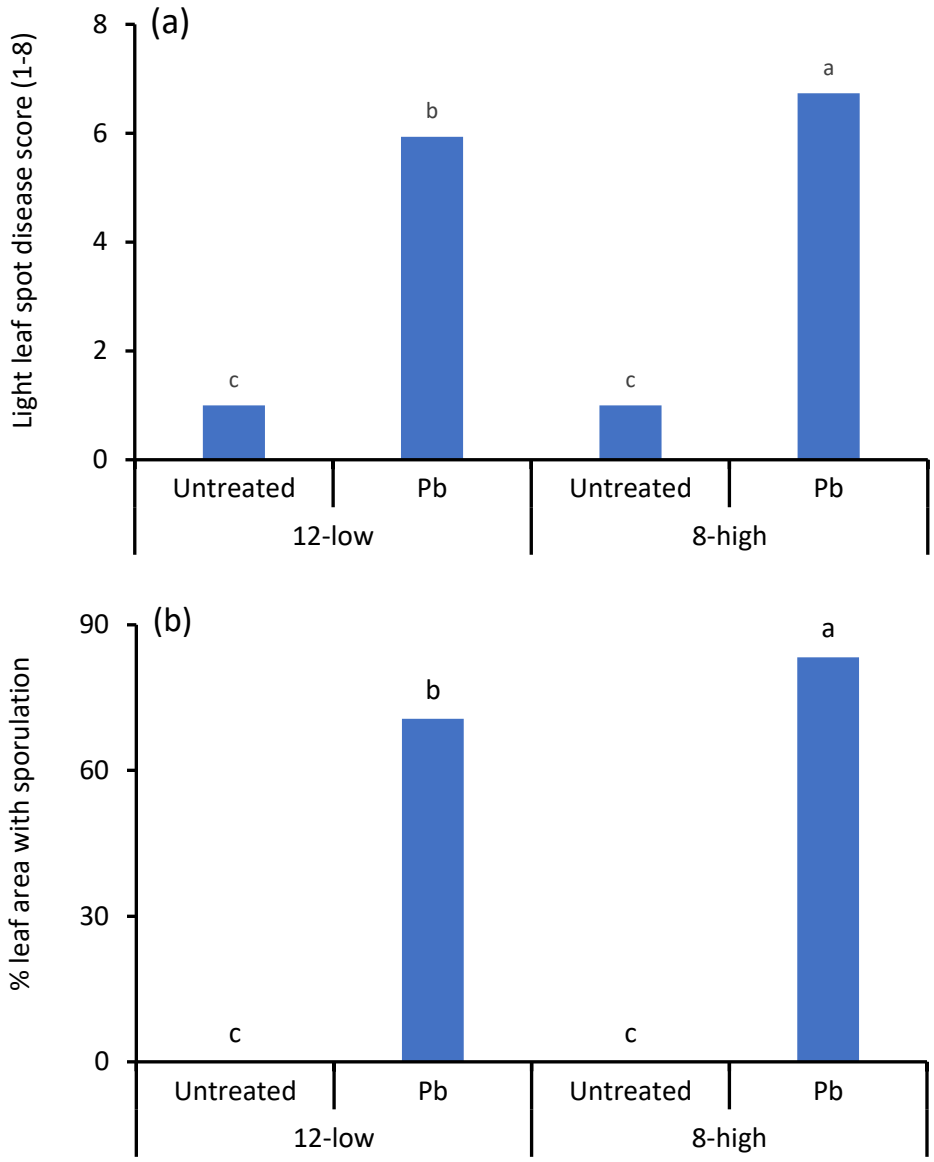

Figure 3.15: (a) Light leaf spot disease score and (b) percentage leaf area covered with *Pyrenopeziza brassicae* sporulation on plants inoculated under two different lighting conditions.

Controlled-environment experiment where plants were inoculated with *P. brassicae* conidial suspensions under two different lighting conditions (12 hr photoperiod at a light intensity of 249 µmol m^{-2} s^{-1} and an 8 hr photoperiod at a greater light intensity of 326 µmol m^{-2} s^{-1}). Disease severity was measured using (a) disease score on a 1-8 scale (with 1 being no disease) and (b) percentage leaf area covered in *P. brassicae* sporulation. Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (d.f. = 36).

area covered in *P. brassicae* sporulation was estimated at four separate times. A first assessment of % sporulation was done at 18 dpi (days post inoculation). Three more assessments were done at 27, 34 and 41 dpi which showed significant differences between treatments (Figure 3.16 and Figure 3.17). Overall, the second sporulation (following the first incubation period) was the greatest, and with each subsequent leaf washing, the % leaf area with sporulation decreased ($P < 0.001$). The third sporulation (second incubation) was greater than the fourth sporulation (third incubation), and the first sporulation (no incubation) was the smallest. Plants exposed to 8 hr of more intense light also showed a greater % area with sporulation overall, compared to those exposed to 12 hr of less intense light ($P < 0.001$).

Presence of a necrotic response was assessed on the leaf petiole, midrib and lamina. None of the plants presented flecking on the petiole, whereas both inoculated plants, regardless of light treatment, presented flecking on both the midrib and lamina. The untreated controls did not present flecking anywhere on the leaf.

Foliar symptoms were recorded at 3, 8, 14, 16, and 18 dpi and started appearing as early as 3 dpi. The percentage of symptomatic leaves steadily increased throughout the incubation period (Figure 3.18). Additionally, inoculated plants exposed to 8 hr of more intense light consistently had the greatest percentages of distorted and discoloured leaves, compared to the untreated controls for both light treatments. Results from the last assessment then showed significantly greater % yellow/senescent leaves for plants exposed to 12 hr of less intense light ($P < 0.001$), whereas plants exposed to 8 hr of more intense light showed significantly greater % discoloured leaves ($P < 0.003$). Inoculated plants also presented significantly greater % deformed ($P < 0.001$) and % discoloured leaves ($P < 0.001$), compared to the untreated plants, and inoculated plants exposed to 8 hr of more intense light showed slightly greater % dead leaves ($P < 0.05$) compared to the other treatments (Table 3.8).

For each plant, height (cm), wet weight (g) and dry weight (g) (after desiccating the plants for 48 hr at 60°C) were measured (Table 3.9). Plants exposed to

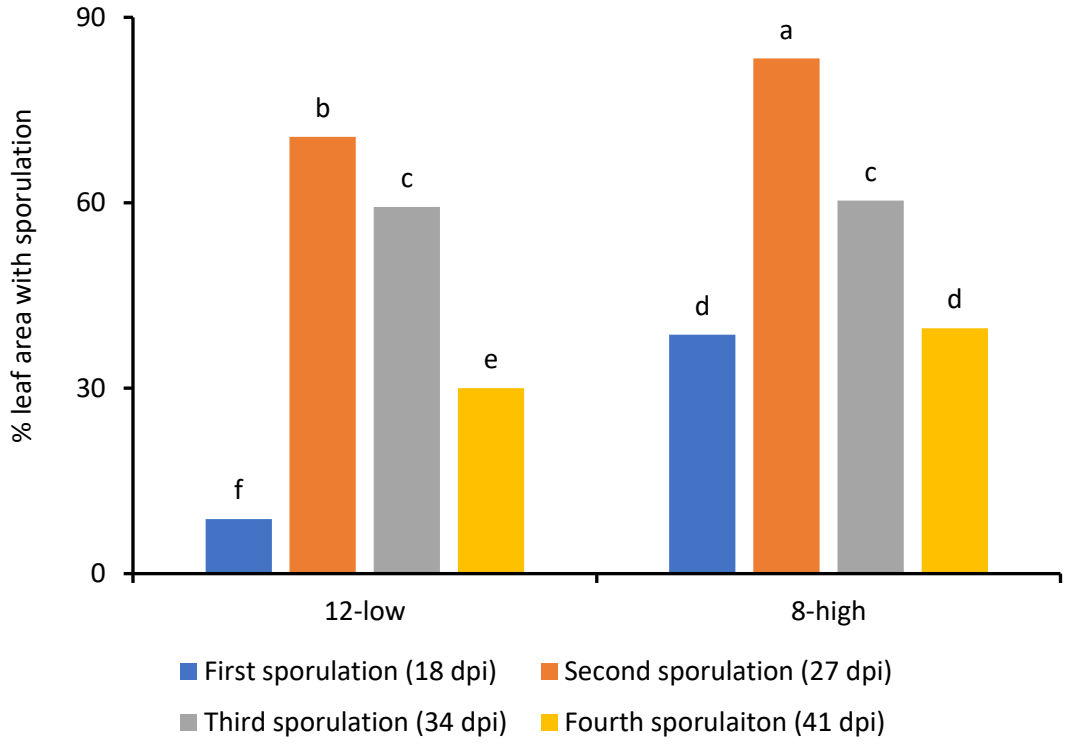

Figure 3.16: First to fourth measurements of percentage leaf area covered in *Pyrenopeziza brassicae* sporulation on plants inoculated under two different lighting conditions.

Controlled-environment experiment where plants were inoculated with *P. brassicae* conidial suspensions under two different lighting conditions (12 hr photoperiod at a light intensity of 249 µmol m^{-2} s^{-1}, and an 8 hr photoperiod at a greater light intensity of 326 µmol m^{-2} s^{-1}). A first assessment of % leaf area covered with sporulation was done at 18 dpi (days post inoculation). A second assessment was done at 27 dpi after an incubation period at 4°C for 9 days, followed by leaf washing (section 2.2.2) to remove sporulation from the leaves. Plants were then further incubated at 4°C for another 7 days, before a third assessment was done at 34 dpi. Finally, following leaf washing and a third incubation period at 4°C for 7 days, a fourth assessment was done at 41 dpi. Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, LSD = 0.08, d.f. = 112).

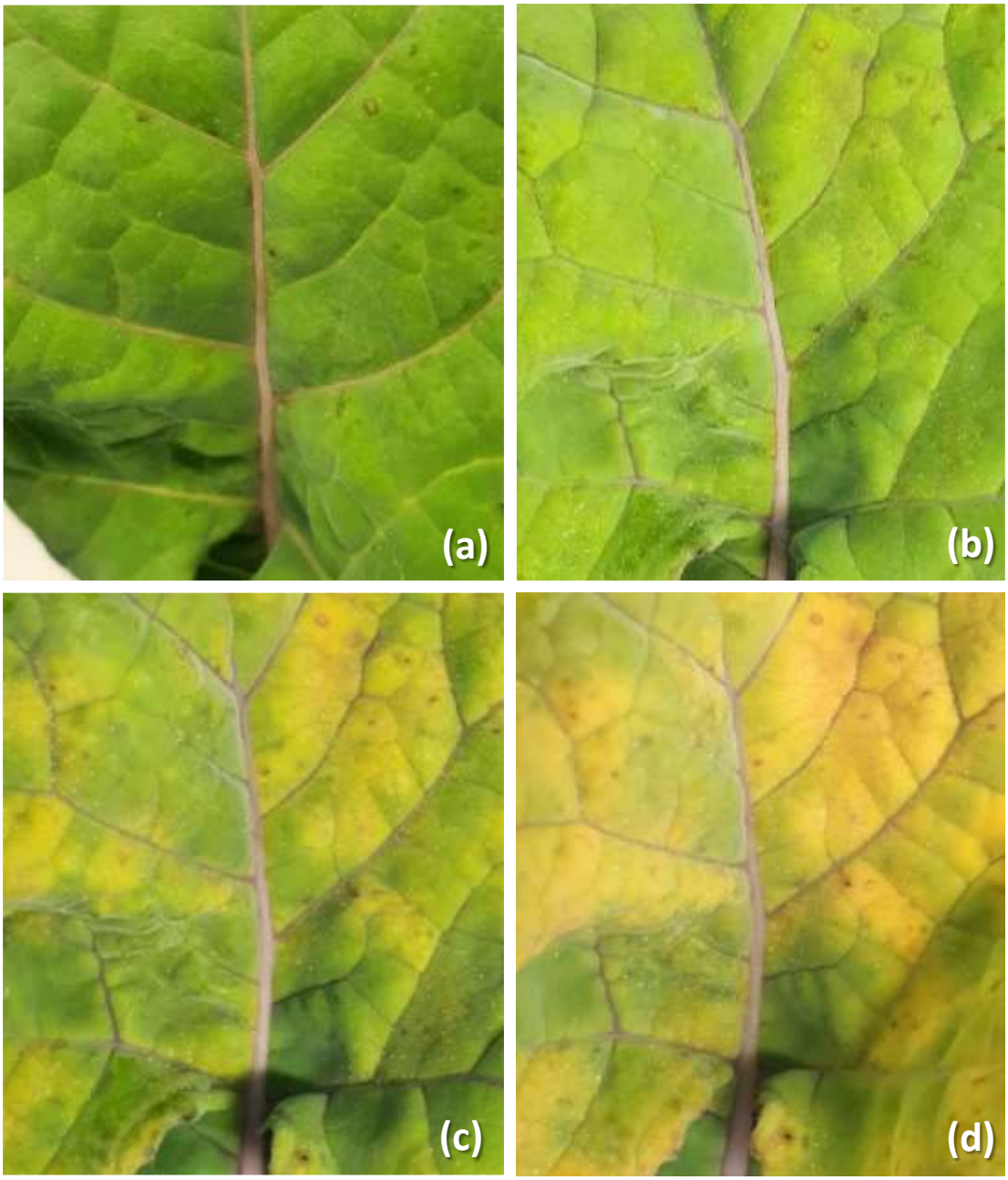

Figure 3.17: Recurring *Pyrenopeziza brassicae* sporulation on oilseed rape leaf at harvest and after three cycles of incubation and leaf washing.

Controlled-environment experiment where plants were inoculated with *P. brassicae* conidial suspensions. The same oilseed rape leaf showing *P. brassicae* sporulation is shown (a) before harvest at 18 dpi (days post inoculation), (b) after incubation at 4°C for 9 days (27 dpi), (c) following leaf washing (section 2.2.2) to remove sporulation from leaves, and second incubation at 4°C for 7 days (34 dpi), (d) following second leaf washing and third incubation at 4°C for 7 days (41 dpi).

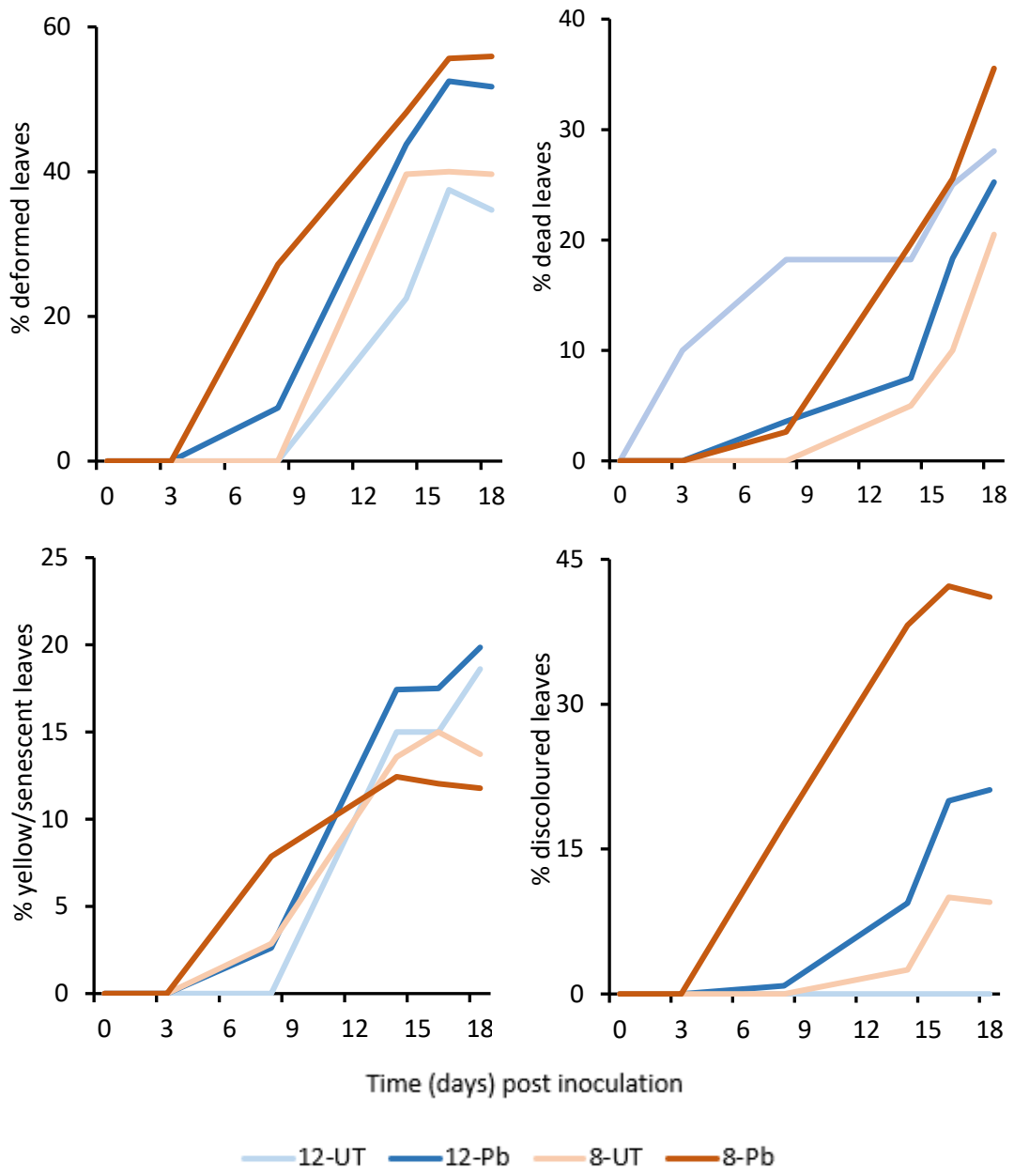

Figure 3.18: Incidence of light leaf spot foliar symptoms (% leaves affected) over time under two different lighting conditions.

Controlled-environment experiment where plants inoculated with *Pyrenopeziza brassicae* conidial suspensions were exposed to two different lighting conditions (12 hr photoperiod at a light intensity of 249 µmol m^{-2} s^{-1} and an 8 hr photoperiod at a greater light intensity of 326 µmol m^{-2} s^{-1}). Shown are inoculated plants (Pb) and untreated controls (UT). Foliar symptoms were measured as (a) % deformed leaves, (b) % dead leaves, (c) % yellow/senescent leaves, and (d) discoloured leaves, at 3, 8, 14, 16, and 18 days post inoculation.

Table 3.8: Light leaf spot foliar symptoms on oilseed rape plants inoculated with *Pyrenopeziza brassicae* conidial suspensions under two different lighting conditions.

Controlled-environment experiment where plants inoculated with *P. brassicae* conidial suspensions were exposed to two different lighting conditions (12 hr photoperiod at a light intensity of 249 µmol m^{-2} s^{-1} or an 8 hr photoperiod at a greater light intensity of 326 µmol m^{-2} s^{-1}). Foliar symptoms were assessed as % deformed leaves, % dead leaves, % yellow/senescent leaves and % discoloured leaves. Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (d.f. = 36).

	Proportion of leaves with symptoms			
Treatment*	% deformed leaves	% dead leaves	% yellow leaves	% discoloured leaves
12-low-UT	30.0 b	23.0 a	16.4 a	0.0 c
12-low-Pb	48.9 a	24.5 a	13.5 a	15.7 b
8-high-UT	32.0 b	21.2 a	4.7 b	8.2 bc
8-high-Pb	51.9 a	35.8 b	1.6 b	30.1 a

*Treatment name structure: photoperiod duration (12 hr or 8 hr) – light intensity (low or high) – inoculation (untreated (UT) or inoculated (Pb))

Table 3.9: Height, wet weight and dry weight data of oilseed rape plants inoculated with *Pyrenopeziza brassicae* conidial suspensions under two different lighting conditions.

Controlled-environment experiment where plants inoculated with *P. brassicae* conidial suspensions were exposed to two different lighting conditions (12 hr photoperiod at a light intensity of 249 µmol m^{-2} s^{-1} or an 8 hr photoperiod at a greater light intensity of 326 µmol m^{-2} s^{-1}). For each plant, height (cm), wet weight (g) and dry weight (g) (after desiccating the plants for 48 hr at 60°C) were assessed. Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (d.f. = 36).

Plant height and weight data

Treatment*	Height (cm)	Wet weight (g)	Dry weight (g)
12-low-UT	20.5 a	17.5 b	1.9 b
12-low-Pb	19.9 a	20.7 a	2.7 a
8-high-UT	18.5 b	16.2 b	2.5 ab
8-high-Pb	17.5 b	15.9 b	2.4 ab

*Treatment name structure: photoperiod duration (12 hr or 8 hr) – light intensity (low or high) – inoculation (untreated (UT) or inoculated (Pb))

8 hr of more intense light were significantly shorter than plants exposed to 12 hr of less intense light ($P < 0.001$), but there were no significant differences between untreated and inoculated plants for either light treatment. Wet weight data showed that inoculated plants exposed to 12 hr of less intense light weighed significantly less than the other treatments ($P < 0.003$), whereas dry weight data showed no differences ($P > 0.09$) between treatments.

3.4 Discussion

3.4.1 Effects of weather conditions on *Pyrenopeziza brassicae* air-borne ascospore release

In this study, patterns of *P. brassicae* air-borne ascospore release over three cropping seasons in relation to weather data suggested that most *P. brassicae* ascospores were generally released in the period of July to November following rainfall, and greater ascospore release in autumn related to greater release in spring/summer. Additionally, stubble from the previous year, rather than just the latest harvest, may act as a source of ascospore inoculum.

There were two major periods of ascospore release each season, one in October-November and a later one in June-July, regardless of the overall number of apothecia, suggesting these are the annual *P. brassicae* ascospore release periods. Ascospore release generally occurred at temperatures ranging between 15-17°C alongside rainfall, which supported evidence from previous studies. Gilles *et al.* (2001c) concluded that the optimal temperature for development of apothecia was between 5-18°C with continuous wetness and that apothecial maturation is inhibited at temperatures above 22°C. Previous studies also detected *P. brassicae* airborne ascospores in April-May (McCartney and Lacey, 1990) or in June-July (Gilles *et al*. 2001b), although usually not within the same study. Temperature and rainfall are established parameters incorporated into models created to predict light leaf spot epidemics (Gilles *et al*., 2000a; Welham *et al*., 2004), but rely on survey data based on foliar disease assessment in crops with already established infections, while models that used airborne ascospore release data (Papastamati *et al*., 2002) only used data collected in the autumn-winter months. Ascospore release data

reported here were measured within the same study over three continuous seasons, providing guidance for follow-up studies to monitor ascospore release and weather parameters for entire cropping seasons, with particular focus on the summer months.

Conversely, ascospore release may be inhibited by lower temperatures in winter, but a sufficiently large number of apothecia can lead to early ascospore release in spring. Ascospores were few in number to completely absent between November and March, when temperatures were lower, despite continuous precipitation in December and January. In the second cropping season (2021/2022), however, which had the greatest ascospore release out of the three seasons, ascospore release started in March and largely occurred until July, despite the cropping season having some of the lowest rainfall. There is evidence suggesting that mature apothecia on leaf debris may release ascospores in spring and contribute to subsequent disease cycles (Boys *et al*., 2007; Gilles *et al*., 2000a). These results suggested that a high enough number of apothecia in autumn can lead to early ascospore release in spring, possibly overcoming the effect low temperatures which are believed to slow ascospore development (Gilles *et al*., 2001c). These results are, however, contrasting by those in the third cropping season (2022/2023) where, despite overall reduced ascospore detection, ascospore release occurred in November-December during a frost period. Frost may accelerate the appearance of light leaf spot symptoms (Gilles *et al*., 2001d), but in relation to existing disease rather than promoting new ascospore release. These results show that, despite evidence linking environmental factors to ascospore release, more information about ascospore release patterns with accompanying weather data are needed.

One-year old stubble, rather than just stubble from the latest harvest, may serve as ascospore inoculum in the current cropping season. For all three cropping seasons assessed, the end of one cropping season and the start of the next one, marked by newly harvested oilseed rape stubble being scattered around the sampler area, occurred at the beginning of August. This means that ascospores released in the summer, specifically in June-July, were sourced from stubble obtained in the previous season (i.e. ascospores released in July

2022 were sourced from oilseed rape stubble harvested in July 2021). This suggested that the production of *P. brassicae* ascospores may be longer than initially anticipated. Oilseed rape stubble from the latest harvest is currently believed to source the *P. brassicae* ascospore inoculum initiating epidemics in the upcoming season (Boys *et al*., 2007; Gilles *et al*., 2001b; Karandeni Dewage *et al*., 2018). There is limited understanding of how older stubble may contribute to disease severity under UK conditions. Cheah and Hartill (1985) reported that *P. brassicae* apothecia on dry cauliflower residues in New Zealand were viable for up to 50 weeks once formed and discharged ascospores when wetted, while McCartney and Lacey (1990) reported ascospore release from apothecia in laboratory conditions for up to 3 weeks when subjected to both wet and dry cycles. Cultural management strategies already recommend the separation in time and space of *Brassicas* crops from one another by implementing crop rotation systems, as well as ploughing soil to bury leftover stubble (FRAG-UK, 2017; Jankovska-Bortkevič *et al*., 2022; SAC, 2009). If these management strategies are correctly implemented, years-old oilseed rape stubble may not be present in the field as new crops are sowed, and the potential of years-old oilseed rape stubble as primary inoculum of new light leaf spot epidemics may have therefore remained unrecognized. Ascospore release in spring has previously been speculated to result from apothecia growing on dead rosette leaves on the ground (McCartney and Lacey, 1990), but this could not be the case in the present study, as the stubble was harvested from a field and brought to a different site, meaning the stubble was the only available source of inoculum. Findings by Cheah and Hartill (1985) may have been confirmed for the first time under UK conditions and follow-up studies testing the survival of *P. brassicae* ascospores may provide further insight in their contribution to disease cycles.

Generally, wind-dispersed ascospores of *P. brassicae* released in autumn from apothecia on crop debris are thought to be the primary inoculum initiating light leaf spot disease epidemics (Gilles *et al*., 2001b). Gaining insight into ascospore release patterns is therefore crucial to reliably predict the potential severity of an epidemic and to correctly time fungicide sprays. Studies monitoring air-borne ascospore release using air samplers have been done for

Leptospheria spp. (Kaczmarek *et al*., 2009; 2024; Lob *et al*., 2013; Van de Wouw *et al*., 2010) and for *P. brassicae* (Calderon *et al*., 2002; Gilles *et al*., 2001b; McCartney and Lacey, 1990) as a method to predict disease epidemics. Two methods of ascospore detection were typically used: ascospore counting by light microscopy and DNA quantification through qPCR. *L. maculans* spores are recognizable through microscopy (Fitt, personal communication), whereas identification of *P. brassicae* ascospores under the microscope is more difficult due to the unreliable distinction of *P. brassicae* spores from those of other fungal pathogens (Inman *et al*., 1992). For this study, microscopy techniques were omitted and ascospore quantification was done using qPCR, which is considered more reliable due to the usage of species-specific primers that amplify the highly conserved ITS region of the *P. brassicae* genome. However, this means that there was only one criterion for detection of *P. brassicae* ascospores. Since samples for qPCR analysis were selected at alternating dates, it is possible that there were greater numbers of *P. brassicae* ascospores at dates that were excluded from the analysis. Despite this limitation, monitoring ascospore release over a continuous three-year period allowed for greater insight into spore release patterns, especially in the spring and summer months, compared to previous studies. Overall, the hope would be to combine the data obtained in this study with spore release data and weather data from other locations and cropping seasons. Furthermore, much like when selecting for cultivar resistance, monitoring spore release of several important fungal pathogens together would make a most robust disease forecasting tool, as fungal pathogens in the environment rarely exist and operate by themselves.

3.4.2 Effects of pathogen factors on light leaf spot symptom development

Studies on the effects of plant age and *P. brassicae* inoculum type on light leaf spot symptom development suggested that plants aged four-weeks-old (four or five true leaves) at the time of inoculation with *P. brassicae* spores at 10^5 spores/ml concentration remain the optimal conditions for light leaf spot studies on oilseed rape in controlled-environment and glasshouse experiments.

Preference for inoculation with a single-spore *P. brassicae* isolate or a population remains study-dependent.

Light leaf spot disease severity increased with plant age. Plants aged one- to three-weeks-old developed less severe disease, while symptoms were most visible in plants aged four- and six-weeks-old and remained indistinguishable between the latter two age groups. In controlled-environment experiments done on oilseed rape, plants are typically inoculated with *P. brassicae* at the leaf production stage when they have four or five true leaves (1,4-1,5 growth stage, Sylvester-Bradley, 1985). Studies on cotyledons (1,0 growth stage) have also been done (Bradburne *et al*., 1999), but results did not correlate well to those of studies done on adult plants. Work done by Karolewski *et al*. (2006) concluded that plants should ideally be at the 1,4-1,7 growth stage when inoculated, ensuring leaves do not senesce throughout the course of the experiment. Results in this study agree with Karolweski *et al*. (2006), as inoculated six-week-old plants (1,6 growth stage) had a much greater leaf death rate compared to four-week-old plants (1,4 growth stage) for both uninoculated and inoculated treatments. This suggested that, since disease severity was similar for both age groups, four-weeks-old was the preferred age for inoculation, as plants aged six-weeks-old started to lose leaves earlier during the incubation period, which may have affected disease development and later assessments. Generally, changes in host phenology across different developmental stages are known to influence disease development as much as pathogen virulence. Infection at a specific developmental stage may also be influenced by whether a plant fungal pathogen is a biotroph (derive energy from living cells), a necrotroph (derive energy from dead or dying cells) or, more uncommonly, a hemibiotroph (invade living cells before transitioning to a necrotrophic lifestyle to kill and feed off host cells), although there have been disagreements over the classification of fungal pathogens within these three categories (Oliver and Ipcho, 2004; Rajarammohan, 2021; Walters *et al*., 2008). Obligate biotrophic fungal pathogens may attack young plants, as was the case for the study by Farber and Mundt (2017) using *Puccinia striiformis*, causative agent of wheat stripe rust, which concluded that younger plants, as well as younger leaves, had significantly greater disease severity. Other studies

determined that plants experience a ‘window of vulnerability’ during their earlier development stages in which they are more susceptible to pathogen colonization, often attributed to differences in the immune systems of still-developing seedlings compared to mature plants (Gongora-Canul and Leandro, 2011; Sharabani *et al*., 2013). Conversely, infection with necrotrophs may cause more severe symptoms on older plants and leaves (Barna and Györgyi, 1992; Barna, 2021). *P. brassicae*, then, despite being considered a hemibiotroph (as it can be grown on artificial media), is nearer to being an obligate biotroph that grows best on living tissues, as suggested by its tendency to form green islands with high photosynthetic activity during colonization to keep the infected leaf area alive for as long as possible (Fitt, pers. comm.; Stotz *et al*., 2014; Walters *et al*., 2008). In these studies, seedlings did not develop disease and light leaf spot symptom development appeared in plants aged four-weeks-old or older, further suggesting *P. brassicae* requiring living leaf tissue to colonize during the asexual stage of its life cycle (Boys *et al*., 2007; Karandeni Dewage *et al*., 2018). Early plant growth stages may lack the necessary green leaf area required for *P. brassicae* colonization, thereby favouring infection of older plants, although even at the oldest growth stages assessed, the plants were still considered juvenile. Overall, further studies should be done to investigate how plant age impacts *P. brassicae* infection using more developmental stages and to determine a possible period of susceptibility of the host.

Light leaf spot disease symptoms were more visible at greater *P. brassicae* inoculum concentrations, but too high inoculum concentrations may affect comparable disease assessment between cultivars. Karolewski *et al*. (2006) investigated the optimal conidial concentration for inoculation and determined 4×10^3 or 4×10^4 spores/ml as the best among those assessed, whereas severity of symptoms decreased at 4×10^5 spores/ml. This was different to results presented here. While inoculum concentration did not affect disease severity in plants aged one- to three-weeks-old, four-week-old plants inoculated with 10^4 spores/ml developed less severe disease compared to those inoculated with 10^5 spores/ml. Plants inoculated with 10^6 spores/ml compared to 10^5 spores/ml, then, also developed more severe disease symptoms.

Greater inoculum concentration generally led to greater disease severity; however, this may not always be ideal. Generally, too low inoculum concentrations may not produce sufficient symptoms, but too high concentrations may cause very severe disease, providing no discrimination of disease severity between cultivars. Optimal inoculum concentration may therefore depend on the type of study and whether assessing differences between cultivars is an experimental objective. Greater inoculum concentrations are sometimes necessary to ensure sufficient infection occurs, especially in glasshouse or field experiments, where environmental conditions can significantly affect disease development. Currently, 10^5 spores/ml remains the standard concentration for light leaf spot studies (Fell *et al*., 2023; Karandeni Dewage *et al*., 2022).

Greater inoculum concentration may decrease plant height, although results remained inconclusive. In experiment 1, plants inoculated with the greater *P. brassicae* inoculum concentration (10^5 spores/ml) were significantly shorter than those inoculated with the lower concentration (10^4 spores/ml) across all age groups tested. In experiment 2, however, plant heights did not significantly differ between treatments. In field environments, *P. brassicae* infection has been shown to cause growth stunting in oilseed rape plants when compared to healthy plants (Karandeni Dewage *et al*., 2018), but these symptoms have not been reported in controlled environment conditions and their underlying physiological mechanisms remain unknown. Previous studies have discussed the roles of fungal plant-growth regulators such as cytokinins produced by *P. brassicae* as possible key determinants of pathogenicity (Ashby, 1997; Boys *et al*., 2007; Murphy *et al*., 1997). These fungal cytokinins may underlie changes in plant morphology such as leaf deformation, stem elongation and even growth stunting, but this could not be verified here. Experiment 1 did not include untreated controls, glasshouse-grown plants did not show significant height differences between treatments, and the symptom was not reproducible in subsequent experiments. This suggested that the growth stunting symptoms reported may have resulted from environmental conditions, rather than being pathogen-induced, or a combination of both. Overall, the ability to recreate field

disease phenotypes in controlled-environment would be an important tool in investigating mechanisms of *P. brassicae* infection.

P. brassicae populations may be more virulent than single-spore *P. brassicae* isolates, but a hypervirulent isolate may cause greater disease than a hypovirulent population. In this study, the *P. brassicae* population used caused more severe disease than the single-spore isolate, which is in line with expectations. In a field environment, epidemics may be caused by genetically diverse pathogen populations that occur under natural conditions. Pathogen populations with high evolutionary potential and frequent sexual reproduction can lead to the rapid spread of virulent alleles and accelerate the breakdown of host resistance genes. The expectation is therefore for populations with multiple genotypes to cause greater disease, compared to single genetically-fixed *P. brassicae* isolates (Karandeni Dewage *et al*., 2018; McDonald *et al*., 2022). However, it is also possible that a single, hyper-virulent isolate could produce more severe disease than a population of moderately virulent to avirulent isolates. Additionally, other factors like host viability, pathogen concentration and environmental conditions can greatly influence disease severity. To study gene expression in controlled-environment experiments, plants are typically inoculated with a single conidial *P. brassicae* isolate to ensure uniformity (Boys *et al*., 2007). The single-spore *P. brassicae* isolate used in this study was collected in England and has previously been characterized and used in experiments due to its high virulence. Conversely, the *P. brassicae* population used was collected in Scotland, where light leaf spot epidemics are more severe (Karandeni Dewage *et al*., 2018). Although the *P. brassicae* population generally caused more severe disease, the single-spore isolate obtained from a region with less severe epidemics (England) caused disease comparable to a population obtained from a higher-risk area (Scotland). This showed that, although plants in field conditions are exposed to entire pathogen populations comprised of many different isolates, if a population is not selected for virulent alleles, a single virulent isolate may cause more severe disease despite being a single genotype.

3.4.3 Effects of light intensity and duration on light leaf spot symptom development

Studies on the effect of light treatment on the rate and severity of light leaf spot development in controlled-environment experiments showed new evidence suggesting that light intensity may accelerate disease development by influencing and possibly promoting *P. brassicae* colonization.

The rate of light leaf spot symptom development was influenced by light treatment. Plants in treatment 2 (8 hr light/16 hr dark period at a greater light intensity of 326 µmol m^{-2} s^{-1}) developed symptoms at a greater rate than plants in treatment 1 (12 hr light/12 hr dark period at a lower light intensity of 249 µmol m^{-2} s^{-1}), as inoculated plants in treatment 2 consistently developed the greatest percentages of discoloured and dead leaves. The high leaf death rate was the main reason why plants were harvested at 18 dpi, which is 6 days shorter than the standard disease incubation period. Additionally, inoculated plants in treatment 2 showed visible sporulation as early as 16 dpi (data not shown), even though asexual sporulation is best visible following an additional incubation period in a cold and humid environment for 7-9 days. Plant height also differed between the two treatments, as plants in treatment 2 were significantly shorter than those in treatment 1. There were no height differences between treated and untreated plants, however, suggesting the controlled-environment conditions, rather than the pathogen itself, stunted the plant growth. Overall, inoculated plants in treatment 2 developed more severe light leaf spot symptoms at a faster rate compared to treatment 1.

Light intensity, rather than duration, may have had a greater impact on disease development. In the following study, plants exposed to a shorter photoperiod, but greater light intensity, developed more severe symptoms. During preliminary observations (Figure 3.14), *P. brassicae*-inoculated plants directly underneath a lamp, but also receiving diffused light from two additional flanking lamps, developed the most leaf distortion and discolouration, symptoms of extensive *P. brassicae* colonization. These plants were exposed to light from up to three lamps, whereas other inoculated plants, which were only exposed to light from one or two lamps, did not show leaf symptoms, suggesting a slower

rate of symptom development. Although these are only empirical observations, it is possible that the increased light intensity from the three lamps considerably accelerated symptom development on the affected plants, compared to plants exposed to fewer (or no) lamps.

Light treatment may have a greater impact on the pathogen than the host. *P. brassicae* in its endophytic phase may initially colonize the foliar vascular system to facilitate nutrient access, as is suggested by the necrotic flecking phenotype first appearing around leaf veins in resistant responses (Boys *et al*., 2012). This means that improved plant growth, possibly stimulated by optimal light conditions, may provide more opportunity for the pathogen to colonize. In this study, however, despite light strongly influencing plant growth and development, including the modulation of host defences (Ballaré, 2014; Karpinski *et al*., 2003; Roden and Ingle, 2009), plant biomass (estimated by the total number of leaves (data not shown) and dry weight in grams) did not significantly differ between light or inoculation treatments, suggesting that plants across all treatments were similar at harvest. Therefore, the light treatment may have had greater influence on the pathogen, as exposure to light is known to stimulate many developmental and biochemical processes in fungi, including germination, growth, conidial production and synthesis of secondary metabolites (Cerón-Bustamante *et al*., 2023; Keane and Kerr, 1997). A study on the plant pathogenic ascomycete fungus *Colletotrichum acutatum* showed that exposure to visible light during fungal growth increased production of conidia, compared to fungal colonies grown in continuous darkness (De Menezes *et al*., 2015). Other studies on ascomycetes suggest that light controls and promotes conidial production (Flaherty *et al*., 2005; Lauter *et al*., 1991; Lee *et al*., 2006). Increased light exposure in treatment 2 may have therefore positively influenced *P. brassicae* colonization.

P. brassicae continuously re-sporulated with asexual conidia while available living leaf tissue remained. The percentage leaf area covered in *P. brassicae* sporulation was estimated at four separate times, each time following leaf washing to remove the previous sporulation. Results showed that sporulation was able to re-occur at significant levels up to forty days after inoculation and

only stopped when leaf tissue fully senesced. Usually, secondary disease cycles in spring result from fungal hyphae underneath the leaf surface producing acervuli, leading to asexual sporulation and subsequent splash-dispersal of conidia (Boys *et al*., 2007; Fitt *et al*., 1998a). In field conditions, this means that even after spores have been washed off from the leaves, fungal hyphae growing below the leaf cuticle will continue to produce acervuli if there is available green leaf tissue to sustain hyphal colonization. This was verified here in controlled-environment conditions, confirming that inoculated plants may function as a potential source for inoculum production for up to four separate spore harvests through leaf washing. Follow-up studies to test the viability of each separate group of *P. brassicae* conidia may then provide further insight into pathogenicity of secondary disease cycles.

Past studies investigating the influence of environmental factors on light leaf spot development mainly focused on temperature and leaf wetness (Figueroa *et al*., 1995; Gilles *et al*., 2001c), whereas there are no current studies investigating the influence of light treatment. Phenotypic assessments in this study showed that light treatment significantly affected disease severity. Investigating the effect of light treatment on light leaf spot development, however, proved initially challenging due to technical setbacks. The biggest change was the addition of light intensity as a new parameter, although photoperiod (12 hr light against 8 hr light) was the only difference between treatments originally planned. Due to both length of the photoperiod and light intensity differing between the two treatments, it was initially difficult to identify which parameter contributed most to the differences in disease development. The underlying mechanisms of infection cannot be determined through visual assessments alone, as light may affect both host response and pathogen colonisation, but these preliminary findings suggested that light has a significant effect on *P. brassicae* asexual sporulation and growth, and future studies with more rigorous lighting treatments may help understand how light influences disease development pathways.

Chapter 4 Variations in *Pyrenopeziza brassicae* populations

Linear regression analysis and the related tables and figures included in this chapter were done by Dr Aiming Qi at the University of Hertfordshire, Hatfield.

4.1 Introduction

Information on the population structure of plant pathogens is essential to improve disease control strategies. One of the greatest challenges in plant disease management is the introduction of novel resistance measures. Developing new resistant cultivars is a long and complicated multidisciplinary approach and the maintenance of current resistance genes relies on rigorous strategies for deployment of resistance genes in space and time (Mitrousia *et al.,* 2018; Thakur *et al*., 2007). Similarly, producing chemical fungicides with new modes of action is very costly and time-consuming (Leadbeater, 2015), further exacerbated by periodic changes in legislation banning the usage of environmentally unfriendly, yet effective formulations (Hillocks, 2012). The greatest threat posed by fungal pathogen populations is their ability to evolve rapidly in response to host genetic and chemical control measures (McDonald and McDermott, 1993). A pathogen's ability to consistently adapt to new environmental changes stimulates research into novel resistance mechanisms. Understanding the genetic variations within fungal pathogen populations is therefore a key aspect in this persistent arms race between pathogen adaptation and introduction of disease control measures.

Genetic structure is defined by the amount and distribution of genetic variation within and between populations. Fungal populations with high levels of genetic variation are more likely to overcome host resistance strategies compared to populations with little genetic variation. By understanding the genetic structure within a population and its changes upon exposure to different environmental changes over time, it is possible to estimate the evolutionary rate of the pathogen and predict how long a control measure can remain effective (McDonald and McDermott, 1993). Generally, the pathogens that pose the greatest risks of breaking down resistance genes are those with a mixed

reproduction system, potential for allele exchange between populations (gene flow), large population sizes and high mutation rates (McDonald and Linde, 2002).

The evolutionary potential of *Pyrenopeziza brassicae*, the causative pathogen of light leaf spot, poses an increasing risk for oilseed and vegetable *Brassicas*. Work by Majer *et al*. (1998) using AFLP markers on *P. brassicae* populations from the UK, Germany and France suggested that there is a high level of genetic diversity within regions, implying frequent sexual reproduction. *P. brassicae* is heterothallic with two mating types designated as MAT-1 and MAT-2 and sexual reproduction occurs through their interaction, culminating in the development of an apothecium (sexual fruiting body) (Singh and Ashby, 1998). These apothecia release ascospores that travel through wind dispersal and act as primary inoculum initiating light leaf spot epidemics in autumn and late summer (Gilles *et al*., 2001b). Secondary disease infections in spring/summer may then be primarily initiated by splash-borne conidia produced through asexual sporulation (Ashby, 1997). This mixed reproduction system gives *P. brassicae* the potential to evolve through recombination of alleles during the sexual stage and to fix newly combined, advantageous alleles within the population using asexual reproduction (Karandeni Dewage *et al*., 2018).

Despite evidence suggesting the evolutionary potential of *P. brassicae* to overcome resistance genes, there is currently limited information about virulence of *P. brassicae* populations. This includes limited understanding of the mechanisms of host resistance against *P. brassicae* in commercial cultivars. This is a problem, since cultivation of cultivars without knowledge of their genetic background can lead to over-reliance on similar resistance mechanisms over long periods of time, applying selection on local pathogen populations and ultimately causing resistance breakdown (Karandeni Dewage *et al*., 2018). Resistance of new cultivars is best assessed in field experiments through exposure to pathogen populations under natural conditions, such as the field trials done by AHDB for their yearly Recommended Lists. However, the specific genetic components of resistance against pathogens are best assessed in controlled environment experiments using single-spore isolates

tested against a differential host set (Boys *et al*., 2007). There are currently limited studies of isolate-specific resistance against *P. brassicae* (Bradburne *et al*., 1999; Karandeni Dewage *et al*., 2021; Karolewski, 1999), particularly a lack of studies comparing isolates from different countries. Experimental work in this chapter therefore describes glasshouse experiments using *P. brassicae* isolates obtained from different sites across the UK and continental Europe, tested against a differential set of oilseed rape cultivars with varying levels of host resistance against *P. brassicae*. Similarly, field assessments were done to compare the light leaf spot disease severity on the same cultivars at different sites and in different cropping seasons. The hope is that these findings will provide evidence and further insight into the variations of *P. brassicae* populations across geographic areas and their underlying mechanisms of resistance.

Light leaf spot can also infect vegetable *Brassicas* as well as oilseed rape (*Brassica napus*), such as cauliflowers (*Brassica oleracea* var. *botrytis*), cabbages (*B. oleracea* var. *capitata*), sprouting broccoli (*B. oleracea* var. *italica*), Brussels sprouts (*B. oleracea* L. var. *gemmifera*), kale (*B. oleracea* var. *acephala*), turnip (*Brassica rapa* ssp. *rapa*) and Chinese cabbage (*B. rapa* ssp. *pekinensis*) (Cheah *et al*., 1980; Karolewski, 2010; Maddock *et al*., 1981). *P. brassicae* has also been shown to cross-infect different *Brassica* host species (Karandeni Dewage *et al*., 2018; Maddock *et al.*, 1981), with the recommendation that fields with vegetable *Brassicas* should be spatially and temporally separated from fields with oilseed *Brassicas*. However, many of these studies are old and used a limited number of isolates. Since *P. brassicae* populations change over time (Majer *et al*., 1998), more work is needed to study the virulence of current *P. brassicae* populations towards different host species in the UK. Experimental work in this chapter therefore aimed to investigate the potential for cross-infection of *P. brassicae* between two related *Brassica* species, oilseed rape and kale, using *P. brassicae* populations derived from either host species.

Overall, more work is needed to investigate the differences between *P. brassicae* populations, their evolutionary potential, and their ability to cross-

infect different *Brassica* species. Information on the virulence of *P. brassicae* populations is essential to elucidate underlying resistance mechanisms.

4.1.1 Aims and objectives

Experimental work in this chapter aimed to improve understanding of variations in *P. brassicae* populations through glasshouse experiments using single-spore *P. brassicae* isolates or *P. brassicae* populations, as well as field experiments with different cultivars at different sites over different seasons. Four objectives were investigated:

- To establish a *P. brassicae* isolate collection
- To assess light leaf spot severity in field experiments with different cultivars at different locations in different cropping seasons
- To characterize the phenotype of differential interactions between oilseed rape cultivars/lines and single spore isolates of *P. brassicae*
- To investigate cross-infection of *P. brassicae* between *B. napus* (oilseed rape) and *B. oleracea* (kale) using *P. brassicae* populations derived from either *Brassica* species

4.2 Materials and methods

Experimental work in this chapter describes two experiments done under glasshouse conditions and two field experiments. The *Pyrenopeziza brassicae* isolate collection obtained was used to study specific host-pathogen interactions between single-spore *P. brassicae* isolates and a differential set of oilseed rape cultivars/lines. *P. brassicae* populations derived from oilseed rape (*Brassica napus*) and kale *(B. oleracea*) were used to investigate cross-infections between these two *Brassica* species.

4.2.1 Establishment of *P. brassicae* isolate collection

P. brassicae isolates were collected in Hertfordshire, Herefordshire, Cambridgeshire and Lincolnshire. Leaves with light leaf spot symptoms were sampled from crops and single *P. brassicae* isolates were obtained following the method described in section 2.1.2. The *P. brassicae* isolates obtained were

subsequently subcultured onto malt extract agar for spore and mycelium production, following the method described in section 2.1.3. Pre-existing isolates from Scotland and continental Europe obtained from external sources were also included in the collection.

4.2.1.1 DNA extraction

Fungal mycelium grown on cellulose-disc amended malt extract agar (MEA) media (section 2.1.2) was harvested using a sterile scalpel and placed in 2 ml screw-cap tubes. Mycelial samples were then freeze-dried for 48 hr. DNA extraction was then done using a modified CTAB method. The DNA extraction buffer used included three separate buffers. Buffer A was made of 0.35 M sorbitol, 0.1 M Tris-HCl and 5 mM EDTA at ph 8. Buffer B was made of 2% (w/v) CTAB, 1% (w/v) PVP in 1 vol 2x TEN (2 M NaCl, 50 mM EDTA, 0.2 M Tris-HCl at ph 8). Buffer C consisted of 5% Sarkosyl. The DNA extraction buffer was prepared at the point of use by pre-warming 2.5 vol of Buffer B for 5 min in a 65°C water bath. Then, 1% β-mercaptoethanol was added, followed by 2.5 vol Buffer A, 1 vol Buffer B and 0.048 vol Proteinase K. Three autoclaved metal beads were added to 20 mg of freeze-dried mycelium and samples were then ground using a Fast-prep machine at 4.0 m/s for 20 sec. Afterwards, 800 µl of DNA extraction buffer were added to each sample. Samples were then vortexed briefly and incubated for 30 min in a 65°C water bath, making sure to invert sample tubes every 10 min. After incubation, samples were allowed to cool down to 20°C, before adding 800 µl of the bottom phase of cold phenol:chloroform:isoamyl alcohol mixture (25:24:1). Samples were then centrifuged at 13000 rpm for 10 min at 4°C and the top aqueous phase was transferred to a new 1.5 ml Eppendorf tube. Samples were subsequently treated with RNase A and incubated for 30-60 min at 37°C on a hot plate. Afterwards, 60 µl of NaAc and 396 µl of cold isopropanol were added to each sample. Samples were inverted to mix, incubated at 20°C for 5 min and then stored at -20°C for 16 hr to precipitate the DNA. The samples were then centrifuged at 13000 rpm for 30 min at 4°C before removing the supernatant. The DNA pellet was then washed with 400 µl of cold 70% ethanol and further centrifuged at 13000 rpm for 10 min at 4°C. The remaining ethanol was then

removed first with a pipette, then by letting excess ethanol evaporate. Once dry, the pellet was resuspended in 70 µl sterile distilled water and stored at -20°C.

4.2.1.2 DNA quantity and quality measurement

DNA samples stored at -20°C were thawed, briefly vortexed and centrifuged at 10000 rpm for 2 min. The supernatant was then transferred in 60 µl aliquots (corresponding to the amount of water added to re-suspend the DNA minus 10 µl) onto a new 1.5 ml Eppendorf tube to prevent the transfer of fungal dust contamination. DNA concentration was determined with a Qubit™ dsDNA BR assay kit (Thermo Fisher Scientific, UK) and measured using a Qubit™ 3.0 fluorometer (Thermo Fisher Scientific, UK) following the manufacturer's protocol. DNA purity was tested using a Nanodrop ND-1000 spectrophotometer (Labtech International, UK) following the manufacturer's protocol by recording the A 260/ A 280 ratio, where a ratio of 1.8 indicated pure DNA.

4.2.1.3 Mating-type PCR

The three-primer PCR technique (Foster *et al*., 2002) was used to identify the mating type of some *P. brassicae* isolates. Each PCR reaction was a 20 µl standard volume containing 10 µl Redtaq ready mix, 1 µl sterile nuclease-free water, 2 µl of 10 µM PbM-1-3 (5′-GAT CAA GAG ACG CAA GAC CAA G-3′), 2 µl of 10 µM PbM-2 (5′-CCC GAA ATC ATT GAG CAT TAC AAG-3′), 4 µl of 10 µM Mt3 (5′-CCA AAT CAG GCC CCA AAA TAT G-3′) and 1 µl of *P. brassicae* genomic DNA with a concentration between 10-100 ng/µl. A negative control was included by replacing sample DNA with nuclease-free water. The thermal cycling parameters were: initial denaturation at 95°C for 10 min, followed by 30 cycles at 95°C for 1 min, 56°C for 1 min and 72°C for 1 min, followed by a final extension step at 72°C for 3 min. PCR products were visualised using gel electrophoresis, where MAT-1 isolates were expected to produce a 687 bp product and MAT-2 isolates were expected to produce a 858 bp product.

4.2.1.4 RAPD PCR

RAPD (random amplified polymorphic DNA) PCRs were done using conditions modified from Boys (2009). A 20 µl reaction volume was used, consisting of 10 µl Redtaq ready mix, 4 µl 50 µM RAPD primer (which acted as both forward and reverse primer, Table 4.1), 5 µl sterile nuclease-free water and 1 µl of DNA. Thermo cycling parameters were 45 cycles of 30 sec at 93°C (ramp rate 30°C min^{-1}), 40 sec at 37°C (ramp rate 30°C min^{-1}) and 80 sec at 72°C (ramp rate 20°C min^{-1}) followed by a final elongation step of 5 min at 72°C and storage at 4°C. Negative (water) controls were included for all RAPD primers. PCR products were analysed with gel electrophoresis. A 1.5% agarose gel was prepared with 1x TBE buffer and 1% GelRed (Sigma-Aldrich, United States) and allowed to set for 30 min. The gel was then placed in the electrophoresis tank filled with 1x TBE buffer and loaded with 10 µl of sample, a negative control and a 1 kb DNA ladder. Gels were run at 90 V for 90 min and visualised under UV light using the GeneSys software (Syngene).

4.2.1.5 Agarose gel electrophoresis

PCR products were analysed with gel electrophoresis. A 1.5% agarose gel was prepared with 1x TBE buffer and 1% GelRed (Sigma-Aldrich, United States) and allowed to set for 30 min. The gel was then placed in the electrophoresis tank filled with 1x TBE buffer and loaded with 10 µl of sample, a negative control and a 100 bp DNA ladder. Gels were run at 90 V for 90 min and visualised under UV light using the GeneSys software (Syngene).

4.2.2 Field experiments

Two different field experiments were done. The first field experiment (experiment A) was done with three commercial cultivars at two locations over two cropping seasons. The second field experiment (experiment B) was done with ten commercial cultivars at two locations in one cropping season. The individual experiments are described below and summarized in Table 4.2.

Table 4.1: Primers used for RAPD PCR analysis of *Pyrenopeziza brassicae* isolates.

Nine primers were synthesized for RAPD (random amplified polymorphic DNA) PCR analysis of *P. brassicae* isolates. The primers were originally described by Boys (2009).

Primer	Sequence
OPA-05	5'-AGGGGTCTTG-3'
OPA-09	5'-GGGTAACGCC-3'
OPA-20	5'-GTTGCGATCC-3'
OPW-05	5'-GGCGGATAAG-3'
OPW-06	5'-AGGCCCGATG-3'
OPW-09	5'-GTGACCGAGT-3'
OPW-10	5'-TCGCATCCCT-3'
OPAJ-01	5'-ACGGGTCAGA-3'
OPAJ-03	5'-AGCACCTCGT-3'

Table 4.2: Summary of field experiments.

Two field experiments were done. The number of cultivars used, locations, cropping seasons and assessment methods for two major oilseed rape diseases, light leaf spot (LLS) and phoma stem canker are shown.

Field experiment	Number of cultivars	Location	Cropping season/s	Assessment method
A	Three	Huntingdon (Cambridgeshire), Hereford (Herefordshire)	2021/22, 2022/23	Spring: incubate leaves and assess for % leaf area with *Pyrenopeziza brassicae* asexual sporulation, LLS* disease score using a 1-8 scale (with 1 being no disease), presence/absence of necrotic response Summer: LLS stem/pod and phoma stem canker assessments
B	Ten	Harpenden (Hertfordshire), Norwich (Norfolk)	2023/24	Spring: on-site LLS assessment using a 0-7 scale (with 0 being no disease) Summer: LLS stem/pod and phoma stem canker assessments

*LLS – Light leaf spot

4.2.2.1 Choice of oilseed rape cultivars

For field experiment A, three commercial oilseed rape cultivars, Aquila, Flamingo and Acacia were chosen according to their resistance rating to *P. brassicae* and used in the field experiments in Huntingdon, Cambridgeshire and Hereford, Herefordshire over two cropping seasons (2021/22, 2022/23). Specific information about the chosen cultivars can be found in Table 4.3. The cv. Aquila and cv. Flamingo were drilled at both sites in both the cropping seasons. However, cv. Flamingo established poorly at the Huntingdon site in the 2022/23 season; therefore, cv. Acacia was assessed instead. All plots were not treated with fungicides.

For field experiment B, ten oilseed rape cultivars were drilled at two field sites, one in Harpenden, Hertfordshire and another one in Norwich, Norfolk, for the 2023/24 cropping season. Light leaf spot resistance ratings of the chosen cultivars can be found in Table 4.4.

4.2.2.2 Field experiment design

Field experiment A was run by ADAS over two cropping seasons (2021/22, 2022/23) at two different sites, Huntingdon in Cambridgeshire and Hereford in Herefordshire. A summary detailing the dates on which the plots were sown, as well as key assessment dates, is shown in Table 4.5. Plots were typically sown at the end of August or start of September in each season at a seed rate of 120 seeds/m^2 at a depth of 1-2 cm and row width of 37.5 cm. Plots were 12 m in length and 3 m wide. Except for no fungicide spray, the field experiments were managed according to the standard farm practice. None of the fields were inoculated, meaning infection resulted from natural *P. brassicae* inoculum.

Field experiment B was operated by Rothamsted Research in Harpenden, Hertfordshire and by the John Innes Centre in Norwich, Norfolk. Plots in Harpenden were sown on 19 August 2023 at a seed rate of 80 seeds/m^2 at a depth of 2-4 cm and rows were 9 m in length and 1.8 m wide with a row width of 50 cm (Appendix E). Plots in Norwich were sown on 30 August 2023 at a seed rate of 80 seeds/m^2 at a depth of 2-4 cm and rows were 6 m in length and 2.7 m wide (Appendix F). All plots were treated with fungicides (Appendix G).

Table 4.3: List of oilseed rape cultivars used for field experiments in Huntingdon and Hereford.

Field experiments were done over two cropping seasons (2021/22, 2022/23) at two different sites (Huntingdon, Cambridgeshire and Hereford, Herefordshire). Cultivars, their AHDB resistance ratings to *Pyrenopeziza brassicae* (light leaf spot), field location and cropping season used are shown.

Cultivar	Resistance rating for *P. brassicae*	AHDB RL Source*	Field experiment location	Cropping season
Aquila	6	2021/22	Huntingdon (Cambridgeshire), Hereford (Herefordshire)	2021/22, 2022/23
Flamingo	7	2020/21	Huntingdon, Hereford	2021/22, 2022/23 (Herefordshire only)
Acacia	6	2023/24	Huntingdon	2022/23

*AHDB Cereals and Oilseeds recommended list (RL) rating for light leaf spot on a 1-9 scale, where 9 is most resistant (https://ahdb.org.uk/knowledge-library/recommended-lists-archive). RL rating for each cultivar was taken from the most recent records available

Table 4.4: List of oilseed rape cultivars used for field experiments in Harpenden and Norwich.

Field experiments were done in the 2023/24 cropping season at two different sites (Harpenden, Hertfordshire and Norwich, Norfolk). Cultivars, their resistance ratings to *Pyrenopeziza brassicae* (light leaf spot) and the resistance rating source are shown.

Cultivar	Resistance rating for *P. brassicae*	Resistance rating source
Acacia	6	AHDB RL* 2023/24
Apex	5	AHDB RL 2001/02
Campus	6	AHDB RL 2019/20
Castille	4.9	AHDB RL 2012/13
Catana	7	AHDB RL 2015/16
Kielder	7	AHDB RL 2020/21
Kromerska	Unknown	N/A
Poh Bolko	Unknown	N/A
Rocket	7	AHDB RL 1996/97
Yudal	Little resistance	Pilet *et al.,* 1998a

*AHDB Cereals and Oilseeds recommended list (RL) rating for light leaf spot on a 1-9 scale, where 9 is most resistant (https://ahdb.org.uk/knowledge-library/recommended-lists-archive). RL rating for each cultivar was taken from the most recent records available, except for cv. Apex, where the value was sourced from Boys (2009) and cv. Rocket, where the value was sourced from Fitt *et al.* (1998a)

Table 4.5: Drilling dates and assessment dates of field experiments in Huntingdon and Hereford.

Field experiments were done over two cropping seasons (2021/22, 2022/23) at two different sites (Huntingdon, Cambridgeshire and Hereford, Herefordshire). Plots were sown in late summer, light leaf spot foliar assessment was done in Spring, while light leaf spot stem and pod, as well as phoma stem canker assessments were done in summer before harvest.

Event description	Huntingdon		Hereford	
	2021/22	2022/23	2021/22	2022/23
Plots drilled	23 August 2021	1 September 2022	20 August 2021	30 August 2022
Light leaf spot foliar assessment	27 April 2022	18 April 2023	20 April 2022	18 April 2023
Light leaf spot stem and pod assessment Phoma stem canker assessment	6 July 2022	26 June 2023	5 July 2022	23 June 2023

None of the fields were inoculated, meaning infection resulted from natural *P. brassicae* inoculum.

4.2.2.3 Light leaf spot foliar assessment

Light leaf spot foliar assessment was done in April when *P. brassicae* symptoms were observable on leaves. For experiment A, 20 leaves were randomly sampled from each plot, wrapped in dampened tissue paper, placed in sealed polyethylene bags to retain humidity and incubated at 4°C for 7-9 days to promote sporulation. Light leaf spot disease severity was then assessed on a 1-8 scale (with 1 being no disease, section 2.4.1) and by visually estimating the percentage leaf area covered in *P. brassicae* asexual sporulation (section 2.4.2). The presence of necrotic flecking was also recorded.

Additionally, single-spore isolates of *P. brassicae* were obtained from the sampled leaves by using the method described in section 2.1.2. For experiment B, light leaf spot severity was assessed at both sites on 10 April 2024. For each cultivar, ten whole plants were randomly selected from three replicate plots, for a total of 30 plants per cultivar, and were scored for light leaf spot on a 0-7 scale used for on-site assessment (with 0 being no disease, Table 4.6).

4.2.2.4 Light leaf spot stem and pod assessment

Assessment of light leaf spot on stems and pods was done in the summer before crop desiccation. The assessment was done by estimating the percentage area of stem and pods covered in light leaf spot lesions. Additionally, phoma stem base canker was scored using a 0-7 scoring system based on the percentage stem cross-section affected by canker (where 0 = 0%, 1 = <5%, 2 = <25%, 3 = <50%, 4 = <75%, 5 = <100%, 6= 100% with unbroken stem and plant still alive, 7 = 100% with dead stem with a hollow or severely necrotic pith; Fortune *et al*., 2021). For experiment A, 20 randomly selected whole plants were destructively sampled at the stem base for each plot. For experiment B, assessments were done on 1st July 2024 and for each cultivar, ten whole plants were randomly selected from three replicate plots and destructively harvested, for a total of 30 plants per cultivar. Stem, pod and phoma stem base canker assessments were done for all plants.

Table 4.6: Light leaf spot disease severity score used for on-site assessment.

A 0-7 scale disease severity score (with 0 being no disease) was used to assess *Pyrenopeziza brassicae* (light leaf spot) foliar disease in field experiments.

Score	Description
0	No disease observable
1	Trace of disease
3	Diseased leaves with one or a few small lesions Plants with scattered lesions
5	Diseased leaves with a few large lesions or many small lesions Area quite severely affected Half green/half diseased, leaf curling and distortion Evidence of stunting
7	Plants very stunted or dead

4.2.2.5 Statistical analysis

Analysis of variance was done using R (version 4.2.2). The data for percentage leaf area covered in *P. brassicae* sporulation, the % stem area covered in *P. brassicae* lesions and the % pod area covered in *P. brassicae* lesions were transformed using an arc-sine transformation. The presence or absence of a necrotic response in different areas of the leaf was taken as a qualitative measure. Plots were generated using Microsoft Excel using calculated means for the different treatments.

4.2.3 Glasshouse experiment: variation of disease phenotypes

4.2.3.1 Preparation of plant material

Nine oilseed rape cultivars and breeding lines with varying levels and sources of host resistance against *P. brassicae* were used in a glasshouse experiment. Characteristics of each cultivar/line can be found on Table 4.7. Plants were grown in glasshouse conditions following the method described in section 2.3.1 until they were ready for inoculation.

4.2.3.2 Preparation of *P. brassicae* inoculum

Twenty-four single-spore isolates of *P. brassicae* from six different European countries were selected (Table 4.8). The individual isolates were obtained from diseased plant material following the method described in section 2.1.2. Isolates were sub-cultured onto malt extract agar (MEA) media for spore production as described in section 2.1.3. *P. brassicae* inoculum was prepared for each isolate from the MEA cultures following the method given in section 2.2.1.

4.2.3.3 Experimental design and plant inoculation

Plants were arranged in a split-plot design generated using Experiment Design Generator and Randomiser (EDGAR) (Brown, 2005), where replicates were blocks, isolates were the main plot and cultivars were the sub-plot. For each treatment, five replicates were used. It was not possible to assess all 24 *P. brassicae* isolates in one experiment, so isolates were divided into six batches

Table 4.7: Characteristics of oilseed rape cultivars/lines selected to study interactions with *Pyrenopeziza brassicae*.

Differential host-pathogen interactions between *P. brassicae* isolates and nine oilseed rape cultivars or breeding lines were studied in glasshouse experiments. The nine cultivars/lines used, their resistance rating against *P. brassicae*, the source of the rating and a description of the expected resistance response are shown.

Cultivar/ line	Type	Resistance rating for *P. brassicae*	Source	Description
Charger	Commercial cultivar	4	AHDB RL* 2016/17	Recommended in the North region for moderate resistance against *P. brassicae* and *L. maculans*
Bristol	Commercial cultivar	2	AHDB RL 1996/97	Suggested to carry a major resistance gene against *P. brassicae* that was rendered ineffective
Barbados	Commercial cultivar	7	AHDB RL 2021/22	Recommended for good resistance against *P. brassicae*
Imola	Commercial cultivar	Good resistance	Boys *et al.*, 2012	Contains a major resistance gene against *P. brassicae* producing a necrotic response
Q02	DH breeding line	Good resistance	Smooker *et al.,* 2011	DH breeding line developed by crossing a synthetic *B. napus* with oilseed rape cv. Tapidor; produces a necrotic response
Q60	DH breeding line	Good resistance	Smooker *et al.*, 2011	DH breeding line developed by crossing a synthetic *B. napus* with oilseed rape cv. Tapidor
NPZ 05/22	Pre-commercial cultivar	Unknown	Supplied by NPZ	Pre-commercial cultivar with no available pedigree or disease resistance information
NPZ 06/22	Pre-commercial cultivar	Unknown	Supplied by NPZ	Pre-commercial cultivar with no available pedigree or disease resistance information
Yudal	Spring inbred line	Little resistance	Pilet *et al.*, 1998a,b	Korean spring inbred line very susceptible to *P. brassicae* and *L. maculans*

*AHDB Cereals and Oilseeds recommended list (RL) rating for light leaf spot on a 1-9 scale, where 9 is most resistant (https://ahdb.org.uk/knowledge-library/recommended-lists-archive). RL rating for each cultivar was taken from the most recent record available, except for cv. Bristol, where the value was sourced from Karolewski *et al.* (2006)

Table 4.8: Description of *Pyrenopeziza brassicae* isolates used to study specific interactions with *Brassica napus*.

Twenty-four single-spore *P. brassicae* isolates obtained from UK and EU were used in a glasshouse experiment to study specific interactions with different oilseed rape cultivars/lines.

Isolate*	Crop†	Cultivar	Location	Year	Mating type
Aqu-Here-1	OSR	Aquila	Hereford, England	2021	MAT-2
Aqu-Here22-1	OSR	Aquila	Hereford, England	2022	MAT-1
Aqu-Hunt22-1	OSR	Aquila	Huntingdon, England	2022	MAT-1
Charger-H-1	OSR	Charger	Hertfordshire, England	2021	MAT-2
Charger-H-2	OSR	Charger	Hertfordshire, England	2021	Untested
Kale-H-6	Kale	Nero di Toscana	Hertfordshire, England	2021	MAT-2
Kale-H-7	Kale	Nero di Toscana	Hertfordshire, England	2021	Untested
Parkside-L-1	OSR	Parkside	Lincolnshire, England	2021	MAT-2
Parkside-L-3	OSR	Parkside	Lincolnshire, England	2021	MAT-2
Tenor-L-4	OSR	Tenor	Lincolnshire, England	2021	MAT-2
A7	Brussel sprout	Clodius	Fife, Scotland	2013	Untested
A11	Brussel sprout	Braemer	Berwickshire, Scotland	2013	MAT-1
18CAR02	OSR	Unknown	Carlow, Ireland	2018	Untested
18CAR04	OSR	Unknown	Carlow, Ireland	2018	Untested
18CAR05	OSR	Unknown	Carlow, Ireland	2018	Untested
19DEN03	OSR	Unknown	Skanderborg, Denmark	2019	Untested
19DEN19	OSR	Unknown	Skanderborg, Denmark	2019	Untested
19DEN33	OSR	Unknown	Skanderborg, Denmark	2019	Untested
19GERA1	OSR	Unknown	Bleckendorf, Germany	2019	Untested
19GERA3	OSR	Unknown	Bleckendorf, Germany	2019	Untested
19GERC2	OSR	Unknown	Bleckendorf, Germany	2019	Untested
20POL01	OSR	Unknown	Bonin, Ireland	2019	Untested
20POL04	OSR	Unknown	Bonin, Ireland	2019	Untested
20POL14	OSR	Unknown	Bonin, Ireland	2019	Untested

**P. brassicae* isolates from Scotland were obtained by Coretta Kloeppel; isolates from Ireland, Denmark, Germany and Poland were collected by Dr Kevin King and Dr Nichola Hawkins and provided by Rothamsted Research
†OSR – Oilseed rape

and assessed in six separate experiments over a 3-month period. Four isolates were assessed in each batch (Appendix H). Plants were grown in glasshouse conditions. When plants reached growth stage 1,4-1,5 (Sylvester-Bradley, 1985), they were spray-inoculated with conidial suspensions (10^5 spores/ml + 0.005% Tween 80) as described in section 2.3.2.

4.2.3.4 Assessment of light leaf spot symptoms

Plants were harvested at 24 days post inoculation (dpi), placed in polyethylene bags and incubated at 4°C for 7-9 days to induce *P. brassicae* sporulation. Individual plants were then scored for light leaf spot severity using the 1-8 scale and for percentage leaf area covered with *P. brassicae* asexual sporulation (acervuli). Presence of a necrotic response and other symptoms such as total number of leaves, numbers of deformed leaves, dead leaves and discoloured leaves (red, purple, etc.) were also recorded.

4.2.3.5 Statistical analysis

Analysis of variance was done using R (version 4.2.2). Data transformations were applied to all parameters, except for the light leaf spot disease score. The numbers of dead leaves, deformed leaves and discoloured leaves were expressed as percentages by dividing the number of affected leaves by the total number of leaves on each plant. These values, along with the percentage leaf area covered with *P. brassicae* sporulation, were transformed using an arc-sine transformation. The presence or absence of a necrotic response was taken as a qualitative measure.

Correlations between light leaf spot disease score, % leaf area with sporulation, % deformed leaves, % dead leaves and % discoloured leaves were analysed using Microsoft Excel. Plots were generated in Microsoft Excel using calculated means of the different treatments.

Host-pathogen interactions were further analysed using the linear regression analysis described in Karandeni Dewage *et al*. (2021). Resistant/susceptible responses of the cultivars/lines (measured using either the disease score or %

leaf area covered in sporulation) were analysed using the following regression model:

$$Y_{ij} = \mu_i + b_i I_j + \delta_{ij}$$

where Y_{ij} is the mean response of the *i*th cultivar to the *j*th isolate, μ_i is the mean response of the *i*th cultivar/line over all isolates, b_i is the regression coefficient that measures the response of the *i*th cultivar/line to varying levels of virulence in isolates, I_j is the isolate virulence index, which is defined as the mean response of all cultivars/lines with the *j*th isolate minus the overall mean, and δ_{ij} is the sum of deviations from regression of the *i*th cultivar/line with the *j*th isolate. Regression slope parameters and deviation mean squares were taken for each cultivar/line in the analyses of disease response to isolate virulence index. Analysis was done and plots were generated using Genstat (Payne *et al*., 2011).

4.2.4 Cross-infection of *P. brassicae* between *B. napus* and *B. oleracea*

4.2.4.1 Preparation of plant material and experiment design

Susceptible oilseed rape cultivar Charger (LLS resistance rating 4 on a 1-9 scale where 9 is most resistant, AHDB Recommended List 2016/2017) was used for this experiment. For kale, cultivar Nero di Toscana (commercial cultivar with no LLS disease data available) was used. Plants were grown in controlled environment conditions according to the method described in section 2.3.1 until plants reached growth stage 1,4-1,5. Plants were arranged in a split-plot design generated using Experiment Design Generator and Randomiser (EDGAR) (Brown, 2005) where replicates were blocks, host type was the main plot and inoculum type was the sub-plot. For each treatment, eight replicates were used. Uninoculated oilseed rape and kale plants were included as controls.

4.2.4.2 Preparation of *P. brassicae* inoculum and plant inoculation

The *P. brassicae* inoculum Scotland-Pb and Kale-Pb (Table 2.1) were bulked up from diseased leaves of oilseed rape cultivar Charger grown in controlled environment. The diseased leaves were incubated at 4°C for 7-9 days after

sampling. *P. brassicae* inoculum was prepared from the incubated leaves following the method described in section 2.2.2. Plants were spray-inoculated with conidial suspensions (10^5 spores/ml + 0.005% Tween 80) as described in section 2.3.2.

4.2.4.3 Assessment of light leaf spot symptoms

Plants were harvested at 24 dpi, placed in polyethylene bags and incubated at 4°C for 7-9 days to induce *P. brassicae* sporulation. Individual plants were then scored for light leaf spot disease using the 1-8 scale and for percentage leaf area covered with *P. brassicae* asexual sporulation (acervuli). Total numbers of leaves, numbers of deformed leaves, dead leaves and discoloured leaves (red, purple, etc.) were recorded at 3, 8, 11, 14, 17, 22 and 24 dpi for each plant. The presence of a necrotic response in different areas of the leaf (petiole, midrib and lamina) was also assessed. After assessment, the initial *P. brassicae* spores were removed from the plants through leaf washing (section 2.2.2). Plants were then further incubated at 4°C for three weeks and a second sporulation was assessed by estimating the % area of *P. brassicae* sporulation on the leaf.

4.2.4.4 Statistical analysis

Analysis of variance was done using R (version 4.2.2). Data transformations were applied to all parameters, except for the light leaf spot disease score. The numbers of deformed leaves, dead leaves and discoloured leaves were expressed as percentages by dividing the numbers of affected leaves by the total number of leaves on each plant. These values, along with the percentage leaf area covered in *P. brassicae* sporulation before and after leaf washing, were transformed using an arc-sine transformation. Plots were generated in Microsoft Excel using calculated means for the different treatments.

4.3 Results

4.3.1 Establishment of *Pyrenopeziza brassicae* isolate collection

For this collection, 50 single-spore *P. brassicae* isolates were obtained in England over three cropping seasons (2021, 2022 and 2023). Additionally, 16 *P. brassicae* isolates obtained from Scotland (originally collected by Coretta Klöppel) were included. Finally, 42 *P. brassicae* isolates from Germany, Denmark, Poland and Ireland were provided by Dr Kevin King from Rothamsted Research, Harpenden. In total, 109 *P. brassicae* isolates from six different European countries were included in the collection (Appendix I). Of these, the mating types of 41 *P. brassicae* isolates were determined; 21 isolates were MAT-1, while 20 isolates were MAT-2 (Figure 4.1).

RAPD-PCR results remained inconclusive. DNA extraction was done for all the single-spore *P. brassicae* isolates, but the DNA was fragmented due to excessive centrifugation and did not amplify using RAPD markers. DNA extraction was repeated for all the isolates, but amplification using RAPD markers was inconsistent. Both RAPD primers and *P. brassicae* isolates often failed to amplify and the products visualized using agarose gel electrophoresis were insufficient and could not be used to distinguish between *P. brassicae* races.

4.3.2 Field experiments

For field experiment A, leaves sampled presented a range of light leaf spot symptoms, including *P. brassicae* sporulation on the leaf surface and necrotic flecking on various parts of the leaf (Figure 4.2). For the 2021/22 season, there were no significant differences in disease score ($P > 0.92$) or % leaf area with sporulation ($P > 0.2$) between cultivars, but cv. Flamingo showed a significantly greater score ($P < 0.001$) and % leaf area with sporulation ($P < 0.001$) at the Hereford site compared to Huntingdon. For the 2022/23 season, there were no significant differences in disease score between cultivars ($P > 0.2$) or between sites ($P > 0.55$) and there were no significant differences in % leaf area with sporulation between cultivars ($P > 0.38$) or between sites ($P > 0.94$). Upon

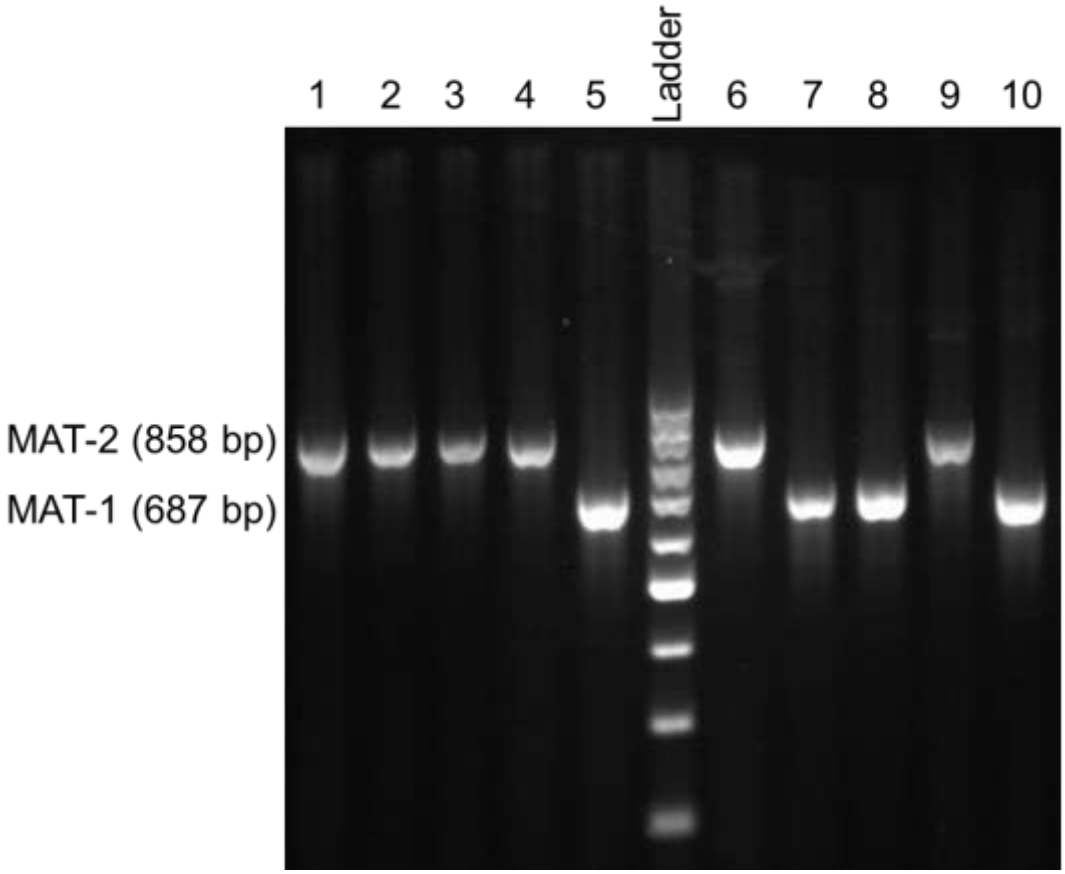

Figure 4.1: Example PCR amplification to identify the mating types of *Pyrenopeziza brassicae* isolates.

PCR amplification using the primers PbM-1-3, PbM-2 and Mt3 (Foster *et al*., 2002) was used to identify the mating types of *P. brassicae* isolates. PCR products were visualised using gel electrophoresis, where MAT-1 isolates produced a 687 bp product (lanes 5, 7, 8 and 10) and MAT-2 isolates produced a 858 bp product (lanes 1, 2, 3, 4, 6 and 9).

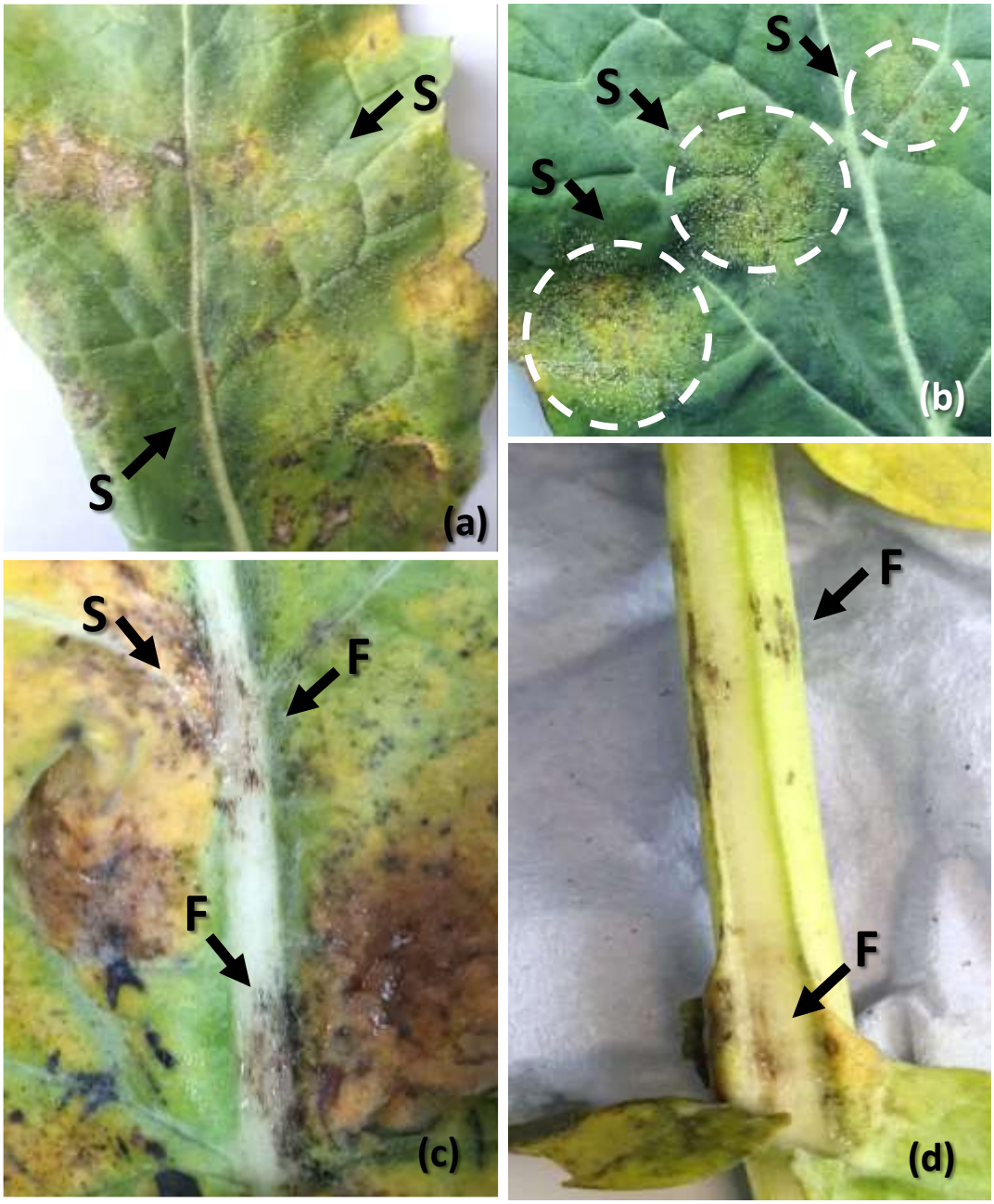

Figure 4.2: Light leaf spot foliar symptoms on oilseed rape leaves sampled from a field experiment.

Leaves of cv. Aquila and cv. Flamingo were sampled from a field experiment in Hereford, Herefordshire on 20 April 2022. The leaves were incubated at 4°C for 9 days to induce *Pyrenopeziza brassicae* (light leaf spot) sporulation (S). Symptoms are shown as (a) *P. brassicae* sporulation covering the leaf lamina of cv. Flamingo and (b) concentric rings of sporulation on cv. Flamingo. Symptoms of necrotic flecking (F) are shown on (c) the leaf midrib of cv. Aquila and (d) the petiole of cv. Aquila.

comparing the two cropping seasons, cv. Aquila showed a significantly greater disease score (Hereford $P < 0.001$; Huntingdon $P < 0.001$) and % leaf area with sporulation (Hereford $P < 0.001$; Huntingdon $P < 0.001$) in the 2021/22 season, compared to the 2022/23 season, at both sites. Cv. Flamingo also showed significantly greater disease score ($P < 0.001$) and % leaf area with sporulation ($P < 0.001$) at the Hereford site in the 2021/22 season, compared to the 2022/23 season (Figure 4.3).

Presence of necrotic flecking anywhere on the leaf was recorded (Figure 4.2). In the 2021/22 season, leaves from cv. Aquila at either site did not show flecking; leaves from cv. Flamingo, however, presented flecking at the Hereford site, but not in Huntingdon. In the 2022/23 season, however, cv. Aquila sourced from Hereford presented flecking but not when sourced from Huntingdon; cv. Flamingo, on the other hand, did not show flecking at either site. Overall, necrotic flecking was recorded from leaves sourced at the Hereford site only, although it was on a different cultivar each year.

Stem and pod assessments were done in the summer on randomly sampled whole plants. Stem lesions were either light brown or purple, often with black flecking around the edge, while pod lesions were mostly small and brown, accompanied by black speckling (Figure 4.4). The % of stem area and % of pod area covered in light leaf spot lesions were assessed, although the % pod area covered was extremely small across all sites and years (min: 0%, max: 7%).

For the 2021/22 season, there were no significant differences in % stem area with lesions ($P > 0.05$) or % pod area with lesions ($P > 0.43$) between cultivars, and plants presented significantly greater % stem area with lesions ($P < 0.001$) and % pod area with lesions ($P < 0.002$) at the Hereford site compared to Huntingdon (Figure 4.5). For the 2022/23 season, plants from Hereford again showed significantly greater % stem area with lesions ($P < 0.001$) and % pod area with lesions ($P < 0.001$) compared to Huntingdon. Cv. Aquila grown in Hereford showed the greatest % stem area with lesions ($P < 0.001$) and % area with pod lesions ($P < 0.001$). Upon comparing the two cropping seasons, there were no overall significant differences between years for cv. Aquila

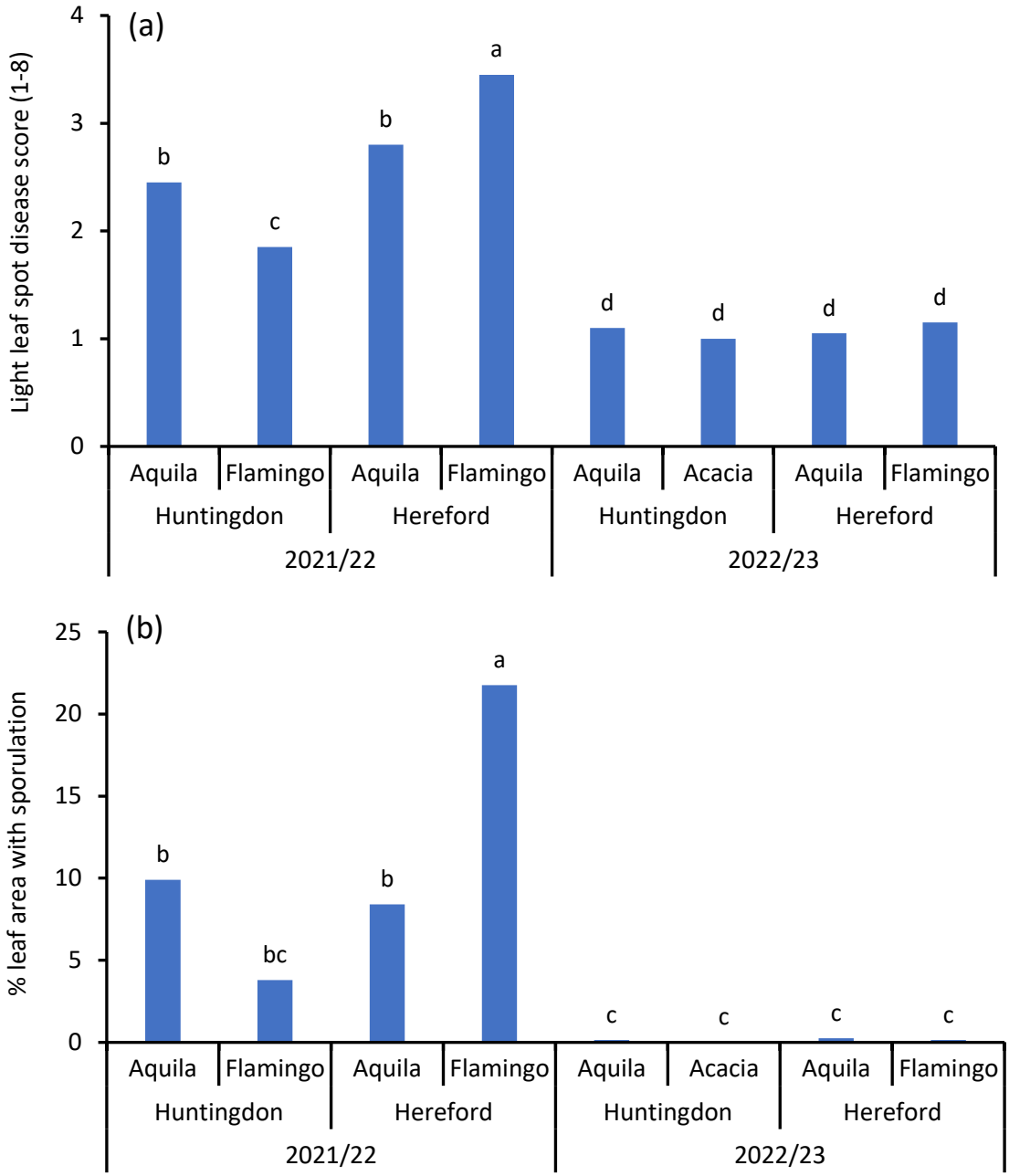

Figure 4.3: (a) Light leaf spot disease score and (b) percentage leaf area covered with *Pyrenopeziza brassicae* sporulation in winter oilseed rape field experiments.

Field experiments were done over two cropping seasons (2021/22 and 2022/23) at two different sites (Huntingdon, Cambridgeshire and Hereford, Herefordshire), using three different oilseed rape cultivars (cv. Aquila, cv. Flamingo and cv. Acacia). Disease severity was measured using (a) disease score on a 1-8 scale (where 1 is no disease) and (b) percentage leaf area covered in *P. brassicae* sporulation. Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, d.f. = 152; (a) LSD = 0.52, (b) LSD = 0.03).

Figure 4.4: Light leaf spot symptoms on stem and pod of oilseed rape plants in a field experiment.

Stems and pods of cv. Aquila and cv. Flamingo were sampled from a field experiment in Hereford, Herefordshire on 23 June 2023. Light leaf spot symptoms on stem and pod are shown as (a) stem lesions on cv. Flamingo, (b) close-up of a stem lesion with *Pyrenopeziza brassicae* sporulation (S) on cv. Flamingo and (c) pod lesions on cv. Aquila.

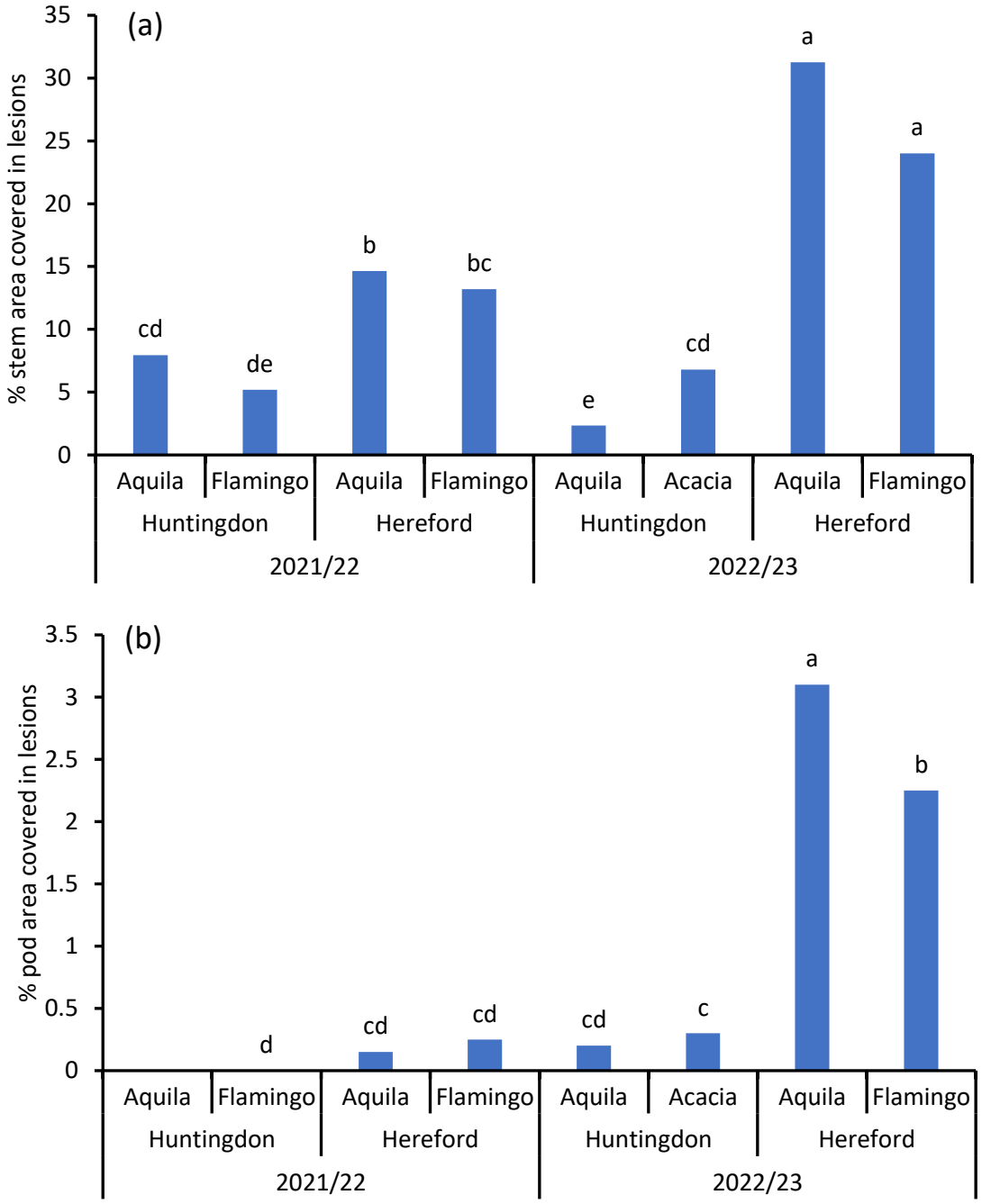

Figure 4.5: Percentage of oilseed rape (a) stem and (b) pod areas covered by light leaf spot lesions in field experiments.

Field experiments were done over two cropping seasons (2021/22 and 2022/23) at two different sites (Huntingdon, Cambridgeshire and Hereford, Herefordshire), using three different oilseed rape cultivars (cv. Aquila, cv. Flamingo and cv. Acacia). Disease severity was measured by (a) estimating the % stem area covered by lesions and (b) estimating the % pod area covered by lesions. Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, d.f. = 152; (a) LSD = 0.09, (b) LSD = 0.02).

($P > 0.29$), but cv. Aquila showed significantly greater % stem area with lesions ($P < 0.001$) and significantly greater % pod area with lesions ($P < 0.001$) in the 2022/23 season at the Hereford site. Similarly, cv. Flamingo also showed significantly greater % stem area with lesions ($P < 0.008$) and % pod area with lesions ($P < 0.001$) at the Hereford site in the 2022/23 season, compared to the 2021/22 season.

Phoma stem base canker was also assessed on a 0-7 score (with 0 as 0% area affected). For the 2021/22 season, there were no significant differences between cultivars ($P > 0.86$) or locations ($P > 0.86$). For the 2022/23 season, canker score was significantly greater in Hereford ($P < 0.001$) compared to Huntingdon and cv. Flamingo showed the overall greatest canker score ($P < 0.001$). Upon comparing the two cropping seasons, there were no overall differences between years ($P > 0.93$), but the Hereford site in the 2022/23 season presented the greatest canker scores for both cv Aquila and cv Flamingo (Figure 4.6).

Light leaf spot foliar assessment was done for field experiment B at two sites using ten cultivars for the 2023/2024 cropping season. Plants at the two field sites were at different developmental stages, as plants in Harpenden were at the stem elongation to early flowering stage (Figure 4.7a), while the plants in Norwich were already at full flowering stage (Figure 4.7b). Disease symptoms nonetheless appeared on both sites but were most visible in Norwich, with leaves showing visible sporulation, leaf distortions and even stem lesions (Figure 4.8). Plants in Norwich overall presented significantly greater disease scores compared to Harpenden ($P < 0.001$). There were also significant differences between cultivars ($P < 0.001$), with cv. Poh Bolko having the greatest disease score and appearing to be the most susceptible, followed by cv. Castille and cv. Yudal. Conversely, cv. Campus appeared the most resistant with the smallest disease score, followed by cv. Catana and cv. Kielder (Figure 4.9).

Stem and pod assessments were again done for field experiment B in the summer on randomly sampled whole plants. Plants in Norwich showed significantly greater % stem area with lesions ($P < 0.002$) and % pod area with

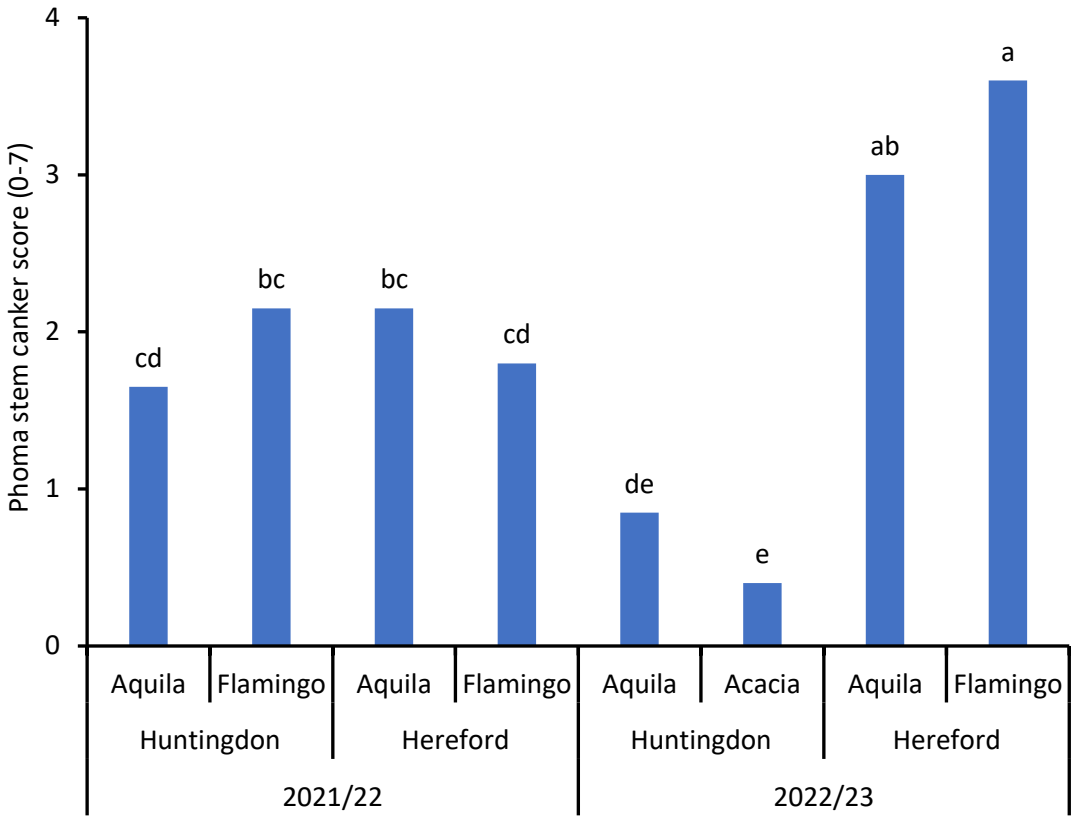

Figure 4.6: Phoma stem base canker score on different cultivars in winter oilseed rape field experiments.

Field experiments were done over two cropping seasons (2021/22 and 2022/23) at two different sites (Huntingdon, Cambridgeshire and Hereford, Herefordshire), using three different oilseed rape cultivars (cv. Aquila, cv. Flamingo and cv. Acacia). Phoma stem canker severity was measured on a 0 to 7 scale (with 0 as 0% area affected). Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, LSD = 1.11, d.f. = 152).

Figure 4.7: Whole view of field experimental sites in Hertfordshire and Norfolk.

Field experiments were done in Harpenen, Hertfordshire and Norwich, Norfolk in the 2023/24 cropping season using ten different oilseed rape cultivars. Plants at the two sites were at different developmental stages. (a) Plants in Harpenden were at the stem elongation to early flowering stage (growth stage 3,5, Sylvester-Bradley, 1985). (b) Plants in Norwich were at full flowering stage (growth stage 4,5). Photos were taken on 10 April 2024.

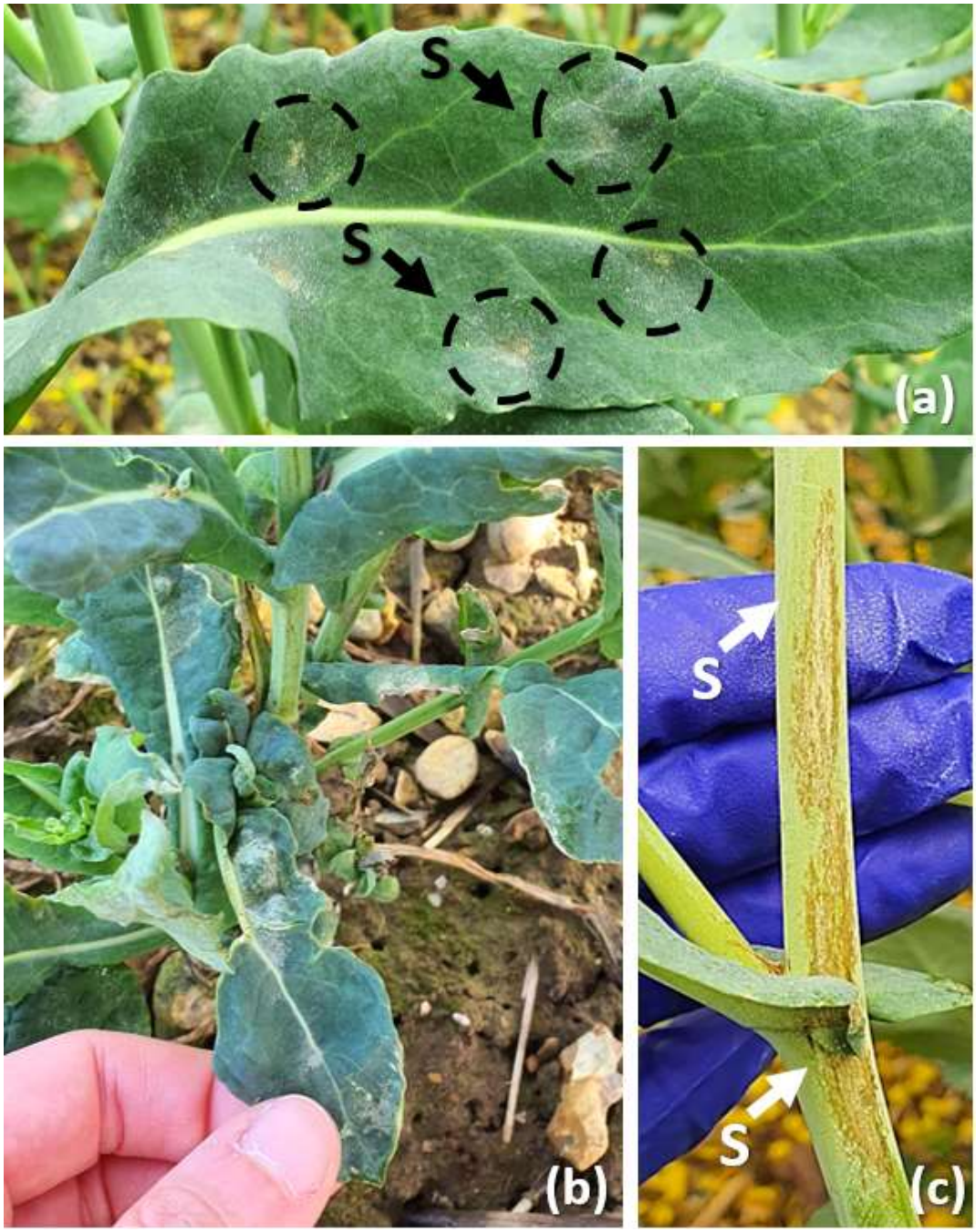

Figure 4.8: Light leaf spot symptoms on oilseed rape leaves and stems in field experiments.

A light leaf spot foliar assessment was done in field experiments in Harpenden, Hertfordshire and Norwich, Norfolk on 10 April 2024. Observed symptoms included (a) visible *Pyrenopeziza brassicae* (light leaf spot) sporulation (S) forming concentric rings on cv. Castille in Norwich, (b) leaf distortions on cv. Apex in Harpenden and (c) stem lesions with visible sporulation on cv. Yudal in Norwich.

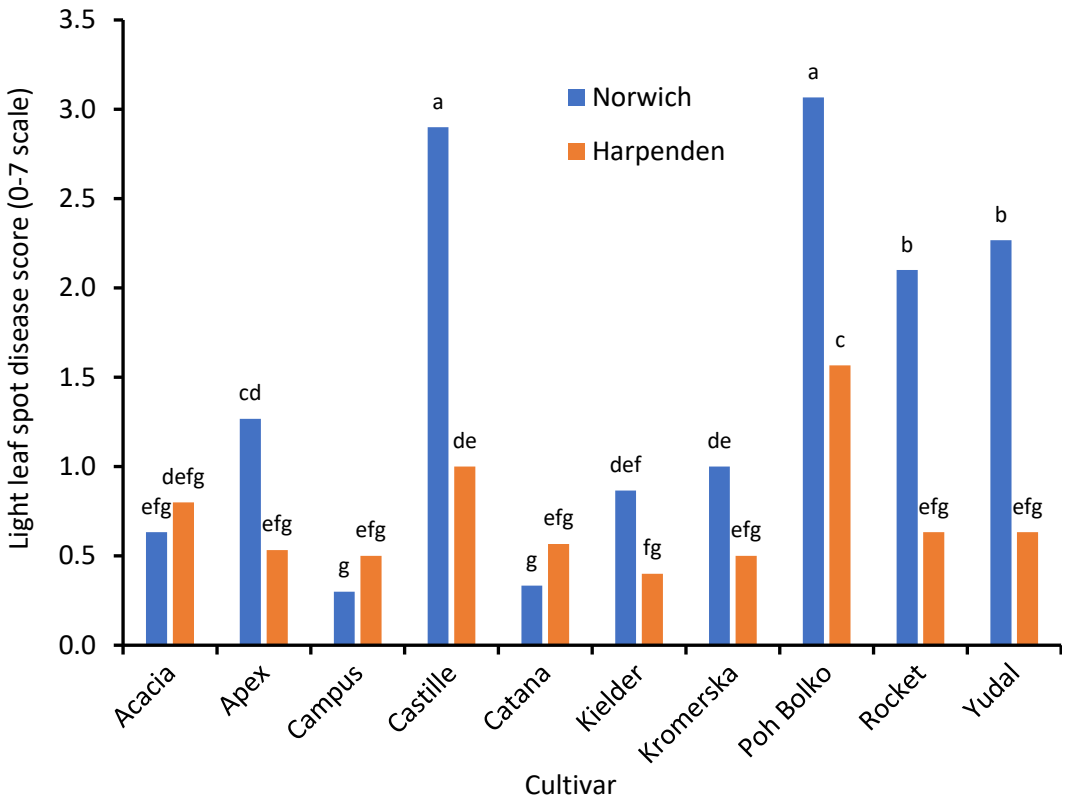

Figure 4.9: Light leaf spot disease score (0-7) in oilseed rape field experiments.

Field experiments were done in Harpenden, Hertfordshire and Norwich, Norfolk in the 2023/24 cropping season using ten different oilseed rape cultivars. A foliar assessment was done on 10 April 2024. Light leaf spot disease score was measured on a 0-7 scale (with 0 being no disease). Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, LSD = 0.53, d.f. = 580).

lesions (P < 0.005) compared to Harpenden. There were also significant differences between cultivars for both % stem area with lesions (P < 0.001) and % pod area with lesions (P < 0.001), with cv. Poh Bolko again having the greatest percentages of both stem and pod lesions, followed by cv. Castille and cv. Apex. Cv. Yudal showed the smallest percentages of stem and pod lesions, along with cv. Catana and cv. Rocket (Figure 4.10). Phoma stem base canker scores, conversely, were significantly greater in Harpenden compared to Norwich (P < 0.001) and showed significant differences between cultivars (P < 0.001), with cv. Yudal showing the greatest disease score, followed by cv. Kielder and cv. Castille. Cv. Campus showed the smallest disease scores, followed by cv. Acacia and cv. Apex (Figure 4.11). It should be noted, however, that in Harpenden, only thirteen plants of cv. Yudal could be assessed, as the plots had poorly established. Additionally, many of the plants at both sites were affected by other diseases, mainly powdery mildew and verticillium, which caused some of the plants to die and complicated light leaf spot and phoma stem canker disease assessments.

4.3.3 Glasshouse experiment; variation of disease phenotypes

In the glasshouse experiment, there was significant variation in light leaf spot severity between the different cultivar/lines inoculated with *P. brassicae* isolates. Two main parameters were used to measure disease severity: light leaf spot disease score (1-8) and visual estimation of percentage leaf area with *P. brassicae* sporulation. Presence of a necrotic response, foliar symptoms such as the % of deformed, dead and discoloured leaves were also recorded. Overall, 216 different host-pathogen interactions were observed, which presented a range of different symptoms. *P. brassicae* asexual sporulation was observed on the leaf surface where, generally, sporulation on less susceptible cultivars/lines was observed mostly along the leaf veins, whereas susceptible cultivars/lines showed sporulation covering the entire leaf. Leaf discolouration from green to red, purple or yellow was also observed. Finally, black necrotic response was observed on different parts of the leaf (Figure 4.12).

The results for light leaf spot disease score and % leaf area with sporulation showed that there were significant differences between cultivar/lines

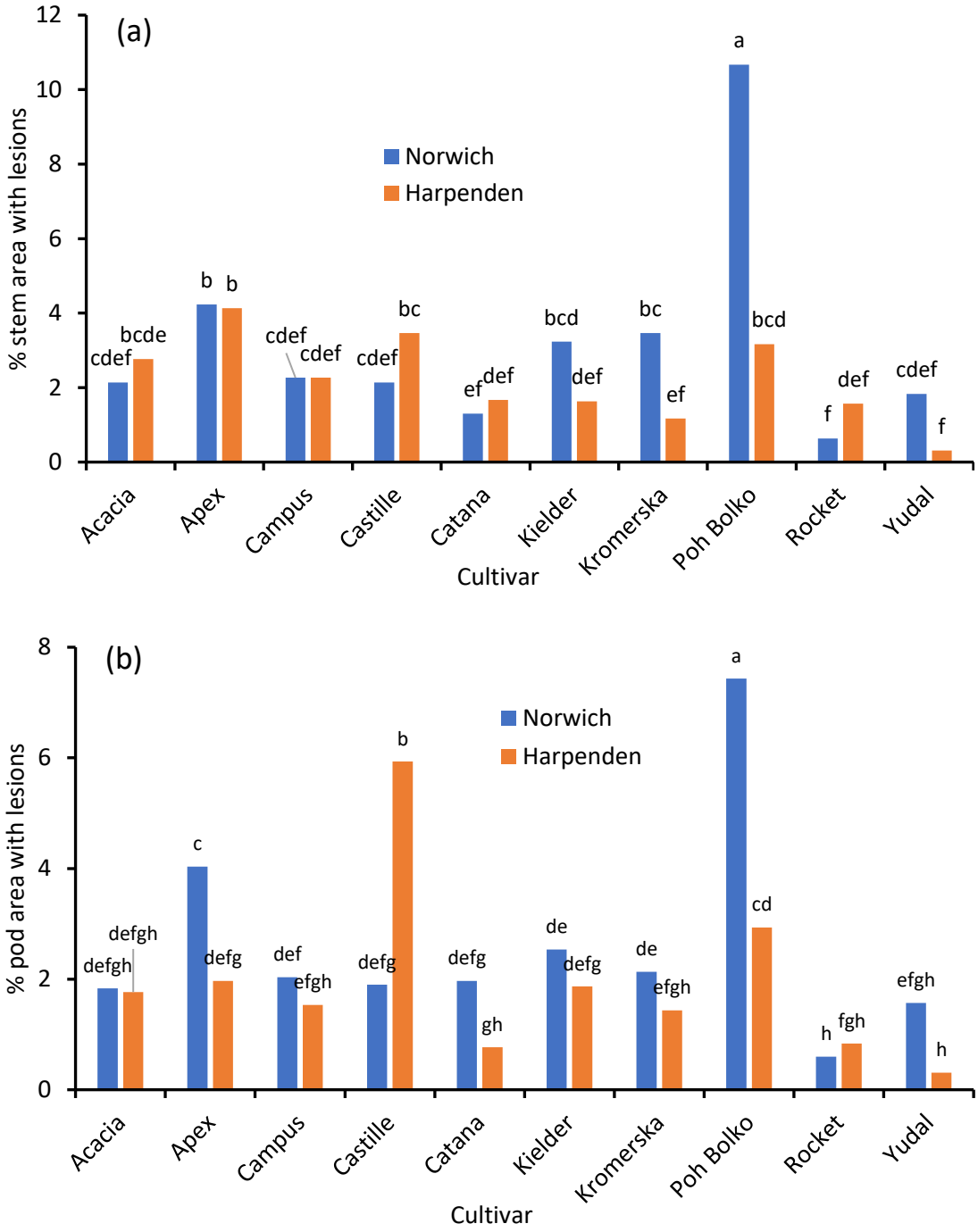

Figure 4.10: Percentages of oilseed rape (a) stem and (b) pod areas covered by light leaf spot lesions in field experiments.

Field experiments were done in Harpenden, Hertfordshire and Norwich, Norfolk in the 2023/24 cropping season using ten different oilseed rape cultivars. Disease severity was measured by (a) estimating the % stem area covered by lesions and (b) estimating the % pod area covered by lesions. Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, d.f. = 563).

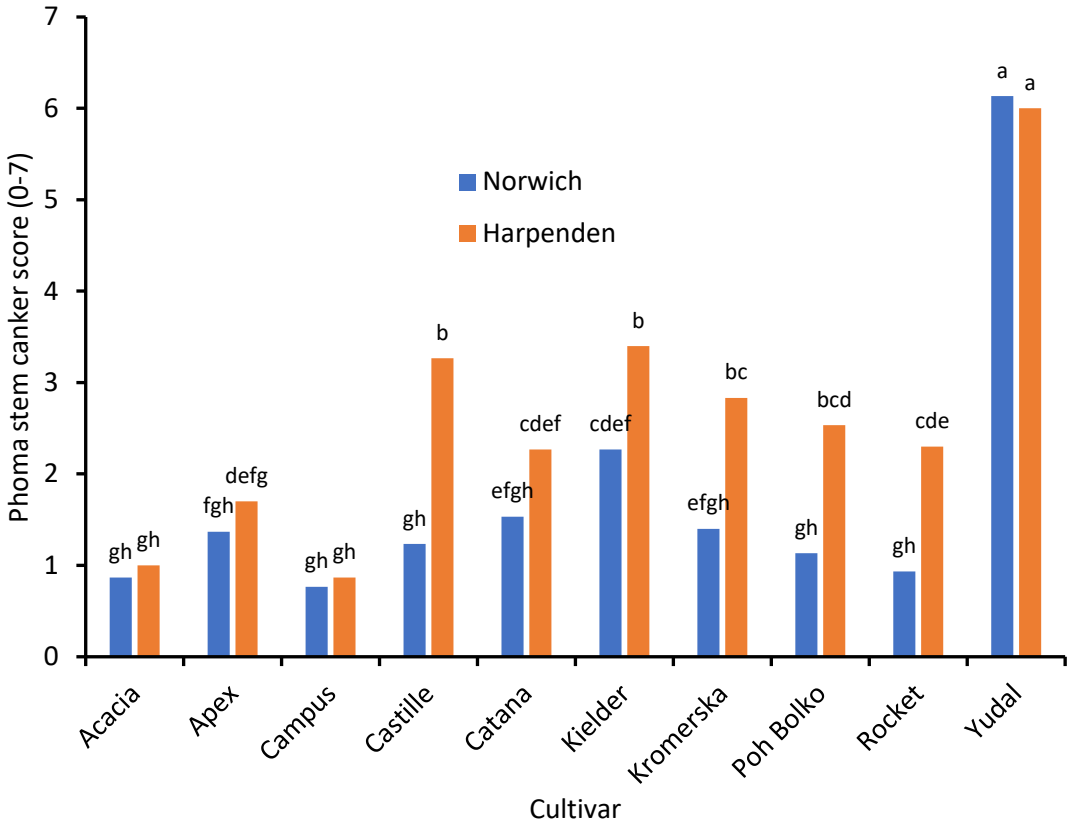

Figure 4.11: Phoma stem base canker score on different cultivars in winter oilseed rape field experiments.

Field experiments were done in Harpenden, Hertfordshire and Norwich, Norfolk in the 2023/24 cropping season using ten different oilseed rape cultivars. Phoma stem canker severity was measured on a 0 to 7 scale (with 0 as 0% area affected). Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, d.f. = 563).

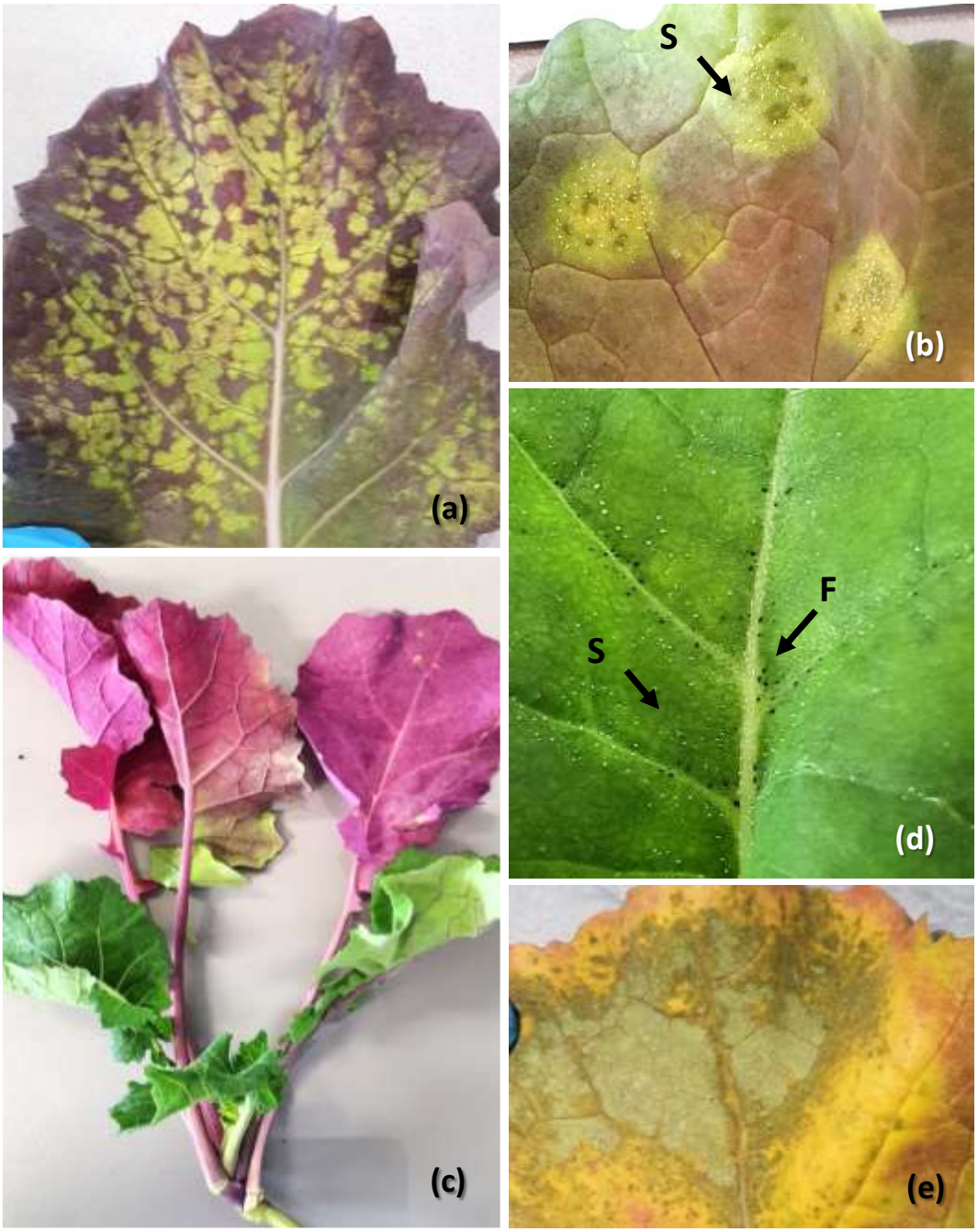

Figure 4.12: Light leaf spot symptoms observed on different oilseed rape cultivars/lines inoculated with single-spore *Pyrenopeziza brassicae* isolates.

Oilseed rape cultivars were inoculated with single-spore *P. brassicae* isolates in a glasshouse experiment to phenotype different host-isolate interactions. Different disease symptoms were observed, including (a) green islands without sporulation (S) on cv. Yudal, (b) green islands with sporulation on cv. Bristol, (c) leaf discolouration (purple) on cv. Yudal, (d) sporulation and presence of necrotic flecking (F) on leaf veins of breeding line Q60 and (e) leaf lesion on cv. Barbados.

($P < 0.001$), between isolates ($P < 0.001$) and significant cultivar/line-isolate interactions ($P < 0.001$) (Table 4.9 and Table 4.10). There was good correlation between the two parameters tested (Figure 4.13). Overall, cultivar Imola, breeding line Q02 and line NPZ 06/22 had the smallest mean scores and % leaf area with sporulation values and appeared more resistant, whereas cultivars Bristol, Charger and Yudal had the greatest mean disease scores and % leaf area with sporulation values and appeared more susceptible. Out of the 24 *P. brassicae* isolates tested, resistance against isolates Kale-H-6, Kale-H-7 and Tenor-L-4 was most commonly observed, even among susceptible cultivars/lines. Conversely, isolates 19DEN33, A7, and 20POL04 caused the most severe disease, even on moderately resistant cultivars/lines. There were overall measurable differences between different *P. brassicae* isolates, even when they were inoculated onto the same cultivar (Figure 4.14). Finally, upon comparing light leaf spot disease severity by country, isolates sourced from England and Ireland caused significantly less disease ($P < 0.001$) compared to isolates sourced from Scotland, Denmark, Poland and Germany. Isolates from Denmark had the greatest mean disease score and % leaf area with sporulation out of all countries.

A necrotic response was also observed, particularly on cultivar Imola, on which 13 out of 24 *P. brassicae* isolates produced a black flecking phenotype. Cultivars Charger, Yudal and Barbados, as well as lines Q60, NPZ 05/22 and NPZ 06/22, showed a necrotic response for less than four isolates. Cultivar Bristol and line Q02 did not produce a necrotic response for any of the isolates tested.

A regression model was used to identify the differential cultivar/line-isolate interactions. The calculated slope parameters and mean square (MS) deviation for each cultivar/line in analyses of disease score or % leaf area with sporulation against isolate virulence index are given in Table 4.11 and Table 4.12, respectively. Regression slope and the MS deviation plotted against mean disease score of each cultivar/line (Figure 4.15) showed that cultivar Imola, breeding line Q02 and line NPZ 06/22 had smaller regression coefficients compared to the other cultivars/lines, suggesting that these host genotypes

Table 4.9: Light leaf spot disease score on different oilseed rape cultivars/lines inoculated with different *Pyrenopeziza brassicae* isolates.

Glasshouse experiment where nine oilseed rape cultivars/breeding lines were inoculated with each of 24 single-spore *P. brassicae* isolates to investigate specific host-pathogen interactions. Light leaf spot severity was assessed using a disease score on a 1-8 scale (where 2 is most resistant). Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (alpha = 0.05, isolate d.f. = 92, cultivar d.f. = 717). Bold values indicate interactions that produced a necrotic response.

Isolate	Cultivar									Isolate mean
	Q02	Imola	NPZ 06/22	Q60	NPZ 05/22	Barbados	Yudal	Bristol	Charger	
Kale-H-7	1.5	1.0	1.8	1.0	1.8	1.0	2.0	1.4	1.6	1.48 n
Kale-H-6	1.4	1.4	1.8	1.4	1.8	1.3	2.2	2.0	1.8	1.7 mn
Tenor-L-4	1.6	1.7	1.6	1.6	2.0	1.6	2.0	1.8	2.0	1.77 lmn
19DEN03	1.5	1.8	2.0	1.4	2.0	1.5	2.0	2.2	2.0	1.83 klm
A11	1.2	2.0	2.0	2.2	1.8	1.5	2.6	3.0	2.2	2.03 jkl
18CAR02	1.4	2.0	2.0	2.4	2.2	1.5	2.6	2.5	2.6	2.14 ijk
18CAR05	2.2	2.0	2.0	2.2	2.2	1.8	2.2	2.0	2.6	2.15 hijk
Aqu-Here-1	1.4	**1.8**	2.0	1.4	2.2	2.8	2.6	3.2	3.3	2.28 ghij
Park-L-3	1.5	**2.0**	2.3	2.2	**2.0**	2.8	2.2	3.8	3.5	2.44 fghi
18CAR04	1.6	**2.0**	3.0	2.2	2.0	2.8	3.0	2.5	3.2	2.45 fghi
Aqu-Hunt22-1	1.8	1.8	2.0	**2.2**	2.6	2.7	3.8	2.4	**3.4**	2.48 efgh
20POL01	2.2	**2.0**	2.4	2.2	2.2	3.6	**3.2**	2.2	2.6	2.51 defg
20POL14	1.6	1.6	2.0	2.0	2.6	3.0	2.8	4.0	3.0	2.51 defg
Charger-H-2	1.8	**2.0**	2.2	1.4	2.0	**3.5**	3.6	3.2	**3.5**	2.53 defg
Parkside-L-1	1.4	2.0	1.8	2.0	2.4	2.8	4.0	4.0	3.8	2.63 def
19DEN19	2.2	**2.0**	2.8	2.2	2.6	2.6	3.6	2.4	3.8	2.69 def
Charger-H-1	1.6	**2.0**	2.0	1.8	2.2	3.4	4.0	3.8	**3.5**	2.71 def
19GERA1	1.6	**2.0**	2.2	2.4	2.4	2.6	3.4	3.8	4.2	2.73 cdef
Aqu-Here22-1	2.0	1.6	1.8	**3.8**	2.2	1.5	4.2	3.8	4.0	2.79 cde
19GERC2	2.4	**2.0**	2.2	2.4	2.6	2.5	3.4	3.8	4.0	2.82 cd
19GERA3	2.0	**2.0**	2.8	3.4	3.4	2.2	3.2	4.0	4.4	3.04 bc
A7	1.4	**2.0**	**2.5**	3.5	3.0	**4.0**	3.8	5.0	3.5	3.17 b
20POL04	2.2	**2.0**	2.4	3.2	3.0	4.5	4.4	3.6	3.8	3.2 b
19DEN33	1.8	**2.0**	2.2	3.6	3.6	4.4	**4.0**	5.0	5.2	3.53 a
Cultivar mean	1.73 e	1.86 e	2.15 d	2.24 cd	2.37 c	2.63 b	3.1 a	3.16 a	3.21 a	-

Table 4.10: Percentage leaf area covered with *Pyrenopeziza brassicae* sporulation on different oilseed rape cultivars/lines inoculated with different *P. brassicae* isolates.

Glasshouse experiment where nine oilseed rape cultivars/breeding lines were inoculated with each of 24 different single-spore *P. brassicae* isolates to investigate specific host-pathogen interactions. Light leaf spot severity was assessed by estimating the % leaf area covered with *P. brassicae* asexual sporulation (acervuli). Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (alpha = 0.05, isolate d.f. = 92, cultivar d.f. = 717). Bold values indicate interactions that produced a necrotic response.

Isolate	Cultivar									Isolate mean
	Imola	Q02	NPZ 06/22	NPZ 05/22	Q60	Barbados	Yudal	Charger	Bristol	
Kale-H-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 o
Tenor-L-4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 o
Kale-H-6	0.0	0.0	0.0	0.0	0.0	0.0	1.0	4.0	3.0	0.93 no
19DEN03	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	4.0	0.95 no
A11	0.0	0.0	0.0	0.6	5.2	0.0	8.4	4.0	6.3	2.75 mno
18CAR05	0.0	3.0	0.0	1.0	6.0	0.0	2.0	8.4	6.7	3.05 lmno
18CAR02	0.0	0.0	0.0	1.6	4.0	1.5	7.0	9.4	10.0	3.71 klmn
Aqu-Here-1	**0.0**	0.0	0.0	0.6	0.0	10.0	2.8	17.5	15.0	4.93 jklm
18CAR04	**0.0**	0.0	8.8	0.0	6.0	8.8	12.0	10.2	8.8	5.86 ijklm
Aqu-Hunt22-1	0.0	0.0	0.2	5.0	**0.8**	10.0	20.0	**16.0**	7.0	6.07 ijkl
Park-L-3	**0.0**	2.5	1.8	**0.2**	0.2	15.0	2.0	19.3	17.5	6.12 hijkl
Charger-H-2	**0.0**	0.0	0.2	0.0	0.0	**14.5**	18.0	**17.5**	14.4	6.77 ghijk
20POL01	**0.0**	4.0	3.6	2.4	2.4	22.0	**14.0**	11.2	5.4	7.22 fghij
20POL14	0.0	0.0	4.0	6.0	3.4	13.0	8.0	11.0	30.0	8.38 efghi
Charger-H-1	**0.0**	0.0	0.2	0.6	0.6	14.0	25.0	**17.0**	25.0	9.40 defgh
19GERA1	**0.0**	1.0	1.2	3.0	6.4	10.0	16.0	30.0	22.0	9.96 defg
19DEN19	**0.0**	2.6	6.0	11.0	6.0	6.4	26.0	24.0	9.0	10.11 def
19GERC2	**0.0**	3.0	4.0	8.0	4.0	12.5	18.0	30.0	22.0	11.25 cde
Parkside-L-1	**0.0**	0.0	0.2	8.0	1.0	12.0	23.8	27.5	37.0	11.53 cde
Aqu-Here22-1	0.0	0.6	0.0	0.2	**24.0**	0.0	27.0	26.0	26.3	11.76 cd
19GERA3	0.0	1.4	5.8	14.0	18.0	6.0	15.8	32.0	30.0	13.67 c
20POL04	**0.0**	2.6	1.8	8.0	13.4	33.8	31.0	22.0	21.0	14.41 c
A7	**0.0**	0.0	**5.0**	15.0	27.5	**42.5**	21.0	23.8	48.0	19.88 b
19DEN33	**0.0**	4.2	2.0	16.0	19.0	40.0	**27.0**	56.0	52.0	24.02 a
Cultivar mean	0.0 d	1.04 d	1.89 d	4.22 c	5.98 c	11.79 b	13.43 b	17.38 a	17.97 a	-

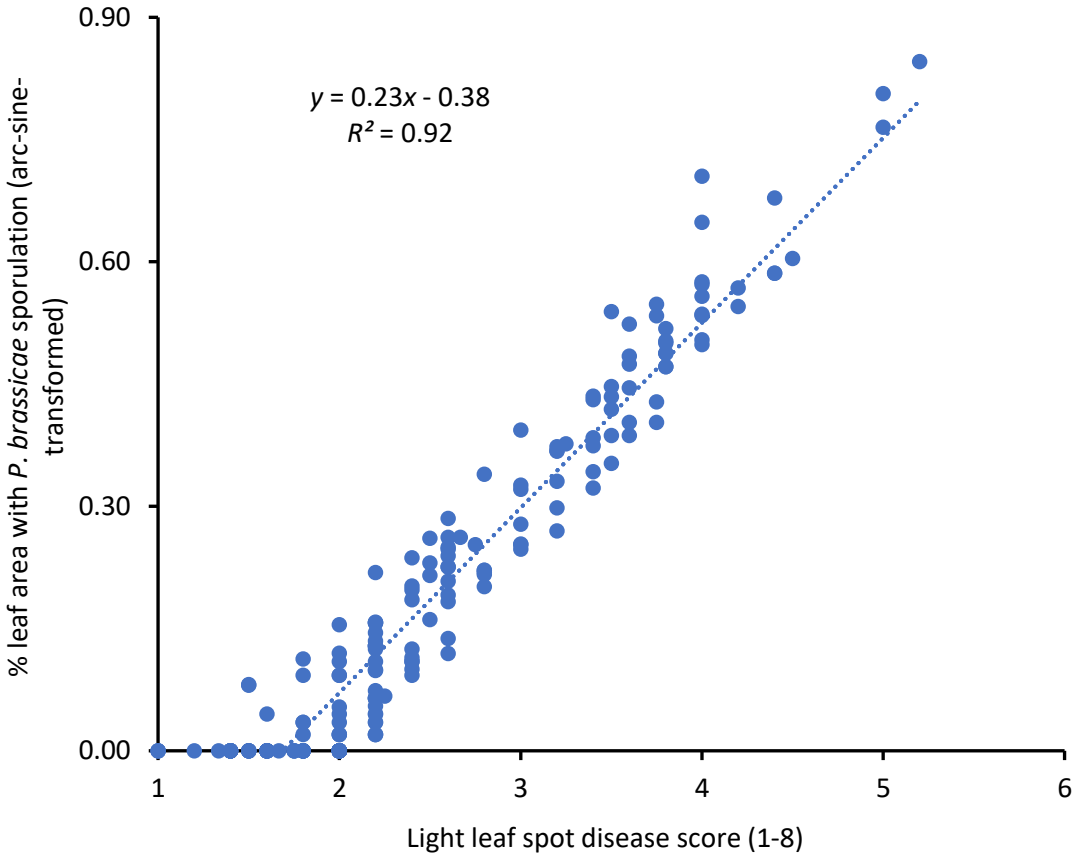

Figure 4.13: Correlation between light leaf spot disease score (1-8) and percentage leaf area covered with *Pyrenopeziza brassicae* sporulation.

Glasshouse experiment where nine oilseed rape cultivars/breeding lines were inoculated with each of 24 different single-spore *P. brassicae* isolates to investigate specific host-pathogen interactions. Plants were assessed for light leaf spot severity using a disease score on a 1-8 scale (where 1 is no disease) and by visual estimation of the % leaf area covered with *P. brassicae* sporulation after samples were incubated at 4°C for 7-9 days (sample correlation coefficient = 0.96, $P > 0.001$, $n = 216$). Percentage leaf area with sporulation values are presented on an arc-sine-transformed scale.

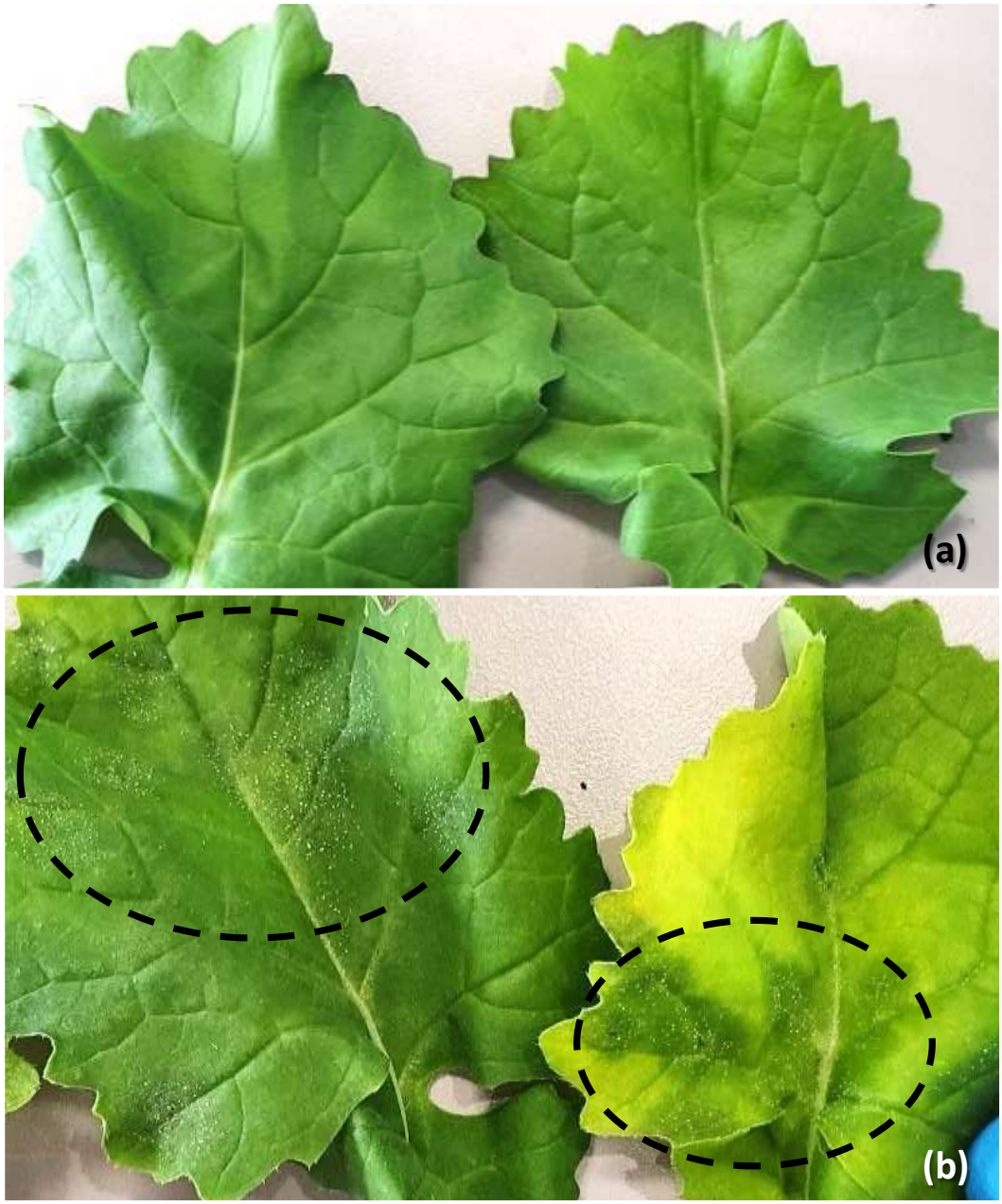

Figure 4.14: Comparison of light leaf spot symptom development on the same oilseed rape breeding line inoculated with two different *Pyrenopeziza brassicae* isolates.

Glasshouse experiment where nine oilseed rape cultivars/breeding lines were inoculated with each of 24 different single-spore *P. brassicae* isolates to investigate specific host-pathogen interactions. Breeding line Q60 (thought to contain quantitative resistance against *P. brassicae*) was inoculated with two different *P. brassicae* isolates. When inoculated with (a) a single-spore isolate Kale-H-7 (Table 4.8), line Q60 presented no symptoms, but when inoculated with (b) a different single-spore isolate Aqu-Here22-1 (Table 4.8), extensive *P. brassicae* sporulation on the leaf lamina occurred (encircled areas).

Table 4.11: Estimated parameter values of μ, b and error mean square (MS) for each cultivar/line in the response of disease score to isolate virulence index.

Glasshouse experiment where nine oilseed rape cultivars/breeding lines were inoculated with each of 24 different single-spore *Pyrenopeziza brassicae* isolates to investigate specific host-pathogen interactions. A regression model was used to identify differential responses of different cultivars/lines to the calculated virulence index for each isolate.

	Parameter value		
Cultivar/line	Mean (μ_i)*	b_i†	Error MS (δ_{ij})‡
Barbados	2.63	1.55	0.38
Bristol	3.16	1.64	0.31
Charger	3.21	1.65	0.13
Imola	1.86	0.30	0.04
NPZ 05/22	2.37	0.81	0.07
NPZ 06/22	2.15	0.36	0.10
Q02	1.73	0.28	0.10
Q60	2.24	1.21	0.22
Yudal	3.10	1.29	0.19

*μ_i – mean response of the *i*th cultivar/line over all isolates

†b_i – regression coefficient that measures the response of the *i*th cultivar/line to level of virulence in isolates

‡δ_{ij} – sum of deviations from regression of the *i*th cultivar/line with the *j*th isolate

Table 4.12: Estimated parameter values of μ, b and error mean square (MS) for each cultivar/line in the response of % leaf area with sporulation to isolate virulence index.

Glasshouse experiment where nine oilseed rape cultivars/breeding lines were inoculated with each of 24 different single-spore *Pyrenopeziza brassicae* isolates to investigate specific host-pathogen interactions. A regression model was used to identify differential responses of different cultivars/lines to the calculated virulence index for each isolate.

	Parameter value		
Cultivar/line	Mean (μ_i)*	b_i†	Error MS (δ_{ij})‡
Barbados	11.79	1.65	54.78
Bristol	17.97	2.16	36.88
Charger	17.38	1.89	33.24
Imola	0.00	0.00	0.00
NPZ 05/22	4.22	0.74	8.42
NPZ 06/22	1.89	0.17	5.53
Q02	1.04	0.10	1.80
Q60	5.98	1.02	28.95
Yudal	13.43	1.24	46.79

*μ_i – mean response of the *i*th cultivar/line over all isolates

†b_i – regression coefficient that measures the response of the *i*th cultivar/line to level of virulence in isolates

‡δ_{ij} – sum of deviations from regression of the *i*th cultivar/line with the *j*th isolate

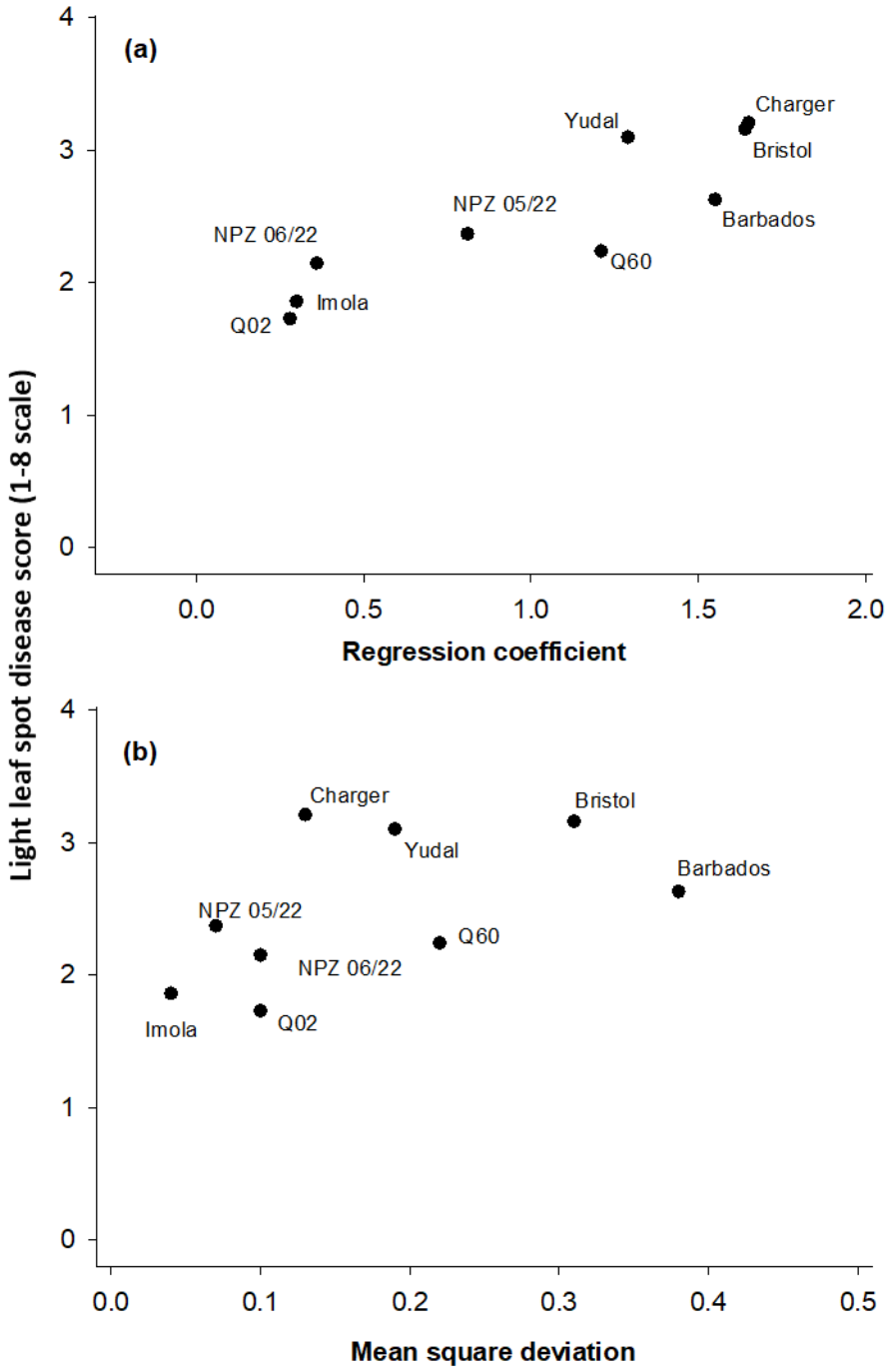

Figure 4.15: Relationships between mean light leaf spot disease score (1-8 scale, with 1 being no disease) and the regression parameters calculated for the interactions between nine oilseed rape cultivars/lines and 24 *Pyrenopeziza brassicae* isolates.

Glasshouse experiment where nine oilseed rape cultivars/breeding lines were inoculated with each of 24 different single-spore *P. brassicae* isolates to investigate specific host-pathogen interactions. A regression model was used to identify differential responses of different cultivars/lines to the calculated virulence index for each isolate. Relationships are shown between disease score and (a) regression coefficient and (b) mean square (MS) deviation.

were more resistant against the *P. brassicae* isolates tested. Conversely, cultivars Charger and Bristol, followed by cultivars Yudal and Barbados, had greater regression coefficients, suggesting that disease severity increased with isolate virulence index and that their host genotypes were more susceptible to the isolates tested (Figure 4.15a). Cultivar Imola, breeding line Q02 and line NPZ 06/22 also showed the smallest MS deviation which, taken as a measure for non-specific interactions, suggested that these cultivar/lines were non-specifically resistant against the isolates tested. Other lines, particularly cultivars Bristol and Barbados, presented greater MS deviation, suggesting the presence of differential interactions (Figure 4.15b). Regression slope and the MS deviation plotted against mean % leaf area with sporulation of each cultivar/line (Figure 4.16) showed similar, more pronounced patterns. Cultivar Imola, breeding line Q02 and line NPZ 06/22 genotypes appeared most resistant against the isolates tested, followed by line NPZ 05/22 and line Q60. Cultivars Yudal and Barbados appeared less resistant to the isolates, followed by cultivars Charger and Bristol, which presented the greatest regression coefficients, suggesting increased susceptibility (Figure 4.16a). Lower MS deviation values for cultivar Imola, lines Q02 and NPZ 06/22 suggested non-specific interactions, whereas the greater MS deviation values of cultivars Charger, Bristol, Yudal and Barbados suggest presence of differential interactions. Percentage leaf area with sporulation was especially large for cultivars Charger and Bristol, suggesting overall greater susceptibility for these two cultivars, but MS deviation values were greater for cultivars Yudal and Barbados, suggesting greater variation in the interactions (Figure 4.16b).

The percentages of deformed, dead and discoloured leaves were calculated and compared between the different host-pathogen interactions. There were significant differences in foliar symptom development between cultivar/lines (% deformed $P < 0.001$, % dead $P < 0.001$, % discoloured $P < 0.001$), between isolates (% deformed $P < 0.05$, % dead $P < 0.001$, % discoloured P 0.001) and significant cultivar/line-isolate interactions (% deformed $P < 0.01$, % dead $P < 0.001$, % discoloured $P < 0.01$). Correlations between disease score and % deformed leaves (sample correlation coefficient = 0.24, $P < 0.01$, $n = 216$), % dead leaves (sample correlation coefficient = 0.22, $P < 0.001$, $n = 216$) and %

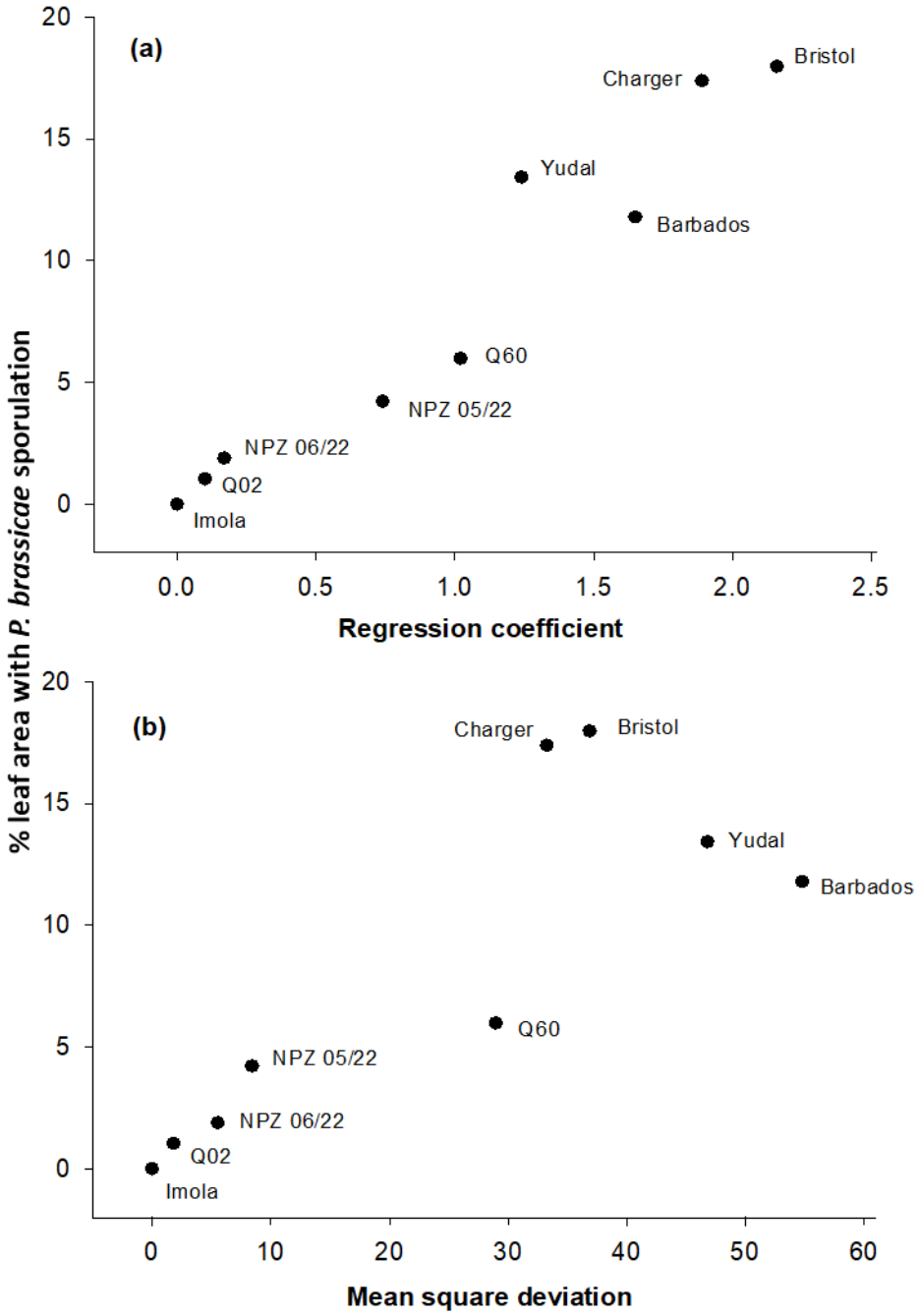

Figure 4.16: Relationships between mean percentage leaf area covered in *P. brassicae* sporulation and the regression parameters calculated for the interactions between nine oilseed rape cultivars/lines and 24 *Pyrenopeziza brassicae* isolates.

Glasshouse experiment where nine oilseed rape cultivars/breeding lines were inoculated with each of 24 different single-spore *P. brassicae* isolates to investigate specific host-pathogen interactions. A regression model was used to identify differential responses of different cultivars/lines to the calculated virulence index for each isolate. Relationships are shown between % leaf area covered in sporulation and (a) regression coefficient and (b) mean square (MS) deviation.

discoloured leaves (sample correlation coefficient = 0.19, $P < 0.01$, $n = 216$) were statistically significant, but the correlations were weak. Correlations between % leaf area with sporulation and % deformed leaves (sample correlation coefficient = 0.18, $P < 0.01$, $n = 216$) and % dead leaves (sample correlation coefficient = 0.16, $P < 0.05$, $n = 216$) were also statistically significant, but the correlations were weak. The correlation between % leaf area with sporulation and % discoloured leaves (sample correlation coefficient = 0.06, $P > 0.31$, $n = 216$) was not significant. Overall, although there was weak correlation between the foliar symptoms, disease score and % leaf area covered with sporulation remained the most accurate parameters to measure disease severity.

4.3.4 Cross-infection of *P. brassicae* between *Brassica napus* and *B. oleracea*

The results for light leaf spot disease score (1-8 scale) and % leaf area with *P. brassicae* asexual sporulation showed that there were significant differences between treatments (Figure 4.17). There were no significant differences between oilseed rape (*B. napus*) and kale (*B. oleracea*) in disease score ($P > 0.16$) or % leaf area with sporulation ($P > 0.37$). However, the *P. brassicae* inoculum derived from oilseed rape caused greater disease scores ($P < 0.001$) and greater % leaf area with sporulation ($P < 0.001$) than the *P. brassicae* inoculum derived from kale.

The percentage leaf area covered in *P. brassicae* sporulation was estimated at two separate times. The first assessment of % leaf area with sporulation was done at 32 dpi (harvested at 24 dpi) followed by an incubation period of 8 days at 4°C). Afterwards, leaf washing was done to remove the first sporulation from the leaves (section 2.2.2). Plants were then further incubated at 4°C for another 25 days so the second assessment of % leaf area with sporulation was done at 57 dpi. There were significant differences between treatments ($P < 0.001$) in both the first and second assessments, and overall, the second sporulation was

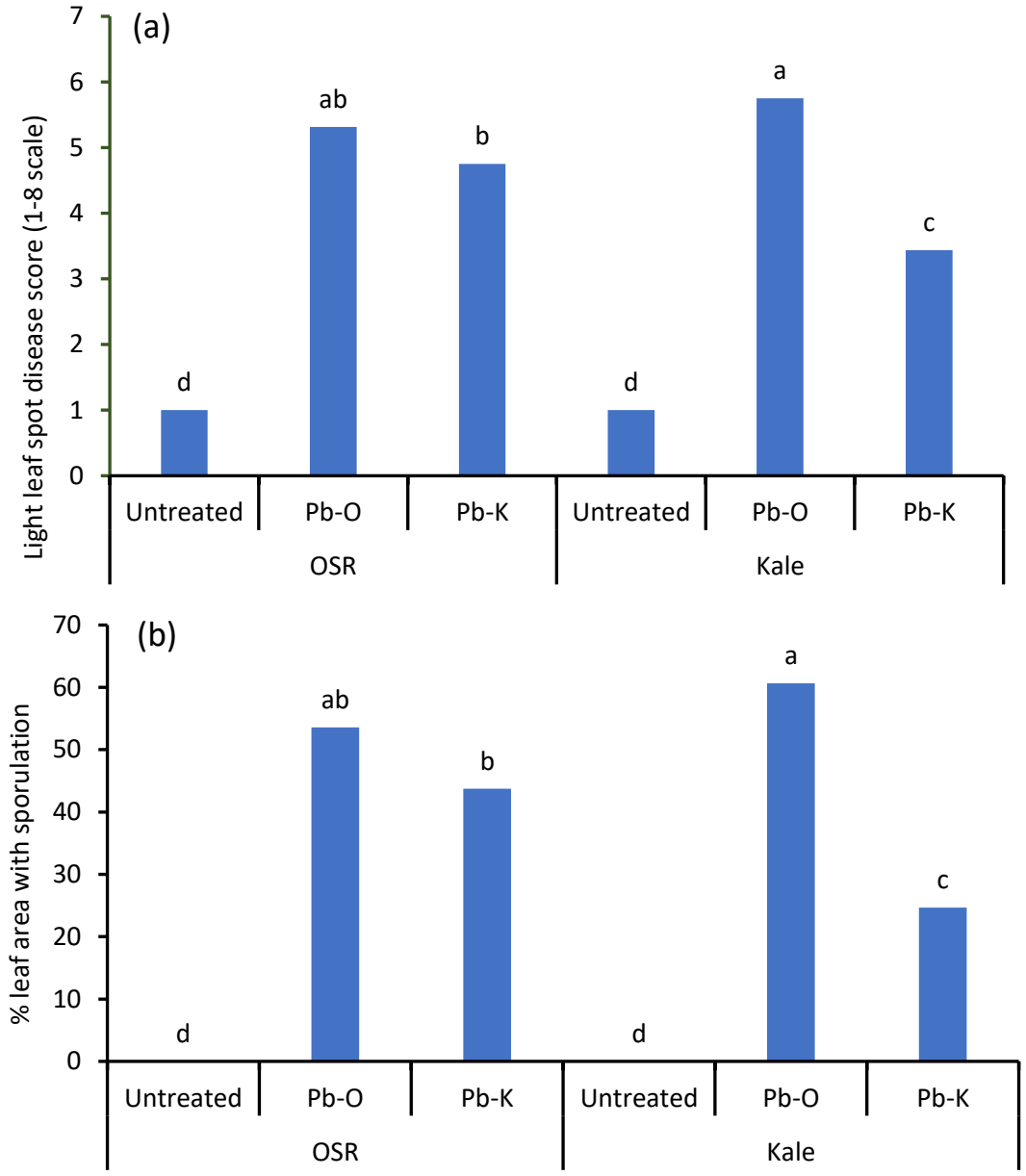

Figure 4.17: (a) Light leaf spot disease score and (b) percentage leaf area covered with *Pyrenopeziza brassicae* sporulation (1-8 scale) on two brassica species inoculated with *P. brassicae* conidial suspensions derived from two different hosts.

Glasshouse experiment where two different *Brassica* hosts (oilseed rape (OSR) and kale) were inoculated with *P. brassicae* conidial suspensions derived from either OSR (Pb-O) or kale (Pb-K) or untreated as control. Disease severity was measured using (a) disease score on a 1-8 scale (where 1 is no disease) and (b) percentage leaf area covered in *P. brassicae* sporulation. Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, d.f. = 28; (a) LSD = 0.09, (b) LSD = 0.17).

less than the first sporulation ($P < 0.001$) (Figure 4.18). An example comparison of the first and second sporulation on kale is shown in Figure 4.19.

Presence of a necrotic response was assessed on the leaf petiole, midrib and lamina of both host types. Kale did not show flecking anywhere on the leaf, regardless of the treatments. In contrast, oilseed rape plants inoculated with either *P. brassicae* population produced flecking on the leaf lamina. The untreated controls did not show flecking anywhere on the leaf.

The percentages of deformed, % dead and % discoloured leaves (by counting the number of affected leaves against the total number of leaves) were recorded at 3, 8, 11, 14, 17, 22 and 24 dpi, with the final assessment done at harvest (Figure 4.20). Results from the last assessment showed that kale had a significantly greater % deformed leaves ($P < 0.002$) compared to oilseed rape, but that oilseed rape had a significantly greater % dead leaves ($P < 0.01$). Both oilseed rape and kale inoculated with either *P. brassicae* inoculum had a significantly greater % dead leaves compared to the untreated controls ($P < 0.001$) but there was no difference in % deformed leaves between inoculated and control plants ($P > 0.55$). There were no significant differences in the % discoloured leaves for any of the treatments ($P > 0.95$) (Table 4.13).

4.4 Discussion

4.4.1 Differences in *Pyrenopeziza brassicae* populations between geographic regions

Overall, results from field experiments suggested differences in *P. brassicae* pathogen races between geographic areas. Results from field experiment A showed significant differences between sites and seasons. Results from field experiment B showed significant differences between sites and cultivars.

These results suggested that *P. brassicae* pathogen races in Norwich, Norfolk may be more virulent than those in Harpenden, Hertfordshire despite fungicide applications. There were differences in disease development between cultivars and, in some cases, disease severity on the same cultivar differed between sites. Cultivars Poh Bolko, Castille and Yudal, which were the three most

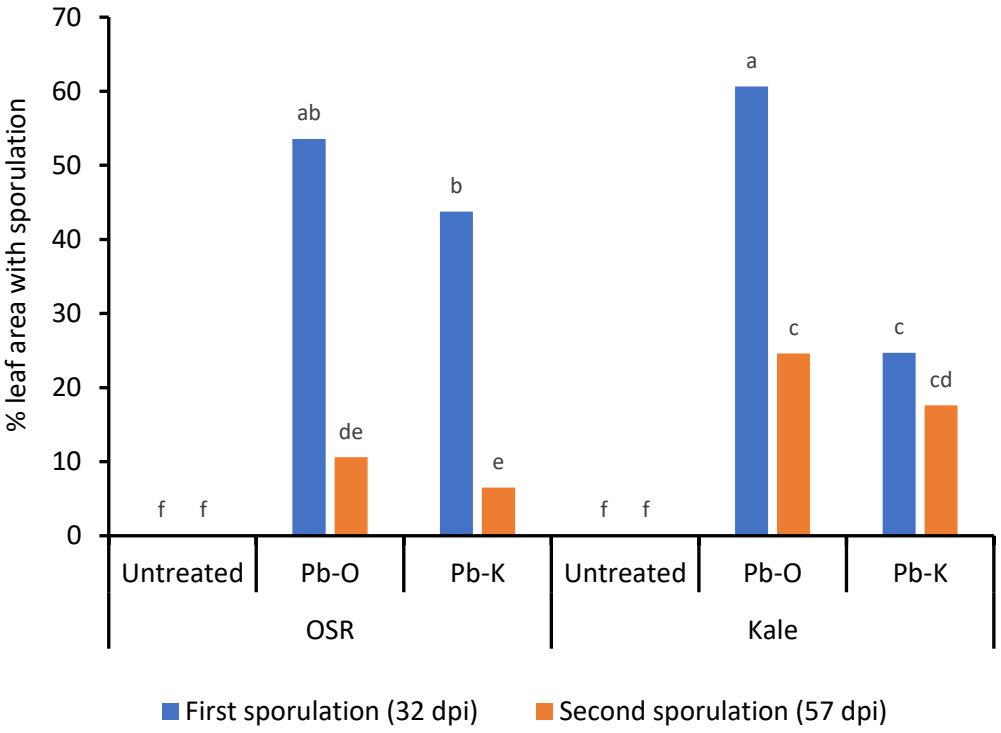

Figure 4.18: First and second measurements of percentage leaf area covered with *Pyrenopeziza brassicae* sporulation on two brassica species inoculated with *P. brassicae* conidial suspensions derived from two different hosts.

Glasshouse experiment where two different brassica hosts (oilseed rape (OSR) and kale) were inoculated with *P. brassicae* conidial suspensions derived from either OSR (Pb-O) or kale (Pb-K) or remained untreated. Plants were harvested at 24 days post inoculation (dpi) and incubated at 4°C for 8 days. First assessment of % leaf area covered in sporulation was done at 32 dpi (blue bars). Afterwards, leaf washing was done to remove the conidia produced. Plants were then further incubated at 4°C for another three weeks. At 57 dpi, a second assessment of % leaf area covered in sporulation was done (orange bars). Fisher's protected LSD post hoc tests are presented as letters, where columns with the same letters are not significantly different (alpha = 0.05, LSD = 0.13, d.f. = 84).

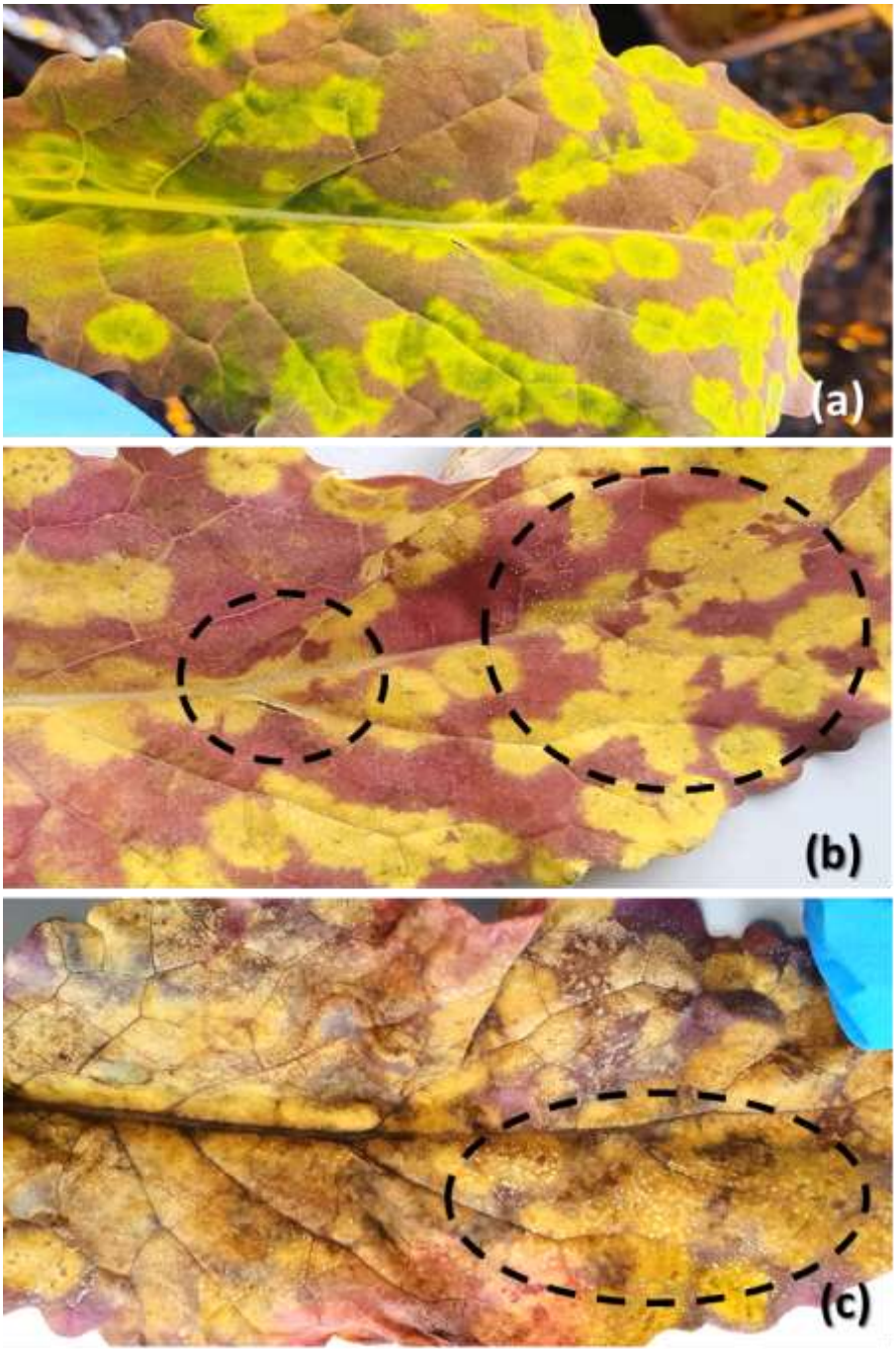

Figure 4.19: Light leaf spot disease symptoms and recurring *Pyrenopeziza brassicae* sporulation on a kale leaf.

Glasshouse experiment where kale was inoculated with a *P. brassicae* conidial suspension. The same kale leaf showing green islands associated with *P. brassicae* infection (a) at harvest at 24 dpi (days post inoculation) with no visible sporulation, (b) after incubation at 4°C for 8 days with sporulation visible on the leaf (32 dpi) (encircled areas) and (c) after leaf washing to remove previous *P. brassicae* sporulation and further incubation at 4°C for 25 days, presenting newly emerged *P. brassicae* sporulation (57 dpi).

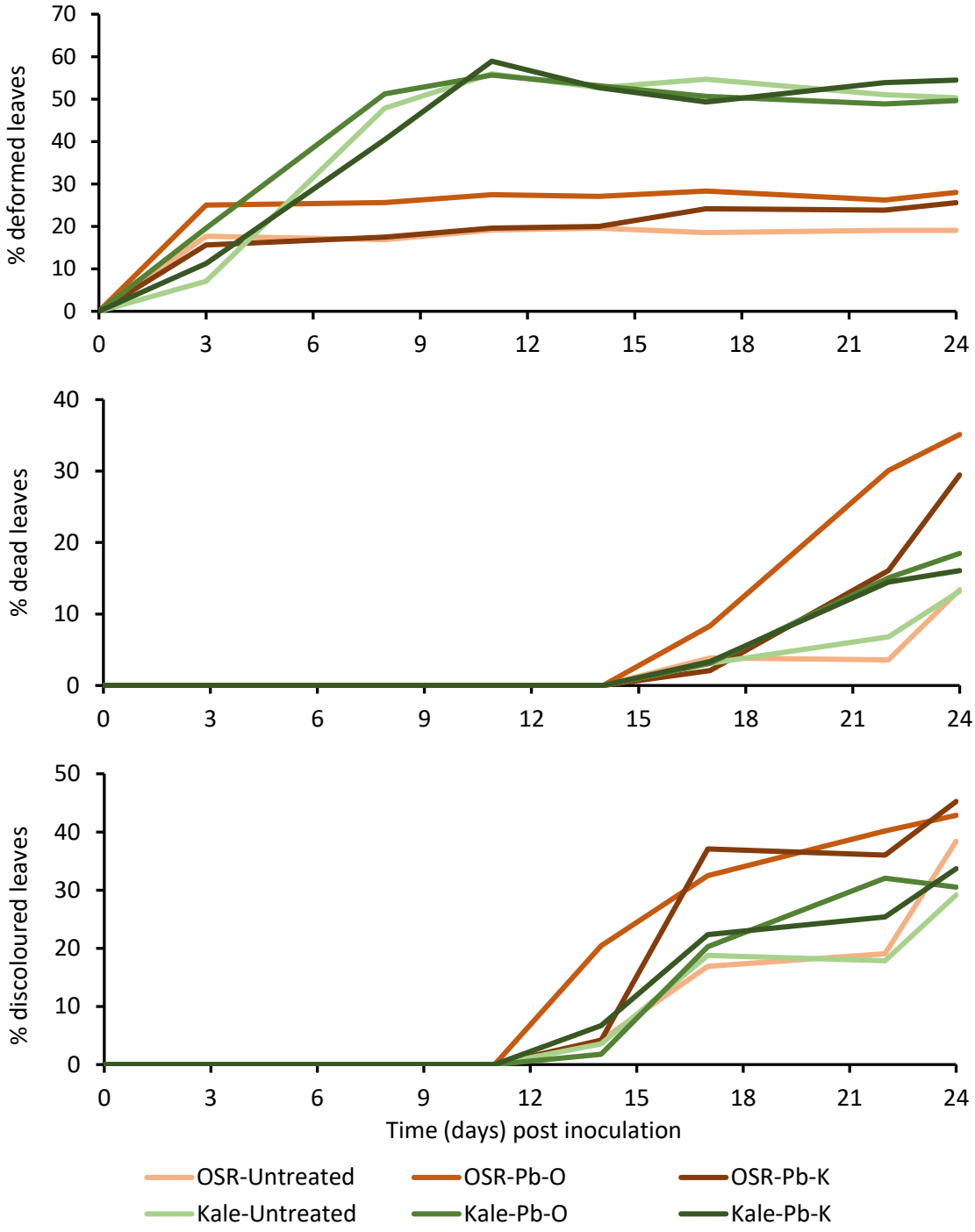

Figure 4.20: Incidence of light leaf spot foliar symptoms over time on two different *Brassica* hosts.

Glasshouse experiment where two different brassica hosts (oilseed rape (OSR) and kale) were inoculated with *Pyrenopeziza brassicae* conidial suspensions derived from either OSR (Pb-O) or kale (Pb-K) or untreated as control. Foliar symptoms were measured as (a) % deformed leaves, (b) % dead leaves and (c) % discoloured leaves at 3, 8, 11, 14, 17, 22 and 24 dpi (days post inoculation).

Table 4.13: Light leaf spot foliar symptoms on two brassica species inoculated with *Pyrenopeziza brassicae* conidial suspensions derived from two different hosts.

Glasshouse experiment where two different brassica hosts (oilseed rape (OSR) and kale) were inoculated with *P. brassicae* conidial suspensions derived from either OSR (Pb-O) or kale (Pb-K) or remained untreated. Foliar symptoms were assessed as % deformed leaves, % dead leaves and % discoloured leaves. Fisher's protected LSD post hoc tests are presented as letters, where values with the same letters are not significantly different (alpha = 0.05, d.f. = 28; % deformed leaves LSD = 0.129; % dead leaves LSD = 0.125; % discoloured leaves LSD = 0.119).

	Proportion of leaves with symptoms		
Treatment*	% deformed leaves	% dead leaves	% discoloured leaves
OSR-UT	16.96 b	13.39 bc	38.39 ab
OSR-Pb-O	22.92 b	35.71 a	37.80 ab
OSR-Pb-K	16.07 b	30.06 a	44.05 a
Kale-UT	48.89 a	10.42 c	29.17 bc
Kale-Pb-O	49.23 a	18.62 b	28.96 c
Kale-Pb-K	48.95 a	14.86 bc	33.07 abc

*Treatment name structure: Host plant (oilseed rape (OSR) or kale) – inoculum type (untreated (UT), derived from OSR (Pb-O) or derived from kale (Pb-K))

susceptible cultivars, presented significantly greater disease scores in Norwich compared to Harpenden. Due to differences in the crop developmental stages between sites, however, disease may have been more severe in Norwich, as plants there also showed stem lesions, which are typically associated with disease in the summer. Additionally, cv. Yudal showed less severe disease in the summer assessment, but these assessments may have been inaccurate due to other diseases like powdery mildew and verticillium causing extensive plant damage and death, preventing accurate detection of light leaf spot symptoms. Conversely, there were no differences in disease scores between locations among the more resistant cultivars Campus, Catana and Kielder. Cultivar Acacia, then, which was used in both field experiments, showed little disease across three sites. This suggested that the underlying resistances of these cultivars may be stable across different environmental conditions and should be considered for further studying.

Regional differences in light leaf spot disease incidence may also be attributed to differences in weather. Disease incidence was greater in Hereford, Herefordshire compared to Huntingdon, Cambridgeshire. Historically, Huntingdon had a mean winter rainfall (December-February) of 50.3 mm between 1991 and 2021, while Hereford had a mean rainfall of 66 mm in the same period, despite mean temperatures remaining similar between the two sites (10.4°C in Huntingdon and 9.8°C in Hereford) (Climate Data, 2024). This suggests that the increased rainfall in Hereford compared to Huntingdon may have contributed to increased disease incidence. Results reported here matched with survey data collected by Welham *et al*. (2004) over a ten-year period between 1987 and 1999. In that study, the Herefordshire region reported a significantly greater disease incidence than the Cambridgeshire region and Herefordshire also reported twice the amount of monthly winter rainfall (December-February) compared to Cambridgeshire, despite mean temperature remaining similar between both regions. These results equally suggested that the overall more humid climate in Herefordshire may have promoted disease development compared to less humid regions. Weather is known to affect light leaf spot disease development, particularly humidity (Fitt *et al*., 1998a,b; Gilles *et al*., 2001b,c; Welham *et al*., 2004). Detailed weather parameters from on-site

weather stations could be included in future studies along with disease data to predict disease incidences between regions and how these effects may impact *P. brassicae* pathogen virulence.

Presence of a necrotic response was equally site-specific and appeared only in association with severe disease, suggesting that the phenotype may be isolate-specific, as well as requiring a greater disease severity to appear. Black flecking appeared on both cv. Aquila and cv. Flamingo at the Hereford site only, but in different seasons. Since Hereford showed more severe disease symptoms overall, appearance of necrotic flecking may be related to disease severity, meaning that a certain disease threshold needs to be reached before a necrotic response is triggered. However, foliar disease severity for cv. Aquila in the 2021/22 season was the same in both Huntingdon and Hereford but flecking appeared only in leaves sampled in Hereford. These results instead suggested that differences in the pathogen populations between locations, rather than differences in pathogen virulence, caused flecking to appear at one site but not the other. Overall, patterns in the appearance of necrotic flecking suggested differences in *P. brassicae* races between the two sites, but the underlying mechanisms that trigger the appearance of the phenotype, whether pathogen race-specific or related to overall disease severity or a combination of both, remain unclear.

These results suggest that there were no significant relationships between light leaf spot and phoma stem canker disease severity. Integrated disease management strategies should aim to improve control of several concurrent diseases, as pathogens rarely exist in isolation. Both phoma stem canker and light leaf spot are economically important diseases of oilseed rape in the UK, although in the present study, there were no significant correlations between the diseases. Phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) is considered a monocyclic disease (ascospores are the primary inoculum), while light leaf spot is a polycyclic disease (ascospores are the primary inoculum and conidia are secondary inoculum, which cause additional disease cycles later in the cropping season). This means that *P. brassicae* has a greater evolutionary potential than *Leptosphaeria* spp. due to asexual reproduction

increasing the frequencies of selected allele combinations for *P. brassicae,* leading to greater potential for resistance breakdown and greater potential for major economic yield losses caused by light leaf spot (Huang *et al*., 2021; McDonald and Linde, 2002). Findings by Fortune (2022) also suggested that interspecific interactions between *L. maculans* and *L. biglobosa* may indirectly affect *P. brassicae*; however, this has not been reported under field conditions and the pathogens are not known to compete interspecifically. Both diseases operate differently, despite affecting the same crop; nonetheless, both should be assessed in relation to one another, as there is not much benefit in having cultivars with resistance against only one disease. There have been numerous advances made in understanding phoma stem canker and the virulence of pathogenic *L. maculans* races, but light leaf spot remains comparatively understudied.

Limited studies currently exist comparing light leaf spot field disease severity between sites and cropping seasons to suggest differences in pathogenic *P. brassicae* races (Evans *et al*., 2017; Klöppel, 2015). Knowledge of the genomics of pathogenic races is paramount for the management of crop diseases to guide cultivar deployment and prevent resistance breakdown but changes in the virulence of *P. brassicae* populations can only be surmised through the suspected breakdown of host resistance over time, which may happen from one season to another for major gene-mediated resistance, or through gradual erosion of minor gene-mediated resistance (Boys *et al*., 2007). Here, disease severity differed between cropping seasons, but more seasons are needed to find observable patterns. Information about light leaf spot disease epidemiology also remains limited. Differences in disease development between cropping seasons may be influenced by numerous factors such as weather, crop establishment, apothecial release timing and proximity to infected stubble (Evans *et al*., 2017; Gilles *et al*., 2001a,b). Foliar disease in spring induced by asexual reproduction is likely to influence severity of stem and pod disease later in the season (Boys *et al*., 2007), although this was not observed here, but by then disease is already likely to lead to substantial yield losses. Several light leaf spot disease modelling frameworks (Gilles *et al*., 2001d; Papastamati *et al*., 2002; Welham *et al*., 2004) and light leaf spot

disease forecasting tools (Rothamsted Research, available at http://resources.rothamsted.ac.uk/light-leaf-spot-forecast) have been proposed, but none are currently effectively implemented to predict epidemics due to lack of reliable data. The following studies remained in line with previous findings suggesting differences in regional *P. brassicae* populations and the hope is that, combined with the reported cultivars with stable resistance, future work may help improve disease management.

4.4.2 Differential interactions between *Brassica napus* cultivars/lines and single-spore *P. brassicae* isolates

Results of glasshouse experiments done to study the disease phenotype of various host-pathogen interactions and gain further insight into the *P. brassicae-B. napus* pathosystem revealed significant differences between cultivar/lines, between isolates and significant cultivar/lines-isolate interactions, identifying resistant host genotypes and virulent pathogen strains.

Cultivar Imola, breeding line Q02 and line NPZ 06/22 showed non-specific and less sensitive resistance against the isolates tested. This remained consistent for both disease score and % leaf area covered in asexual *P. brassicae* sporulation assessments. These assessment techniques showed the strongest correlation and were therefore considered the most reliable parameters to measure disease severity. Considering secondary disease cycles in spring are often initiated by splash-dispersed conidia (and ascospores), whose spread contributes to pre-harvest disease severity and occurrence (Boys *et al*., 2007; Fitt *et al*., 1998a), host resistance that prevents or limits asexual sporulation presents a valuable resource. The regression parameters from these three cultivar/lines showed both low levels of sporulation and low MS deviation values, suggesting good underlying resistance that is stable against a broad range of isolates. Cultivar Imola in particular did not show sporulation for any of the isolates tested and instead produced necrotic flecking against 13 out of 24 isolates. Necrotic flecking is considered a phenotype of resistance (Boys *et al*., 2012), although in this study, flecking appeared equally in susceptible interactions and many resistant responses did not include black flecking. Results here are in line with a similar study by Karandeni Dewage *et al.* (2021)

that concluded that necrotic flecking and limited asexual sporulation may operate under different, independent mechanisms. Nonetheless, these host genotypes are effective potential sources for resistance breeding in oilseed rape.

Conversely, cultivars Charger, Bristol, Yudal and Barbados were most susceptible to the isolates tested. Cultivar Yudal is one of the parents of the DY DH mapping population that has previously been studied for quantitative resistance against *P. brassicae* (Pilet *et al*., 1998b). In the study by Pilet *et al*. (1998b), cv. Yudal was considered highly susceptible to *P. brassicae*, but Karandeni Dewage *et al*. (2021) instead concluded Yudal to be moderately to partially resistant to *P. brassicae*. The results reported here agree with Pilet *et al*. (1998b), but such discrepancies demonstrate that disease phenotypes can vary between experimental conditions and pathogen populations. Resistance may also break down over time, as was suggested to be the case with the major gene-mediated resistance in cultivar Bristol (Boys *et al*., 2007). Previous studies linked *P. brassicae* infection to changes in plant morphology, namely stem elongation and leaf deformation, due to imbalance in plant growth regulators following fungal infection (Ashby, 1997). There is evidence suggesting that host resistance recognition occurs at later stages of *P. brassicae* colonization, whereas leaf distortion is associated with early colonization (Karandeni Dewage *et al*., 2021). This means that resistant and susceptible cultivars/lines alike will have similar amounts of leaf deformation, accounting for the poor correlation between foliar morphology features and more established methods of light leaf spot severity assessment (disease score and % sporulation).

Next, there were significant variations in disease score and % leaf area with sporulation between isolates, suggesting differences in virulence between *P. brassicae* isolates. Between the six European countries tested, isolates sourced from England and Ireland presented the smallest disease severity across all cultivars. This was contrasted with isolates sourced from Germany, which presented the greatest mean disease score and % leaf area with sporulation out of all countries. Isolate 19DEN33, which was overall the most

virulent isolate tested, was sampled in Denmark, whereas the least virulent isolates, Kale-H-7 and Tenor-L-5, were sampled in England. These present interesting findings; light leaf spot severity in northern Europe is increasing (Karandeni Dewage *et al*., 2018) and light leaf spot is already well established in the UK as the most economically damaging disease of oilseed rape. The cultivars/lines tested for this study were grown in UK field conditions, so it is possible that they were overall less resistant to non-UK isolates due to lack of prior exposure. These results highlight the need to monitor *P. brassicae* not only in the UK but in other areas in northern Europe. Additionally, all isolates from this study were grown in culture media. *P. brassicae* isolates can lose their pathogenicity if they are subcultured *in vitro* and should ideally be routinely passaged through plants to retain their pathogenicity (Boys *et al*., 2012). This was not feasible here due to the number of isolates involved, so it is possible that their virulence under natural conditions was underestimated. It should also be considered that the number of isolates studied for each country was small, so comparisons may change depending on the specific strains assessed and follow-up studies should ideally include a greater number of representative isolates for each country.

Understanding pathogen population structures is paramount to improve plant disease management. Investigating various host-pathogen responses to differentiate resistant and susceptible phenotypes can help identify candidate host plant material for resistance breeding, as well as gain insight in location-specific pathogen race structures. The present study showed significant disease phenotypes of host-pathogen interactions. This presented a good basis for further investigations studying host defence mechanisms, as well as pathogen virulence factors. By combining the phenotypic data obtained in this study, it was planned to do molecular marker analyses on the single-spore *P. brassicae* isolates used and establish patterns between disease phenotype and pathogen genotype. The original plan was to use random amplified polymorphic DNA (RAPD) markers according to the method described by Boys (2009), but this was not possible due to technical complications. DNA extraction, quantification and quality control, as well as preliminary rounds of RAPD PCR were done, but no further work could be done. RAPD was chosen as the

preferred technique due to the availability of characterized primers (Boys, 2009) and because no prior knowledge of the target gene sequence was needed. However, the technique also has disadvantages, namely its low reproducibility (Kumar and Gurusubramanian 2011). Other methods considered included amplified fragment length polymorphism (AFLP) markers, which have been used on *P. brassicae* by Majer *et al*. (1998), or more recently, repetitive sequence-based PCR (or rep PCR) DNA fingerprinting was used by Carmody *et al*. (2020) to identify a phylogenetically distinct lineage of *P. brassicae* in North America. Unfortunately, no information about the genetic structures of the *P. brassicae* isolates used in this study could be determined.

Such limited information about the virulence of *P. brassicae* populations remains a problem. Studies on *L. maculans* (phoma stem canker) have shown that over-reliance on the same resistance mechanisms applies selection on pathogen populations. This can cause selection for isolates with avirulent alleles, which has previously led to the breakdown of resistance genes and subsequent phoma stem canker epidemics (Rouxel *et al*., 2003; Sprague *et al*., 2006). To prevent this from happening, pathogen population monitoring schemes are paramount to guide cultivar deployment (Stachowiak *et al*., 2006) and are currently operating in several regions for *L. maculans* (e.g. CanolaCouncil in Canada, available at https://www.canolacouncil.org/). Unfortunately, no such schemes exist for *P. brassicae* due to overall lack of knowledge of virulence among pathogen races. Studies like this therefore show that understanding specific host-pathogen interactions in the *P. brassicae-B. napus* pathosystem can provide a basis for identifying novel sources of host resistance, as well as variations in *P. brassicae* pathogen populations.

4.4.3 Cross-infection of *P. brassicae* between *B. napus* and *B. oleracea*

Results of glasshouse experiments to investigate the ability of *P. brassicae* to cross-infect between different *Brassica* hosts showed that both *P. brassicae* populations used were able to cause disease in both *Brassica* hosts, confirming cross-infectivity. The *P. brassicae* population derived from oilseed rape caused more severe disease than the kale-derived population. The kale-derived population, however, caused less severe disease on kale than it did on oilseed

rape, whereas oilseed rape-derived inoculum affected both host types equally. This suggested that the differences in disease development may be due to differences within the pathogen populations, rather than kale being a more resistant host compared to oilseed rape. Oilseed rape is the third most cultivated arable crop in the UK, making it the primary *Brassica* host for the light leaf spot pathogen. It is therefore possible that *P. brassicae* populations in the UK have evolved to primarily adapt to host resistance found within oilseed rape, which may differ from resistance found within other *Brassica* species. *P. brassicae* populations derived from other hosts may therefore be less virulent due to reduced selection and the reduced need to overcome resistance, explaining why the kale-derived inoculum was less virulent than the oilseed rape-derived inoculum. However, while this may explain the differences in disease severities in kale, oilseed rape was affected by both populations equally. This discrepancy may be attributed to the individual cultivars used in this study. Oilseed rape cultivar Charger was chosen due to its established susceptibility to *P. brassicae*, whereas the resistance rating of the chosen kale cultivar (Nero di Toscana) remains unknown. The susceptibility threshold for cv. Charger is small, meaning that even less virulent *P. brassicae* populations may cause visible disease which is indistinguishable from disease caused by more virulent races. Cv. Nero di Toscana, conversely, may be less susceptible to *P. brassicae* infection compared to cv. Charger, making the differences in disease response more apparent, as a less virulent population would not cause severe disease.

Disease symptoms, including foliar morphologies, further differed between hosts. Kale had a consistently greater percentage of deformed leaves compared to oilseed rape, but differences in foliar symptoms remained otherwise inconclusive. The presence of a necrotic response in oilseed rape, but not in kale, however, suggested that the *B. oleracea* genotype does not include the molecular mechanisms underlying the black flecking phenotype, which remains a response primarily associated with *B. napus*.

Results showed that sporulation was able to re-occur for both inoculum types, on both hosts, up to 60 days post-inoculation, upon which further tests could

not be done due to senescing leaf tissue. Amounts of re-sporulation on kale in particular were significantly greater for both inoculum types compared to oilseed rape. This suggested that for both *Brassica* species, sporulation remains sustainable if there is available green leaf tissue for the pathogen to colonize. Since asexual sporulation in spring causes secondary disease cycles (Boys *et al*., 2007; Fitt *et al*., 1998a), it is of great value to understand how re-sporulation cycles differ between *Brassica* species and how they impact additional epidemics.

The ability of *P. brassicae* to cross-infect between major *Brassica* species has been previously demonstrated. Work done by Maddock *et al*. (1981) and Simons and Skidmore (1998) reported cross-infectivity between *P. brassicae* isolates on brassicas that differed from the pathogen's original host. Similar findings were also reported in more recent studies (Boys, 2009; Klöppel, unpubl. data). However, these studies used either single-spore *P. brassicae* isolates, which provided a limited representation of wild *P. brassicae* populations, or *P. brassicae* populations derived from *Brassicas* other than kale. This study used field *P. brassicae* populations derived from either oilseed rape or kale and demonstrated cross-infectivity between hosts for both populations. The differences in symptom development between interactions may be attributed to reduced virulence in the kale-derived population. Follow-up studies using a differential set of both oilseed rape and kale cultivars with varying resistance levels may confirm pathogen population virulence levels in more host-pathogen interactions. However, even if kale was less affected by kale-derived inoculum, disease still occurred; therefore, even if infection will not put the host at risk, less severely affected hosts can still act as reservoirs of pathogens and exacerbate epidemics, highlighting the need to study resistance in other hosts.

Chapter 5 Identification of quantitative resistance against *Pyrenopeziza brassicae*

QTL analyses of the glasshouse experiments (GH-E and GH-S) described in this chapter and frequency distribution graphs included in this chapter were done by Dr Hao Wang at the Huazhong Agricultural University, China.

5.1 Introduction

The importance of oilseed rape (*Brassica napus* L.) as an arable crop has been increasing globally due to its many applications including vegetable oil, animal feed and industrial uses. In the UK alone, oilseed rape ranks as the third most important arable crop, with 1.361 million tonnes harvested from 364 thousand hectares in 2022, worth an estimated £877 million (price: £644/t in 2022) (Agriculture in the United Kingdom, 2022). To meet market expectations, selective breeding to produce cultivars with more desirable agronomic traits, which in turn offer a greater economic return, becomes increasingly important.

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, remains the most economically damaging disease of oilseed rape in the UK (Karandeni Dewage *et al*., 2021). Despite current disease management through fungicide applications, risks of fungicide insensitivity development and environmental concerns make plant host resistance ever more important (Bucur *et al*., 2024; Carter *et al*., 2013; 2014). Host resistance against pathogens is generally classified as either major (*R*) gene-mediated, complete qualitative resistance, or minor gene-mediated, partial quantitative resistance (Brun *et al*., 2010; Huang *et al*., 2009; Mitrousia *et al*., 2018). Combining quantitative trait loci (QTL) resistance with major *R* genes could provide more effective and potentially durable resistance (Huang *et al*., 2018; Pilet-Nayel *et al*., 2017). This highlights the need to further understand quantitative resistance and identify resistance QTL against *P. brassicae* for potential new breeding materials.

QTL analysis is the process of identifying genomic regions associated with specific traits by analysing the association of phenotypic data with DNA (or

molecular) markers. DNA markers linked to agronomically important traits can be used as the basis for marker-assisted selection (MAS) in plant breeding. Similarly, MAS can be used for the detection of resistance QTL and information about these resistance loci can then be applied to plant breeding programs (Collard *et al*., 2005; Young, 1996). The accuracy of the QTL detection depends on the quality of the phenotypic data, size of the population and the resolution of the linkage map.

Construction of a linkage map requires a segregating plant population produced by crossing parental lines that differ for the trait/s of interest. Of the different population types suitable for mapping, doubled haploid (DH) populations are widely used in molecular research, as they produce homozygous or 'true-breeding' lines with high genetic stability. Breeding DH lines relies on artificially induced chromosome doubling using anther or microspore culture methods and their generation time is considerably shorter than that for conventional breeding methods. Another advantage is that DH lines can be 'eternally' multiplied and reproduced without genetic change occurring (Collard *et al*., 2005; Starosta *et al*., 2023). This chapter describes an attempt to identify resistance QTL against *P. brassicae* within the DY (Darmor-*bzh* x Yudal) DH mapping population.

The oilseed rape DY DH population was first described by Foisset *et al.* (1996). This population originated from the F_1 of the cross between the parental cultivars Darmor-*bzh* and Yudal. Darmor-*bzh* is a dwarf isogenic line (BC_3F_3) obtained through introduction of the dwarf *Bzh* gene to French winter cultivar Darmor, which itself is derived through back-crosses with winter cultivar Jet Neuf. Yudal is a spring inbred Korean line (F_9) behaving as an early-flowering winter type in temperate climates. The parental lines were chosen due to the high polymorphism level between them and the occurrence of selected segregating agronomic traits, including resistance to pathogens. The DY population has previously been used to identify QTL for resistance against *Leptosphaeria maculans* (phoma stem canker) (Pilet *et al*., 1998a; Huang *et al*., 2016; 2019) because cv. Darmor-*bzh* carries the resistance gene *Rlm9* against *L. maculans*, whereas cv. Yudal is susceptible to the pathogen infection. These studies identified several environmentally stable QTL,

including QTL detected in young plants, that contribute to quantitative resistance in adult plants, providing valuable resources to develop markers for breeding cultivars with durable quantitative resistance against *L. maculans*.

Resistance QTL against *P. brassicae* have also previously been identified in the DY DH population (Pilet *et al*., 1998b). The study identified several resistance QTL against the light leaf spot and phoma stem canker pathogens (Pilet *et al*., 1998a), of which two were 'multiple disease resistance' QTL that may correspond to genes involved in common resistance mechanisms against the two pathogens. However, the study was done in France using an older linkage map, so there is a need to test the DY DH population against more contemporary *P. brassicae* populations from the UK to identify and compare resistance QTL. The most recent study to identify resistance QTL against *L. maculans* within the DY population (Huang *et al*., 2019) used the same linkage map as the work described in this chapter; it will therefore be useful to compare resistance QTL for the two pathogens. Commercially viable cultivars should, ideally, not only include desirable agronomic traits, but also carry resistance against a broad range of pathogens. Since phoma stem canker and light leaf spot are the two most damaging fungal diseases in oilseed rape in the UK (Karandeni Dewage *et al*., 2018), identifying breeding lines that carry resistance QTL for both pathogens would be a valuable step in selecting novel breeding material for commercial cultivars.

5.1.1 Aims and objectives

Experimental work in this chapter aimed to characterise the resistance phenotypes and to map the genetic locus/loci of resistance within the DY DH population against *P. brassicae*. By comparing the findings with previously published literature, the hope is to provide further insight into resistance against *P. brassicae* for resistance breeding. Three separate objectives were investigated:

- To investigate the phenotype of resistance against *P. brassicae* in the DY DH population using two different *P. brassicae* populations from the UK in glasshouse experiments.

- To identify resistance QTL against *P. brassicae* in the DY DH population.
- To compare QTL identified in this study with those identified in previous studies in the DY DH population.

5.2 Materials and methods

This chapter describes experimental work done in two glasshouse experiments, each using a different *Pyrenopeziza brassicae* (light leaf spot) population as inoculum, to assesses the disease phenotype in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population. The phenotypic data obtained, along with the linkage map described by Kumar *et al*. (2018), were then used to identify resistance QTL within the DY DH population.

5.2.1 Phenotyping of resistance against *P. brassicae* in the DY DH population in glasshouse experiments

5.2.1.1 Preparation of plant material

For this work, 190 DH lines from the DY DH population were used in glasshouse experiments (Appendix J). Seeds were provided by Dr Régine Delourme (UMR1349 INRA-Rennes, French National Institute for Agriculture, Food, and Environment, France). Oilseed rape cultivar Charger (AHDB Recommended List (RL) 2016/17 resistance rating 4, susceptible) and cultivar Excel (AHDB RL 2010/11 resistance rating 5, susceptible) were added as susceptible controls. Cultivar Imola (believed to have a major gene for resistance against *P. brassicae*; Boys *et al*., 2012) and DH breeding line Q02 (believed to have quantitative resistance against *P. brassicae*; Karandeni Dewage *et al*., 2022) were added as resistant controls. Seeds for the control lines were from the host material collections in the UH Crop Protection and Climate Change Group. Five replicates were used for each DH line and control cultivars/lines.

5.2.1.2 Preparation of *P. brassicae* inoculum

P. brassicae inoculum obtained from Morley, Norfolk (England-Pb) and Aberdeen (Scotland-Pb) (Table 2.1) were each used for one of the two glasshouse experiments: GH-E with England-Pb and GH-S with Scotland-Pb.

The inoculum was bulked up by re-inoculating oilseed rape cultivar Charger under controlled-environment conditions. Diseased leaves were collected from re-inoculated plants and incubated at 4°C for 7-9 days. *P. brassicae* inoculum was prepared from the incubated leaves following the method described in section 2.2.2.

5.2.1.3 Experimental design and plant inoculation

The same experimental design was used for both GH-E and GH-S glasshouse experiments. Plants were arranged in an alpha design generated using Microsoft Excel (Appendix K). It was not possible to assess all the lines in one experiment, so they were divided into six batches of experiments within the same glasshouse over a three-month period. Each batch consisted of 32 DY lines, including the parental lines. The four control cultivars/lines were repeated in each of the six batches of experiments. Plants were grown in glasshouse conditions following the method described in section 2.3.1. When plants reached growth stage 1,4-1,5 (Sylvester-Bradley, 1985), they were spray-inoculated with *P. brassicae* conidial suspensions (10^5 spores/ml + 0.005% Tween 80) as described in section 2.3.2.

5.2.1.4 Assessment of light leaf spot symptoms

Plants were harvested and assessed between 23-26 days post-inoculation (dpi). Plants were assessed for total number of leaves, numbers of deformed leaves, dead leaves and symptomatic leaves, as well as the presence of a necrotic response. For the second glasshouse experiment, a distinction was made between necrotic responses depending on their location on the leaf (lamina, midrib or petiole). Plants were then placed in polyethylene bags and incubated at 4°C for 7-9 days to induce *P. brassicae* sporulation. After incubation, light leaf spot disease severity was assessed on a 1-8 scale (with 1 being no disease, section 2.4.1) and by visually estimating the percentage leaf area covered in *P. brassicae* asexual sporulation (section 2.4.2).

5.2.1.5 Statistical analysis

The data obtained from the six batches of glasshouse experiments for GH-E and GH-S were combined and lines with no datapoints (due to low seed germination, etc.) were excluded from the analysis. The numbers of dead leaves, deformed leaves and symptomatic leaves were expressed as percentages by dividing the number of affected leaves by the total number of leaves on each plant. These values, along with the percentage leaf area covered with *P. brassicae* sporulation, were transformed using an arc-sine transformation. The effect of batch within GH-E or GH-S was investigated using the data of the four control lines (Charger, Excel, Imola and Q02) across the six batches using analysis of variance in R (version 4.2.2). The number of lines presenting a necrotic response was also counted and used as a qualitative measure. Correlation analyses and figures were done using Microsoft Excel using calculated means of the different treatments. The BLUE (Best Linear Unbiased Estimator) calculation and the frequency distribution graphs were done using Genstat (Payne *et al*., 2011).

Due to significant batch effect between the six batch experiments within GH-E or GH-S, there was a need to transform the data to remove the batch effect. All the datapoints were transformed as relative values. To do the transformation, the disease symptom values of the two susceptible controls (cultivars Charger and Excel) from each experiment were averaged, and all the replicates of each DH line in the same experiment were divided by the averaged value calculated. These transformations were applied to all datapoints. By doing this, the batch effect was removed and all the six batch experiments could be combined into one for QTL analysis.

5.2.2 QTL mapping for resistance against *P. brassicae* in the DY DH population

5.2.2.1 Linkage map for the DY DH population

The linkage map published by Kumar *et al.* (2018) was used for QTL detection. The map consists of 3767 single nucleotide polymorphisms (SNPs) comprising 19 linkage groups covering 2128.2 cM in distance.

5.2.2.2 QTL analysis

QTL analysis was done using QTL IciMapping (version 4.2) (Meng *et al*., 2015) and linkage maps were visualized using MapChart (version 2.32) (Voorrips, 2002). Percentages of dead leaves, deformed leaves and symptomatic leaves, as well as % leaf area covered in *P. brassicae* sporulation were arc-sine transformed and used as quantitative phenotypic data for QTL analysis. Eight traits were analysed: number of dead leaves, number of deformed leaves, number of symptomatic leaves, % dead leaves, % deformed leaves, % symptomatic leaves, light leaf spot disease score (1-8 scale with 1 being no disease) and % leaf area covered in *P. brassicae* asexual sporulation. Composite interval mapping analysis was done using a step length of 1cM, probability in step-wise regression at 0.001 and using an LOD threshold of 2.5.

5.2.2.3 Comparison of QTL detected in this study with those in previous work on the DY DH population

Resistance QTL previously detected within the DY DH mapping population in four separate studies on *P. brassicae* (light leaf spot) and *Leptosphaeria maculans* (phoma stem canker) were compared to the QTL detected in this chapter. The four studies used were two studies by Pilet *et al*. (1998a,b) and two studies by Huang *et al*. (2016; 2019). Information about the studies can be found in Table 5.1. The two studies by Pilet *et al*. (1998a,b), however, used a different naming structure rather than the A1–A10 and C1–C9 designations agreed by the Multinational *Brassica* Genome Project (MBGP) Steering Committee used in the other studies and the 19 linkage groups were instead named DY1-DY19. Additionally, marker and QTL positions were presented on a relative scale and could not be directly compared to the QTL found in other studies. Therefore, composite interval mapping analysis was re-done using the parameters described in section 5.2.2.2 with the original data from those publications (Pilet *et al*., 1998a,b) and the QTL identified were used for comparison studies here. QTL were considered overlapping or co-localised if the QTL positions were within 3 cM of one another.

Table 5.1: Previous studies that detected QTL for resistance against *Pyrenopeziza brassicae* (light leaf spot) and *Leptosphaeria maculans* (phoma stem canker) within the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population.

Study	Experiment type	Experiment location	Pathogen assessed	Number of QTL detected
Pilet *et al.* (1998a)	Field	France	*L. maculans*	20
Pilet *et al.* (1998b)	Field	France	*P. brassicae*	20
Huang *et al.* (2016)	Field	England, France	*L. maculans*	26
Huang *et al.* (2019)	Controlled environment, field	England	*L. maculans*	17

5.3 Results

5.3.1 Variations in phenotype of resistance against *Pyrenopeziza brassicae*

Due to poor germination of some DH lines, of the 190 DY DH lines used in both glasshouse experiments, 165 lines were assessed in the glasshouse experiment GH-E and 179 lines were assessed in the glasshouse experiment GH-S. The parental lines were also assessed in both experiments. For the six batches of experiments within GH-E, analysis of variance on the four control lines showed significant differences in light leaf spot disease score (1-8 scale with 1 being no disease) ($P < 0.001$), % leaf area covered in *P. brassicae* asexual sporulation ($P < 0.001$), % dead leaves ($P < 0.001$) and % symptomatic leaves ($P < 0.001$), but no significant differences in % deformed leaves ($P > 0.4$) between the six experiments. Cv. Imola did not germinate for the second experiment and was excluded from the analysis. For the six batches of experiments within GH-S, analysis of variance on the four control lines showed significant differences in disease score ($P < 0.001$), % leaf area with sporulation ($P < 0.001$), % deformed leaves ($P < 0.001$), % dead leaves ($P < 0.001$) and % symptomatic leaves ($P < 0.005$) between the six experiments. There was no significant difference between GH-E and GH-S in disease score ($P > 0.3$) or % leaf area with sporulation ($P > 0.2$). Due to the significant effect of experiment between the six experiments within GH-E or GH-S, transformation was applied to the datapoints to calculate relative values.

There was significant variation in light leaf spot disease phenotypes between the DY DH lines. *P. brassicae* asexual sporulation was generally confined along the leaf veins on less susceptible lines and the resistant control cultivars/lines (Imola, Q02), whereas more susceptible lines and the susceptible control cultivars (Charger, Excel) showed sporulation covering the entire leaf surface, sometimes showing concentric ring-like sporulation patterns (Figure 5.1d). Leaf deformation or curling and leaf discolouration from green to red, purple or yellow, were also observed (Figure 5.2). Premature leaf senescence and death were also associated with *P. brassicae* infection (Figure 5.2c). A black necrotic response was observed on different parts of the leaf (petiole, lamina and midrib)

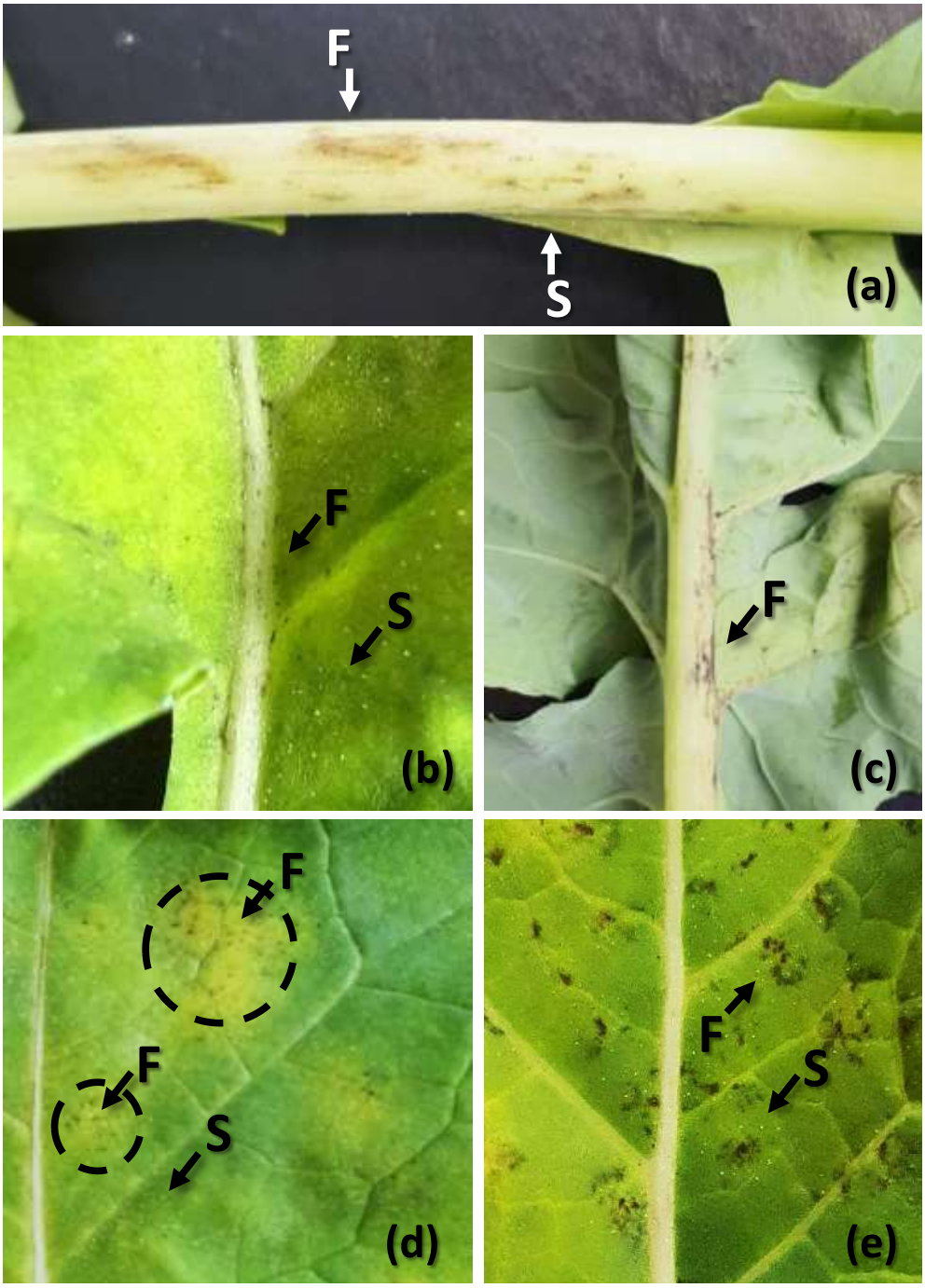

Figure 5.1: Necrotic responses observed on leaves of different lines from the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population inoculated with *Pyrenopeziza brassicae* populations.

Lines of the DY DH mapping population were inoculated with *P. brassicae* populations in glasshouse experiments. Black necrotic flecking (F), which is considered a phenotype of resistance (Boys *et al*., 2012), along with white *P. brassicae* asexual sporulation (S) were observed on the leaf (a) petiole (line DY042), (b) midrib on the adaxial (upper) leaf side (line DY390), (c) midrib on the abaxial (lower) leaf side (line DY260), (d) lamina in concentric rings (line DY069) and (e) lamina as larger lesions (line DY185).

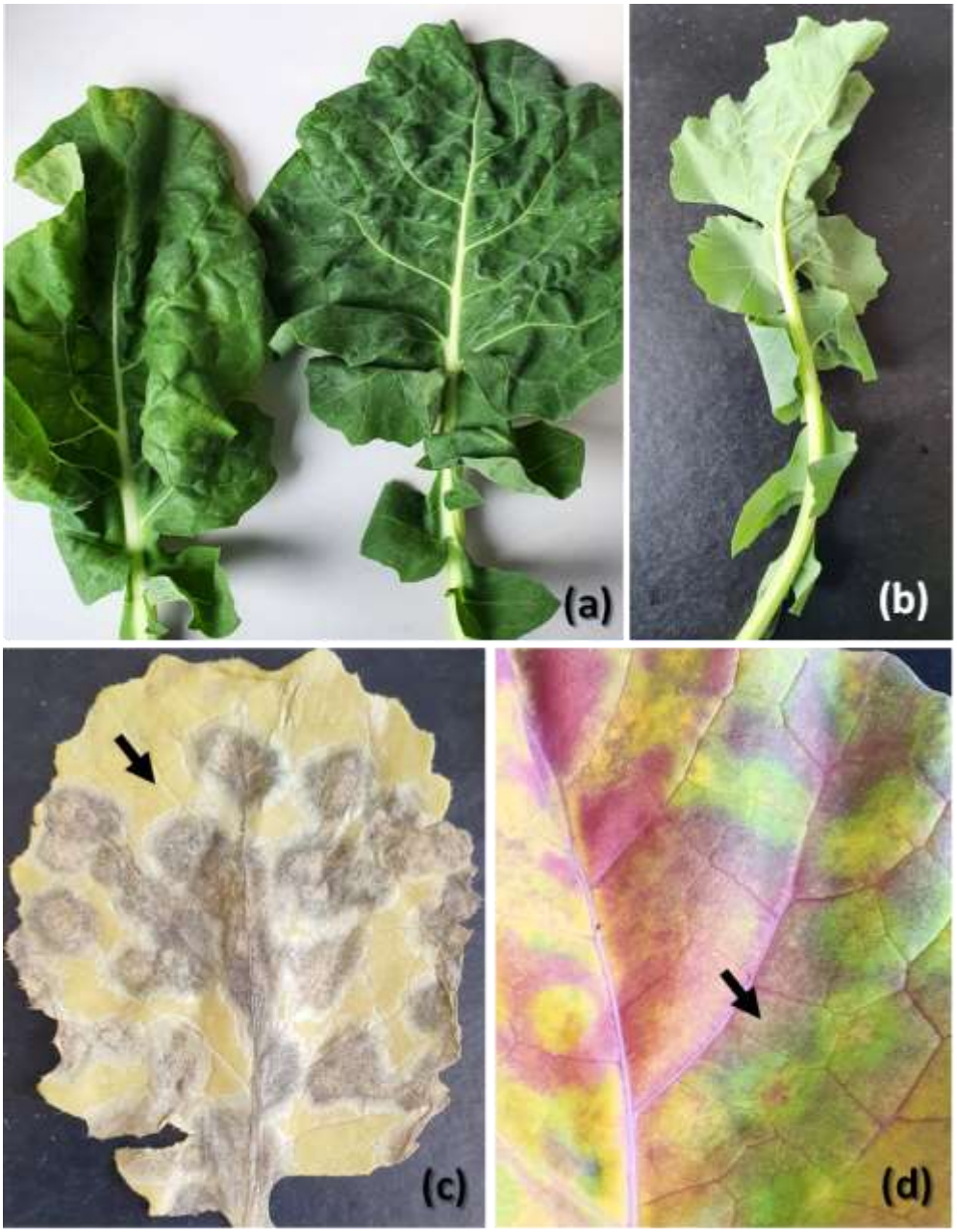

Figure 5.2: Light leaf spot symptoms observed on leaves of different lines from the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population inoculated with *Pyrenopeziza brassicae* populations.

Lines of the DY DH mapping population were inoculated with *P. brassicae* populations in glasshouse experiments. Different disease symptoms on leaves were observed, including (a) leaf deformation (line DY284), (b) petiole distortion (line DY033), (c) premature leaf senescence, with characteristic *P. brassicae* lesions (arrowhead) (line DY314) and (d) leaf discolouration, with characteristic *P. brassicae* lesions (line DY259).

in both resistant and susceptible lines (Figure 5.1). Parental cultivar Yudal showed a resistant response against *P. brassicae* (% leaf area with *P. brassicae* sporulation ranged between 0 and 20%), whereas parental cultivar Darmor-*bzh* showed a susceptible response against *P. brassicae* (% leaf area with *P. brassicae* sporulation ranged between 50 and 90%) in both glasshouse experiments (Figure 5.3). In GH-E, necrotic flecking was only observed on cv. Yudal, whereas in GH-S, necrotic flecking was observed on both cv. Yudal and cv. Darmor-*bzh* (Appendix L).

Results of data analysis reported in this chapter were from the analysis using the transformed values. Results of the analysis using the original un-transformed data can be found in Appendix M. Light leaf spot disease score and % leaf area covered in *P. brassicae* asexual sporulation were the most significant symptoms used to assess light leaf spot disease severity and showed good correlation for both glasshouse experiments (Figure 5.4). Frequency distributions for both disease assessment methods in both glasshouse experiments showed a continuous sinusoidal shape, implying normal distributions (Figure 5.5). The percentages of deformed, dead and symptomatic leaves were also calculated. For GH-E, there was no significant correlation between disease score and % dead leaves (sample correlation coefficient = 0.06, $P > 0.39$, $n = 165$) but there was a modest correlation for % deformed leaves (sample correlation coefficient = 0.21, $P < 0.004$, $n = 165$) and % symptomatic leaves (sample correlation coefficient = 0.35, $P < 0.001$, $n = 165$). Similarly, there was no correlation between % leaf area with sporulation and % dead leaves (sample correlation coefficient = 0.06, $P > 0.44$, $n = 165$), but there was modest correlation for % deformed leaves (sample correlation coefficient = 0.25, $P < 0.001$, $n = 165$) and % symptomatic leaves (sample correlation coefficient = 0.38, $P < 0.001$, $n = 165$). For GH-S, there was modest correlation between disease score and % deformed leaves (sample correlation coefficient = 0.17, $P < 0.01$, $n = 179$), % dead leaves (sample correlation coefficient = 0.49, $P < 0.001$, $n = 179$) and % symptomatic leaves (sample correlation coefficient = 0.57, $P < 0.001$, $n = 179$). Similarly, there was modest correlation between % leaf area with sporulation and % deformed leaves (sample correlation coefficient = 0.21, $P < 0.004$, $n = 179$), % dead leaves

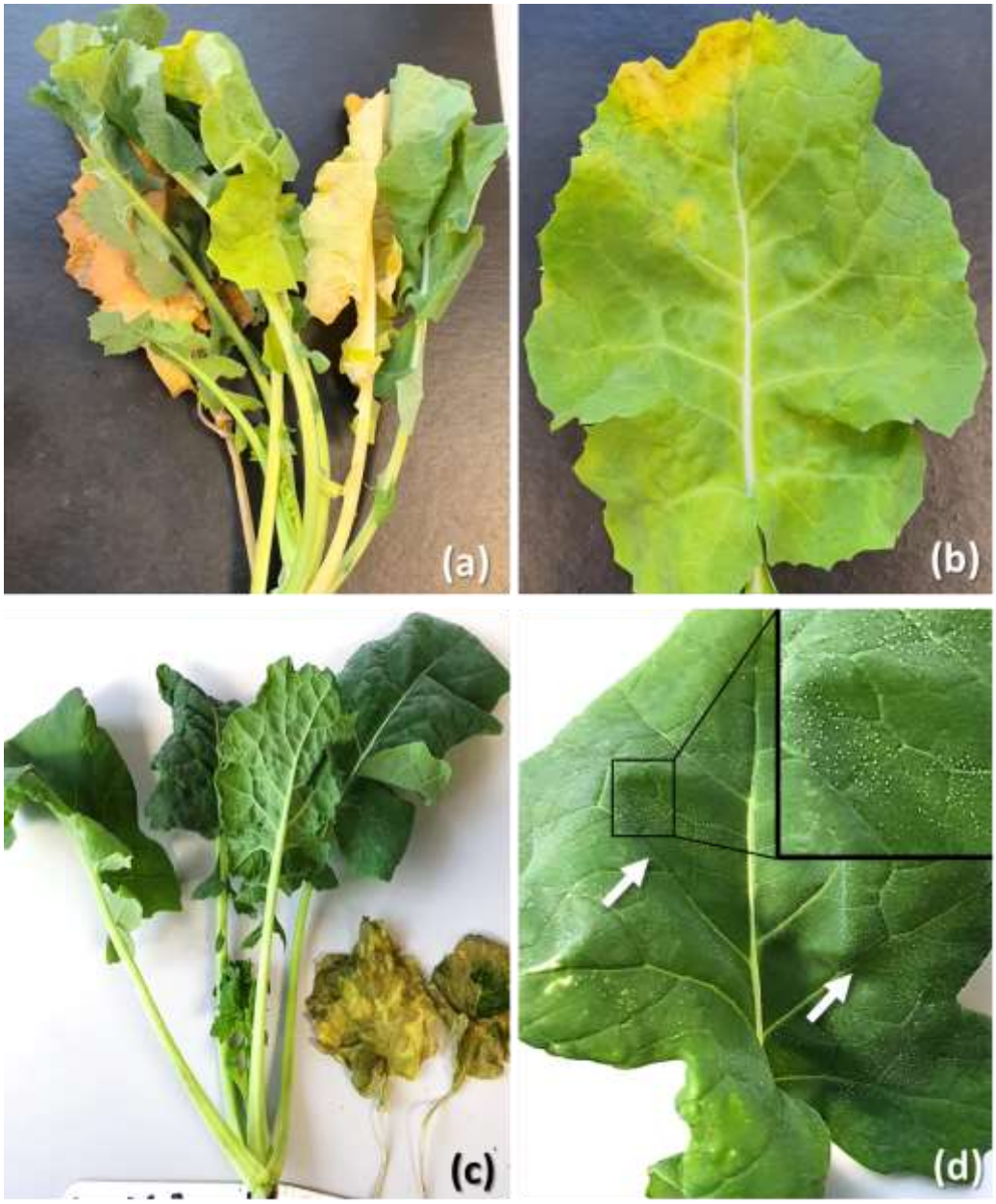

Figure 5.3: Light leaf spot (*Pyrenopeziza brassicae*) symptoms observed on the parental lines of the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population.

Light leaf spot symptoms observed on cultivar Darmor-*bzh* and cultivar Yudal, the parental lines of the DY DH population, following spray inoculation with *P. brassicae* conidial suspensions in a glasshouse experiment. (a & b) cv. Yudal showed a resistant response with limited *P. brassicae* asexual sporulation, while (c & d) cv. Darmor-*bzh* showed a susceptible response, including leaf deformations and extensive *P. brassicae* asexual sporulation (white, salt-like crystals, arrowheads) on the leaf surface.

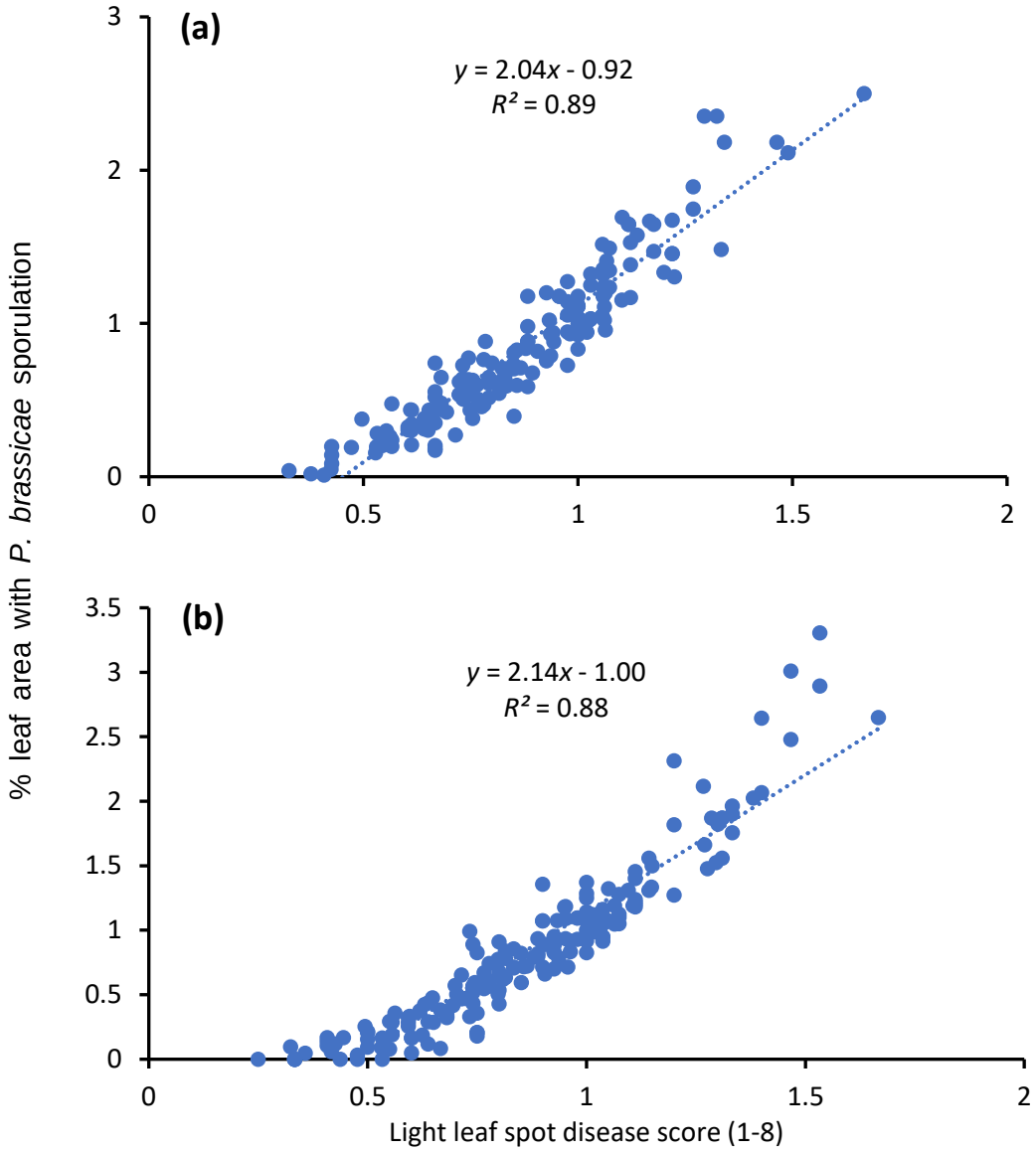

Figure 5.4: Correlation between light leaf spot disease score (1-8) and percentage leaf area covered with *Pyrenopeziza brassicae* sporulation in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in glasshouse experiments (a) GH-E and (b) GH-S.

Lines of the DY DH mapping population were inoculated with two different *P. brassicae* populations in two separate glasshouse experiments (GH-E and GH-S). Plants were assessed for light leaf spot severity using a disease score on a 1-8 scale (where 1 is no disease) and by visual estimation of the % leaf area covered with *P. brassicae* sporulation after samples were incubated at 4°C for 7-9 days. Correlations are shown for the two assessment methods for (a) GH-E (sample correlation coefficient = 0.94, $P < 0.001$, $n = 165$) and (b) GH-S (sample correlation coefficient = 0.93, $P < 0.001$, $n = 179$). Percentages of leaf area with sporulation values are presented on an arc-sine-transformed scale.

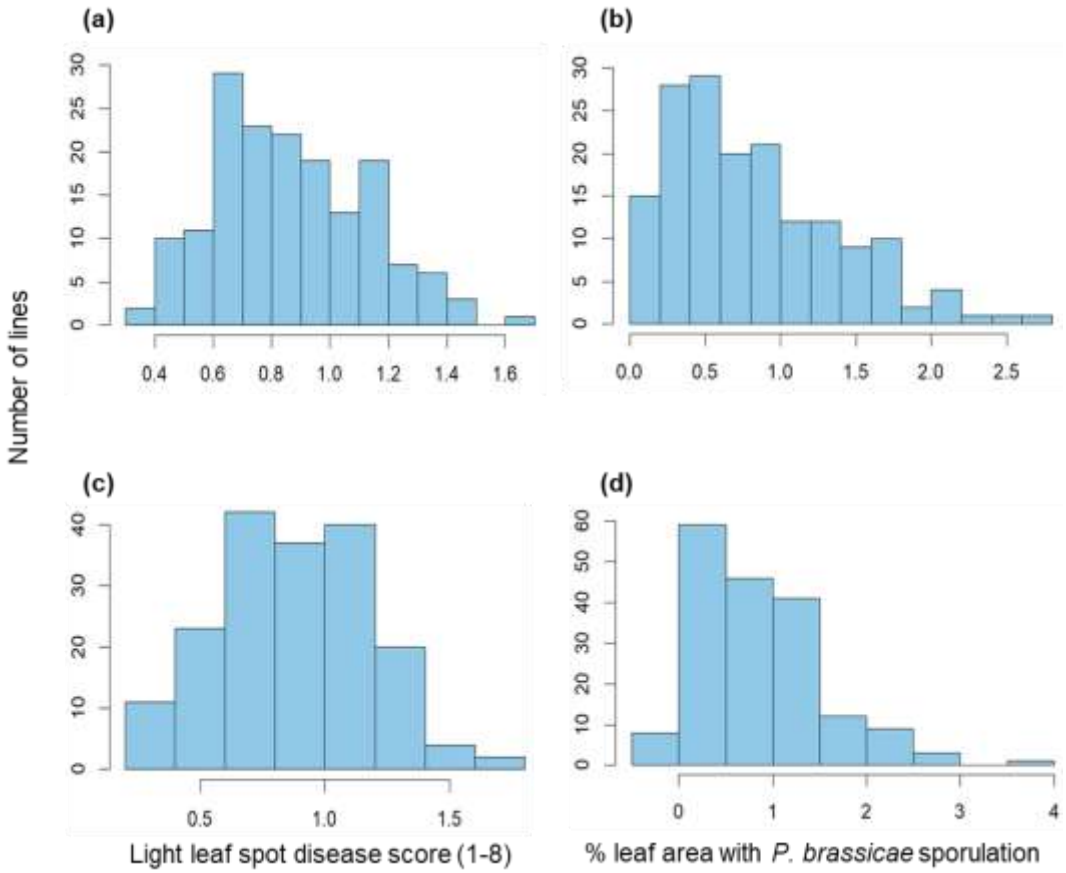

Figure 5.5: Frequency distribution of light leaf spot disease score (1-8) and percentage leaf area covered with *Pyrenopeziza brassicae* sporulation in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in glasshouse experiments (a & b) GH-E and (c & d) GH-S.

Lines of the DY DH mapping population were inoculated with two different *P. brassicae* populations in two separate glasshouse experiments (GH1 and GH2). Plants were assessed for light leaf spot severity using a disease score on a 1-8 scale (where 1 is no disease) and by visual estimation of the % leaf area covered with *P. brassicae* sporulation after samples were incubated at 4°C for 7-9 days. All traits for (a & b) GH-E and for (c & d) GH-S conformed to a normal distribution.

(sample correlation coefficient = 0.44, $P < 0.001$, $n = 179$) and % symptomatic leaves (sample correlation coefficient = 0.52, $P < 0.001$, $n = 179$). Frequency distributions were done for all three leaf symptoms in both glasshouse experiments using both the numbers of affected leaves and the percentage values calculated. Graphs of all six traits assessed showed continuous sinusoidal shapes, implying normal distributions for both GH-E (Figure 5.6) and GH-S (Figure 5.7).

5.3.2 Detection of QTL for resistance against *P. brassicae*

Composite interval mapping (CIM) analysis was done for eight quantitative traits from two glasshouse experiments (GH-E and GH-S) using both the original and transformed datasets. Only QTL obtained from the transformed data are discussed in this chapter; the QTL obtained from the original (un-transformed) data can be found in Appendix N. Using the transformed data, 21 QTL distributed across nine linkage groups for all eight quantitative traits were identified (Table 5.2 and Figure 5.8). For GH-E, there were six QTL identified, which accounted for 38.2% of overall phenotypic variation (ranging from 1.83 to 10.52%). For GH-S, there were 15 QTL identified, with phenotypic variation explained ranging from 1.78 to 11.01%. One QTL identified within GH-S on linkage group A09 was co-localised for the 'light leaf spot disease score' and '% *P. brassicae* sporulation' traits (Figure 5.8). There were no overlapping QTL between the two glasshouse experiments. Additionally, within the same glasshouse experiments, there tended to be overlapping QTL between quantitative traits derived from the same measurements, such as overlapping QTL between both the number and percentage of dead leaves traits.

5.3.3 Comparison of QTL with those found in previous studies

Using the new map and previous data (Pilet *et al*. 1998a,b), several QTL have been identified in the DY DH mapping population in this study. Using the data from Pilet *et al*. (1998a), 11 QTL for resistance against *L. maculans* (phoma stem canker) were identified here across nine linkage groups (A01, A06, A07, A08, A09, C02, C04, C07, C08, C09) with CIM analysis using data from field

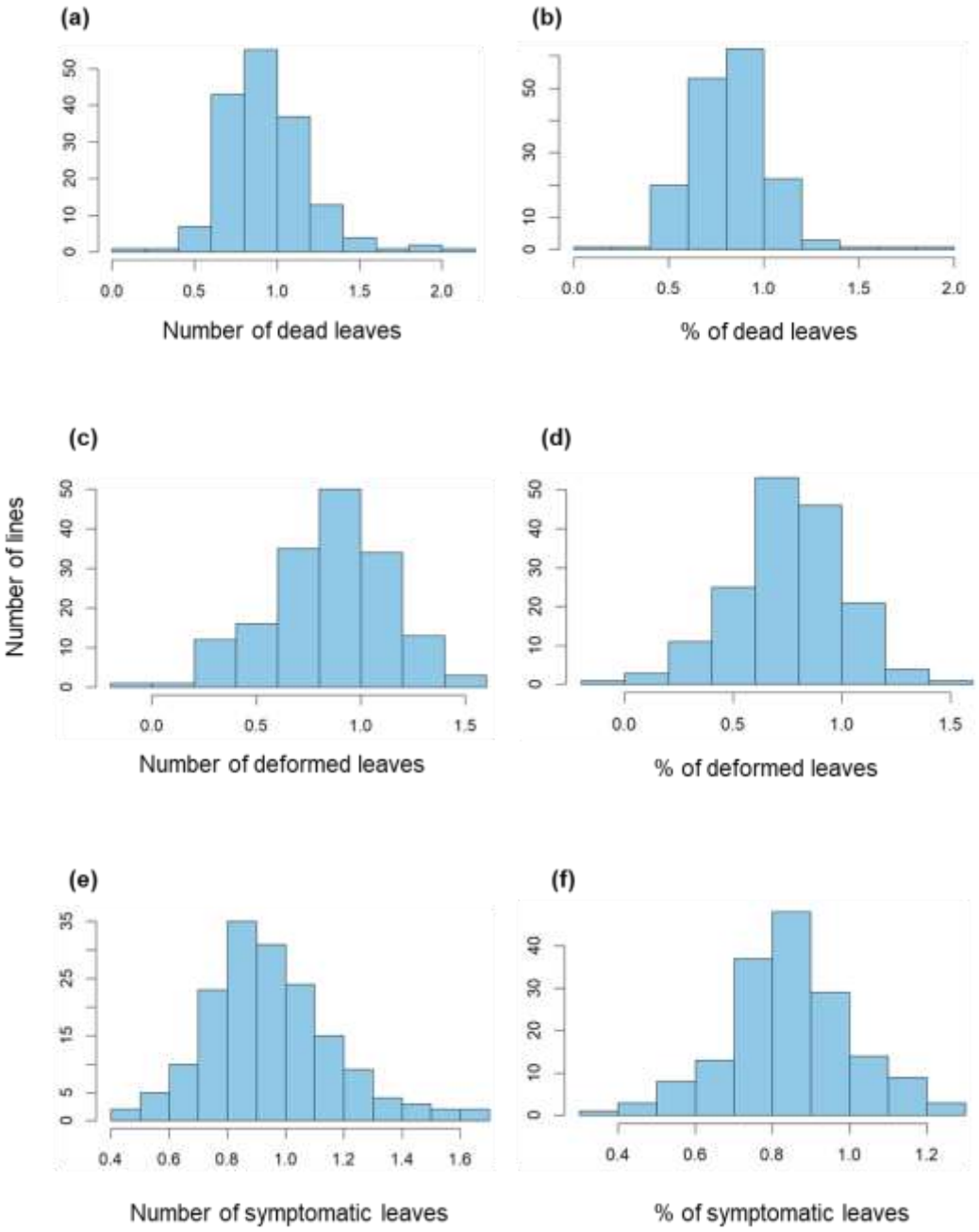

Figure 5.6: Frequency distribution of transformed values of light leaf spot (*Pyrenopeziza brassicae*) foliar symptoms in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in a glasshouse experiment (GH-E).

Lines of the DY DH mapping population were inoculated with a *P. brassicae* population from England (England-Pb, Table 2.1) in the glasshouse experiment (GH-E). Light leaf spot foliar symptoms were assessed. Measurements of (a & b) dead leaves, (c & d) deformed leaves and (e & f) symptomatic leaves conformed to a normal distribution.

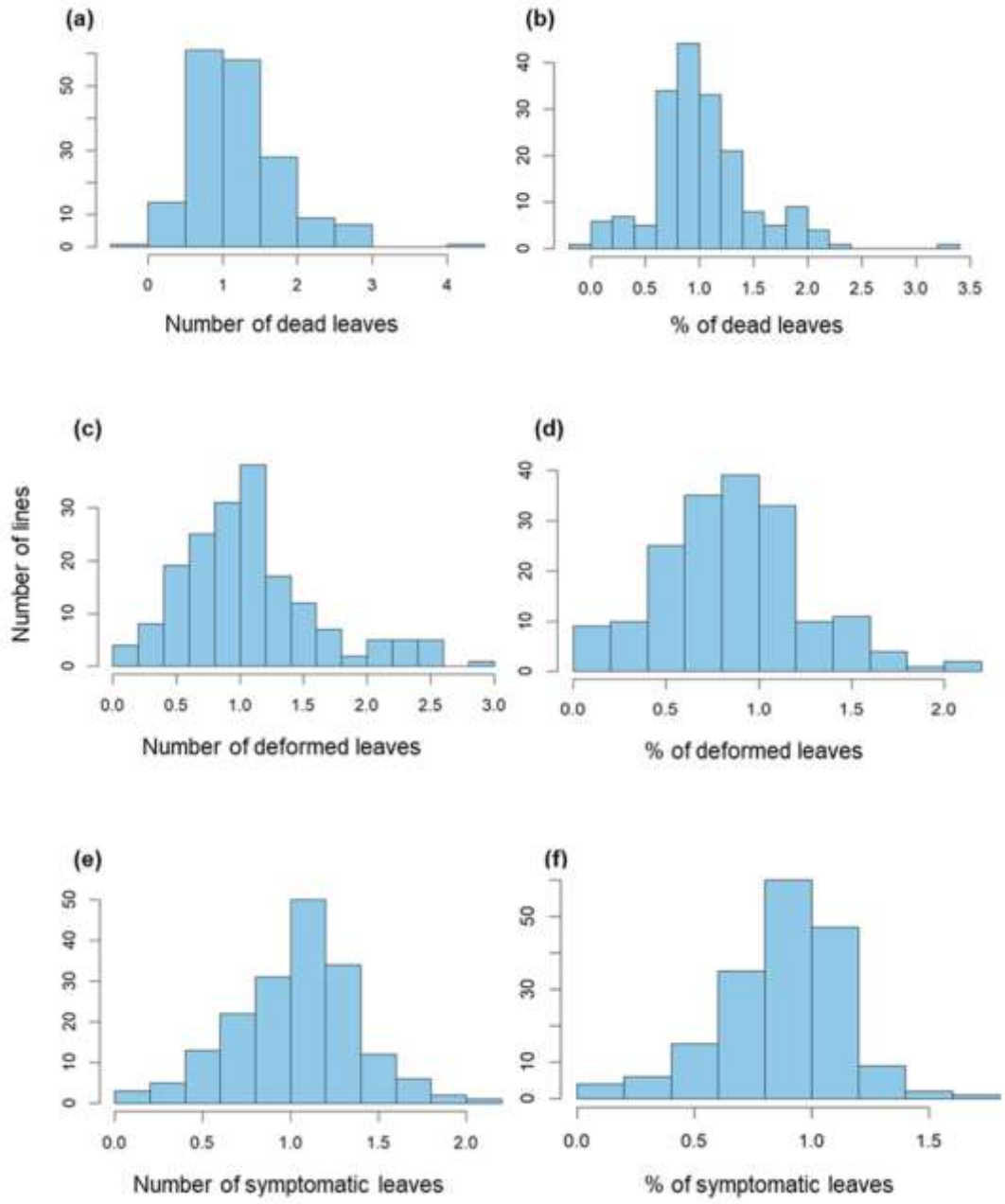

Figure 5.7: Frequency distribution of light leaf spot (*Pyrenopeziza brassicae*) foliar symptoms in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in a glasshouse experiment (GH-S).

Lines of the DY DH mapping population were inoculated with a *P. brassicae* population from Scotland (Scotland-Pb, Table 2.1) in a glasshouse experiment (GH-S). Light leaf spot foliar symptoms were assessed. Measurements of (a & b) dead leaves, (c & d) deformed leaves and (e & f) symptomatic leaves conformed to a normal distribution.

Table 5.2: QTL for resistance against *Pyrenopeziza brassicae* (light leaf spot) detected in glasshouse experiments within the oilseed rape DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population determined by composite interval mapping analysis.

Experiment*	Quantitative trait	Linkage group†	QTL position (cM)‡	Support interval (cM)	Peak LOD	R^2 (%)§	Additive effect
GH-E	Nr dead leaves	C02	37.6	37.4-37.8	2.5	6.7	0.07
	Nr deformed leaves	A04	50.1	49.9-50.3	4.0	10.5	-0.09
		C01	44.7	43.9-45.6	2.8	7.1	0.08
	% dead leaves	A09	96.1	95.8-96.4	12.8	1.8	0.16
		A09	97.7	97.1-98.3	19.4	2.7	-0.20
	% deformed leaves	C01	53.1	52.9-53.2	3.5	9.3	0.08
GH-S	Nr dead leaves	A02	104.3	104.2-104.4	3.1	7.4	-0.17
		C02	48.5	47.5-49.6	3.5	8.2	0.18
	Nr deformed leaves	A06	113.2	112.9-113.5	2.7	6.6	0.21
	Nr symptomatic leaves	A03	126	125.7-126.3	2.8	6.7	0.09
		C03	74.2	73.7-74.8	3.2	7.8	-0.10
	% dead leaves	A02	104.3	104.2-104.4	3.8	4.0	-0.14
		C02	48.5	47.5-49.6	5.3	5.6	0.16
		C02	90.1	90-90.2	3.1	9.6	-0.22
	% deformed leaves	A06	112.4	112-112.9	3.1	9.6	0.17
		A07	89.4	88.9-90	2.9	3.8	0.11
	% symptomatic leaves	C03	74.9	74.8-75.1	3.1	7.8	-0.07
	Light leaf spot disease score	A03	129.6	129.5-129.8	3.2	11.0	0.17
		A09	124.5	124.3-124.7	4.5	5.3	0.12
	% *P. brassicae* sporulation	A09	118	117.7-118.3	6.6	1.8	-0.27
		A09	124.2	123.8-124.7	15.6	4.8	0.45

* Phenotypic data from the DY DH population were collected from two separate glasshouse experiments: GH-E, where plants were inoculated with a *P. brassicae* population from England and GH-S, where plants were inoculated with a *P. brassicae* population from Scotland

† Linkage groups are labelled according to the standard *Brassica napus* chromosome/linkage group A1–A10 and C1–C9 designations agreed by the Multinational *Brassica* Genome Project (MBGP) Steering Committee (http://www.brassica.info/resource/maps/lg-assignments.php)

‡ The marker closest to the position of the maximum effect of the QTL

§ Proportion (%) of the phenotypic variance explained by the QTL

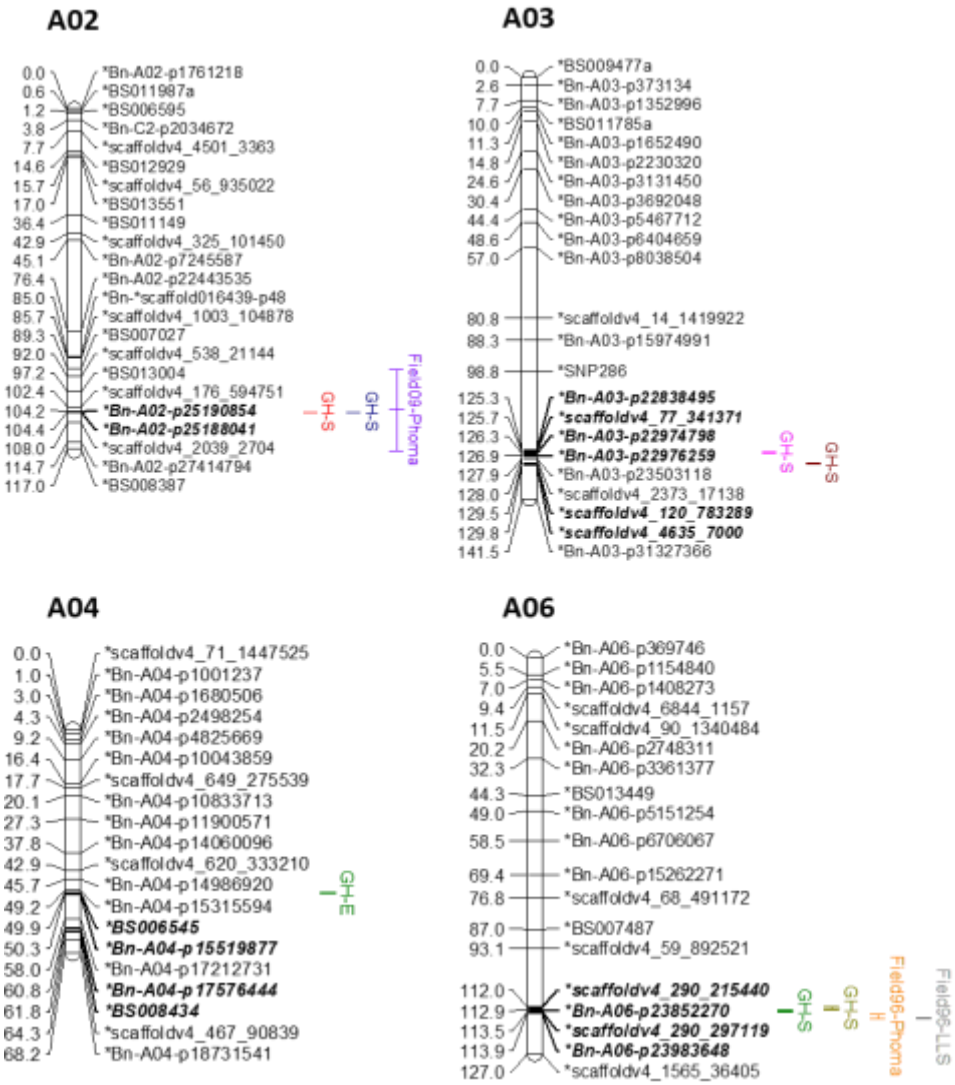

Figure 5.8: Genetic linkage map positions in cM of QTL for resistance against *Pyrenopeziza brassicae* (light leaf spot (LLS)) identified in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in two glasshouse experiments (GH-E and GH-S) using composite interval mapping analysis.

Phenotyping of the DY DH population was done in two glasshouse experiments with two different pathogen populations (GH-E and GH-S). Composite interval mapping analysis was done for the transformed dataset and 21 QTL were identified across nine linkage groups. Only linkage groups with identified QTL are shown and flanking markers for each QTL are indicated in bold letters. Individual quantitative traits used to identify each QTL are differentiated by colour: number of dead leaves (red), number of deformed leaves (dark green), number of symptomatic leaves (pink), % dead leaves (dark blue), % deformed leaves (light brown), % symptomatic leaves (light green), light leaf spot disease score (dark brown), % leaf area covered in *P. brassicae* asexual sporulation (light blue). Co-localising QTL from previous studies (Pilet *et al*., 1998a, orange; Pilet *et al.*, 1998b, grey; Huang *et al*., 2016, purple; Huang *et al*., 2019, black) are also shown.

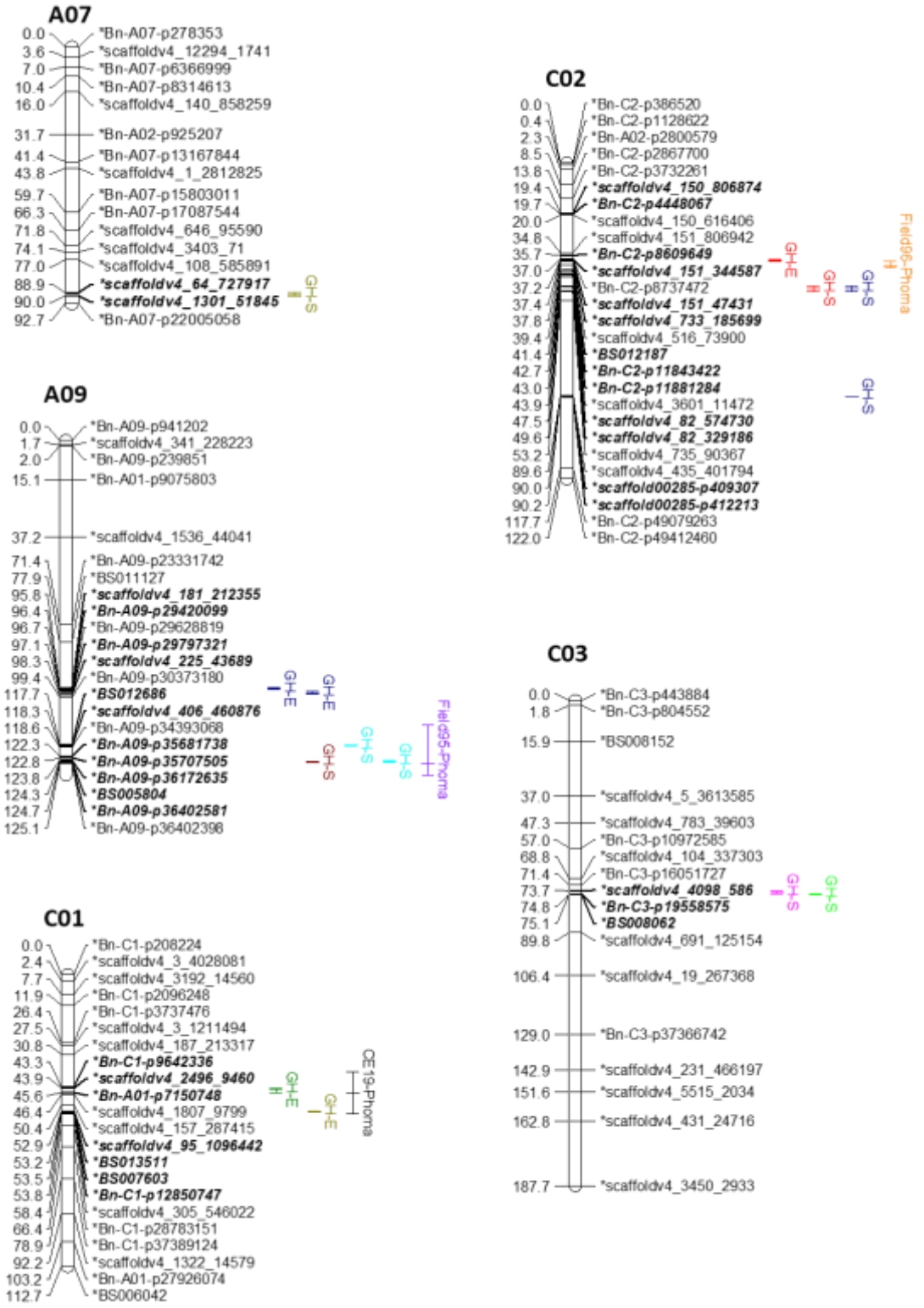

Figure 5.8 continued

experiments (Table 5.3). Next, using the data from Pilet *et al.* (1998b), 19 QTL for resistance against *P. brassicae* were identified here across seven linkage groups (A01, A02, A06, A07, A09, C05, C07) with CIM analysis using data from field experiments (Table 5.4). Huang *et al*. (2016) identified 26 QTL for resistance against *L. maculans* across 13 linkage groups (A01, A02, A03, A04, A06, A07, A08, A09, C01, C02, C04, C07, C08) using data from field experiments. Huang *et al.* (2019) identified 17 QTL for resistance against *L. maculans* across six linkage groups (A02, A03, A04, A10, C01, C09) using data from controlled environment experiments. The present study identified 21 QTL for resistance against *P. brassicae* across nine linkage groups (A02, A03, A04, A06, A07, A09, C01, C02, C03) for two glasshouse experiments with two different pathogen populations. Four QTL found in the present study overlapped with those found in the previous studies (Figure 5.8). There was one QTL on linkage group A06 that co-localised with a QTL identified within both studies by Pilet *et al*. (1998a,b). This QTL had a positive effect, suggesting that the allele of resistant parent cv. Yudal contributed to resistance. Additionally, there were two QTL on linkage groups A02 and A09 that co-localised with QTL identified by Huang *et al*. (2016), one QTL on linkage group C01 that co-localised with one identified by Huang *et al.* (2019) and another QTL on linkage group C02 that co-localised with one found by Pilet *et al*. (1998a).

5.4 Discussion

Phenotypic analysis from two glasshouse experiments suggested segregation of resistance against *Pyrenopeziza brassicae* within the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population. There were significant differences in the assessed traits among the breeding lines tested. There was good correlation between light leaf spot disease score on a 1-8 scale (with 1 being no disease) and visual estimation of percentage leaf area covered with *P. brassicae* asexual sporulation, but otherwise poor to modest correlation for the leaf symptoms (deformed, dead and symptomatic leaves) for both glasshouse experiments. These results suggested that leaf symptoms, while related to disease severity, were not as accurate as disease score and

Table 5.3: QTL for resistance against *Leptosphaeria maculans* (phoma stem canker) detected in field experiments within the oilseed rape DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population determined by composite interval mapping analysis using the data from Pilet *et al.* (1998a).

Quantitative trait*	Linkage group[†]	QTL position (cM)[‡]	Support interval (cM)	Peak LOD	R^2 (%)[§]	Additive effect
1995	A01	49.9	49.5-49.8	2.9	4.9	-0.22
	A08	2.1	1.8-2.5	5.0	8.8	-0.30
	A09	57.6	56.9-57.5	4.8	8.5	-0.31
	C02	1.9	1.5-1.9	2.6	4.3	0.21
	C04	46.5	44.1-46.5	5.3	9.4	-0.31
	C09	119.1	117.9-119.1	4.1	7.1	-0.27
1996	A06	114.4	114.3-115.6	14.4	24.4	-0.57
	C02	39.6	37.9-40.3	4.3	6.4	0.29
	C07	77.1	76.8-77.7	4.1	5.9	-0.27
	C08	77.0	76.7-78.2	5.2	7.7	-0.31
	C09	119.1	117.9-119.1	3.6	5.3	-0.25

* A mean disease index was scored on 30 plants using a 0 – 9 score (with 0 being no disease) in field trials done in 1995 and 1996 in Le Rheu, France

[†] Linkage groups are labelled according to the standard *Brassica napus* chromosome/linkage group A1–A10 and C1–C9 designations agreed by the Multinational *Brassica* Genome Project (MBGP) Steering Committee (http://www.brassica.info/resource/maps/lg-assignments.php)

[‡] The marker closest to the position of the maximum effect of the QTL

[§] Proportion (%) of the phenotypic variance explained by the QTL

Table 5.4: QTL for resistance against *Pyrenopeziza brassicae* (light leaf spot) detected in field experiments within the oilseed rape DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population determined by composite interval mapping analysis using the data from Pilet *et al*. (1998b).

Quantitative trait*	Linkage group†	QTL position (cM)‡	Support interval (cM)	Peak LOD	R^2 (%)§	Additive effect
Leaf-95	A02	76.8	76.4-76.7	4.7	8.6	-0.68
	A06	102.8	101.9-104.7	5.2	9.7	-0.76
	A07	44.5	44.1-44.5	6.7	12.9	-0.82
	A09	6.5	5-7	5.2	9.8	-0.71
	A09	69.9	68.9-69.8	5.9	11.2	-0.85
Stem-95	A02	76.8	76.4-76.7	3.2	8.9	-0.45
	A09	14.8	14.4-15.1	4.9	14.2	-0.56
Leaf-96	A01	65.3	64.7-65.3	3.6	5.3	-0.43
	A02	47.7	47.1-48	6.9	10.9	-0.65
	A06	103.4	101.9-104.7	6.4	10.3	-0.65
	A07	49.6	49.2-49.5	5.7	8.9	-0.57
	A09	9.2	8.8-12.9	2.9	4.3	-0.39
	A09	57.6	56.9-57.5	2.6	3.8	-0.38
	C05	17.2	16.9-17.2	3.9	5.9	0.46
	C07	48.4	47.8-48.4	5.7	8.9	0.57
Stem-96	A02	76.8	76.4-76.7	5.1	11.5	-0.60
	A06	115.6	115.2-116.7	6.8	15.8	0.71
	A07	49.6	49.2-49.5	3.5	7.8	-0.49
	A09	70.9	70.5-70.8	2.6	5.7	-0.48

* A visual qualitative light leaf spot disease score was attributed for each field plot using a 1 - 11 scale (with 1 being healthy plot appearance), based on the percentage of plants infected and the intensity of disease on both leaves and stems in field trials done in 1995 and 1996 in Le Rheu, France

† Linkage groups are labelled according to the standard *Brassica napus* chromosome/linkage group A1–A10 and C1–C9 designations agreed by the Multinational *Brassica* Genome Project (MBGP) Steering Committee (http://www.brassica.info/resource/maps/lg-assignments.php)

‡ The marker closest to the position of the maximum effect of the QTL

§ Proportion (%) of the phenotypic variance explained by the QTL

percentage leaf area with *P. brassicae* sporulation. There was distinction in the amount and pattern of sporulation between susceptible and more resistant lines. Less susceptible lines produced sporulation primarily in the leaf midrib and veins, suggesting the pathogen colonized the leaf vasculature for nutrient acquisition first, whereas susceptible lines showed concentric ring-like patterns or, in more severe cases, sporulation covering the entire leaf surface.

Black necrotic flecking may appear on the host only in the case of severe *P. brassicae* infection. Black flecking is considered a phenotype of resistance and a locus associated with this phenotype has been previously characterized (Bradburne *et al*., 1999; Boys *et al*. 2012), but in the present study, black flecking was indiscriminately observed on both susceptible and resistant lines. Disease was generally more severe in the experiment GH-S with inoculum from Scotland compared to the experiment GH-E with inoculum from England. This suggested that the *P. brassicae* population from Scotland was more virulent compared to the one from England. Interestingly, some lines that did not show black flecking in GH-E developed flecking in GH-S, including cv. Darmor-*bzh*, one of the parental lines of the DY DH mapping population, which showed a susceptible disease phenotype in both glasshouse experiments. This suggested that there may be a certain threshold of colonization needed to trigger the black flecking symptoms on susceptible lines. Unfortunately, it was not possible to map the black flecking phenotype to a genomic locus here, but a study by Boys *et al.* (2012) using a different DH mapping population suggested the involvement of a single genomic locus controlling the black flecking phenotype. However, the necrotic response (black flecking) may be affected by background resistance or different pathogen effectors. Overall, the underlying mechanisms that control a necrotic response remain largely unknown and may not only be associated with a resistant response since black flecking was also observed on susceptible lines when the colonization was sufficiently extensive.

Upon comparing the parental lines of the DY DH mapping population, cv. Darmor-*bzh* showed susceptible whereas cv. Yudal showed resistant to *P. brassicae* populations from England and Scotland in the two glasshouse

experiments (GH-E, GH-S). This is in contrast to the study by Pilet *et al*. (1998b), which presented cv. Darmor-*bzh* as moderately resistant and cv. Yudal as susceptible to *P. brassicae* in French field experiments. The study of host-pathogen interactions described in Chapter 4 showed that cv. Yudal was susceptible to most of the *P. brassicae* isolates tested. A study by Karandeni Dewage *et al*. (2021) showed that cv. Yudal (overall sporulation 20.4%) was overall more resistant to *P. brassicae* than Darmor-*bzh* (overall sporulation 45.7%) for the isolates tested. These discrepancies show that disease phenotype can vary between experimental conditions and pathogen populations or specific isolates used, but it is nonetheless interesting how the same line can show different levels of susceptibilities under different conditions with different isolates/populations. This further highlights the need to study candidate resistant genotypes under many different experimental conditions.

There were overlapping QTL found within the glasshouse experiments with different *P. brassicae* populations. Most overlapping QTL were identified for quantitative traits derived from the same quantitative measurements (such as overlapping QTL between both the number of dead leaves and percentage of dead leaves traits), but the overlapping QTL identified on linkage group A09 was found for both 'light leaf spot disease score' and '% *P. brassicae* sporulation' traits. Most QTL did not have an LOD that exceeded 3, meaning that, although significant, many were not particularly strong QTL that only accounted for less than 10% of genotypic variation. Nonetheless, the QTL that co-localised for different quantitative traits could be considered stable and be considered for further mapping studies.

There were several overlapping QTL found between the present study and previous studies done on the DY DH mapping population. Particularly significant was the QTL identified in this study on linkage group A06 which co-localised with the QTL for resistance against *Leptosphaeria maculans* (phoma stem canker) (new QTL analysis using data from Pilet *et al*. (1998a)) and the QTL for resistance against *P. brassicae* (light leaf spot) (new QTL analysis using data from Pilet *et al*., 1998b)). Pilet *et al*. (1998b) compared the QTL for resistance against *P. brassicae* with the QTL for resistance against *L. maculans*

(phoma stem canker) (Pilet *et al*., 1998a). They found two regions on linkage groups DY6 and DY10 (which are linkage groups A06 and A07, respectively) that they considered 'multiple disease resistance' ('MDR') QTL possibly involved in common resistance mechanisms against both fungal pathogens. The QTL on linkage group A06 found in the present study co-localised with one of these 'MDR' QTL, suggesting the QTL is stable multiple disease resistance which is valuable for resistance breeding, as it was now detected almost 30 years apart in both France and the UK. Similarly, QTL on linkage groups A02, A09 and C02 for resistance against *P. brassicae* found in the present study were co-localised with QTL found by Huang *et al*. (2016) for resistance against *L. maculans*; the QTL on linkage group A02 is especially interesting for further study since it was described as environmentally stable QTL in that study. Another QTL on linkage group C01 was co-localised with another QTL found by Huang *et al*. (2019) for resistance against *L. maculans*. Three out of these four studies mapped QTL against *L. maculans* but still co-localised with QTL against *P. brassicae* found in the present study, suggesting these QTL could be involved in resistance against both pathogens.

There were limitations to the study. Despite using the same DH mapping population, there were differences from the previous studies, such as differences in the DH lines and linkage maps used, as well as differences in the experimental conditions. The study by Huang *et al*. (2019) used the same linkage map as the present study, but the other studies (Huang *et al*., 2016; Pilet *et al*., 1998a,b) used older linkage maps with limited common markers between them, which were overall less dense and less robust due to having fewer markers. The QTL identified here using the data from Pilet *et al*. (1998a,b) could not be directly compared to the QTL found in the original studies due to differences in nomenclature of linkage groups and absence of QTL position information. Previous studies also used different breeding lines across experiments compared to the lines used in the present study. Additionally, most of the other studies involved field experiments in both France and England, although the *P. brassicae* populations were derived from fields in England and Scotland, the experimental work in the present study was done in a glasshouse, which may not always reflect field conditions. Due to significant effect of

experiment between the six batches of experiments within GH-E or GH-S, the original data sets were transformed as relative values, which may have also influenced the resulting QTL.

Despite evidence suggesting that combining *R*-gene mediated resistance and quantitative resistance (Huang *et al*., 2018; Pilet-Nayel *et al*., 2017) provides more durable cultivar resistance, the mechanisms of operation of quantitative resistance remain poorly understood (Poland *et al*., 2008). Quantitative resistance is considered more durable than *R*-gene mediated resistance (Brun *et al*., 2010) but is also more challenging to screen quantitative resistance compared to single *R* genes (Balesdent *et al*., 2000; Huang *et al*., 2009). Identifying resistance QTL against pathogens of arable crops remains essential for developing durable disease resistance. The stability of QTL across environments remains a major limitation of QTL mapping studies (Raman *et al.,* 2018), so the identified stable QTL across different environments, experimental conditions and pathogen populations will be an important source of durable resistance for pre-breeding.

Chapter 6 General discussion

The results of the experimental work described in this thesis provide new knowledge to better understand the operation of host resistance within oilseed rape (*Brassica napus*) against the light leaf spot pathogen *Pyrenopeziza brassicae* by investigating factors contributing to disease development, studying specific host-pathogen interactions and by identifying quantitative trait loci (QTL) related to host genetic resistance.

Effects of environmental, pathogen and host factors affecting light leaf spot disease development

Patterns of *P. brassicae* air-borne ascospore release over three cropping seasons in relation to weather data suggested that there are two major periods of ascospore release, one in late autumn and a second one in early summer, at temperatures ranging between 15-17°C and alongside rainfall. Ascospore release was absent in winter and may be inhibited by low temperatures, but a sufficiently large number of apothecia, as was the case during the second cropping season (2021/2022), may lead to early ascospore release in spring. New oilseed rape stems were placed around the Burkard spore sampler in early August in each cropping season and the observed ascospore release in June/July suggests that apothecia on oilseed rape stubble were able to release ascospores up to a year later. Cheah and Hartill (1985) reported similar findings in New Zealand, where *P. brassicae* apothecia on dry cauliflower residues were viable for up to 50 weeks once formed, but this may be the first report under UK conditions. Studies monitoring *P. brassicae* air-borne ascospore release using air samplers have been done (Calderon *et al*., 2002; Gilles *et al*., 2001b; McCartney and Lacey, 1990) and both temperature and rainfall have been incorporated into light leaf spot disease prediction models (Gilles *et al*., 2000a; Welham *et al*., 2004), but there are few studies that continuously monitored ascospore release over multiple cropping seasons under UK field conditions. The present study therefore proved valuable in investigating the effects of weather conditions on *P. brassicae* ascospore release throughout the entire cropping season.

Results of this study showed that plant age and *P. brassicae* inoculum concentration affected light leaf spot disease development in oilseed rape. Plant ages ranging from one- to six-weeks-old were assessed for disease development using a light leaf spot disease score on a 1-8 scale (with 1 being no disease) and by estimation of percentage leaf area covered in *P. brassicae* asexual sporulation. Four-weeks-old (four to five true leaves) was found to be the optimal age for disease development in controlled-environment and glasshouse studies, as younger plants (e.g. one-, two-weeks-old plants) did not develop sufficient disease symptoms, while leaves on older plants (e.g. six-weeks-old plants) senesced before disease assessment. Although greater *P. brassicae* inoculum concentrations correlated with more severe disease symptoms, too great inoculum concentrations may affect comparable disease assessment between cultivars. Therefore, an inoculum concentration of 10^5 spores/ml was found to be the optimal inoculum concentration. *P. brassicae* pathogen populations also caused greater disease compared to a single-spore *P. brassicae* isolate, but disease severity largely depends on whether the genotype of the isolate or population is aggressive or not (McDonald *et al*., 2022). Optimal host age and pathogen inoculum type ultimately depend on the experimental requirements.

Inconclusive results suggested that *P. brassicae* infection may affect host plant height in a controlled-environment. The second experiment of Chapter 3 reported reduced height on plants inoculated with the greater *P. brassicae* inoculum concentration (10^5 spores/ml), but this phenotype could not be recreated in subsequent experiments with the same or different experimental conditions. Changes in plant height due to severe *P. brassicae* infection have been reported in crops (Karandeni Dewage *et al*., 2018) and fungal cytokinins are believed to underlie changes in plant morphology such as leaf deformation, stem elongation and growth stunting (Ashby, 1997; Boys *et al*., 2007; Murphy *et al*., 1997). However, a recreation of the plant growth stunting phenotype in controlled-environment has not been reported. This may be due to the absence of rain in controlled-environment to splash the spores onto key plant growing points (e.g. emerging leaves, meristem, etc.) that may lead to growth stunting

in the field. As the findings presented here were not reproduced, the phenotype may have therefore resulted from environmental conditions.

Light treatment affected the rate and severity of light leaf spot development, providing new evidence suggesting that greater light intensity may promote *P. brassicae* colonization and accelerate disease development. Inoculated plants exposed to a greater light intensity developed foliar symptoms (leaf deformation, senescence and discoloration) at a faster rate and had an overall greater disease severity compared to plants exposed to a lower light intensity. Light may have had a greater effect on the pathogen than on the host, since plant biomass did not change between treatments. Studies on other ascomycete fungi suggested that light promotes conidial production (Flaherty *et al*., 2005; Lauter *et al*., 1991; Lee *et al*., 2006). Follow-up studies are necessary to verify these findings by investigating the effects of photoperiod and light intensity separately and how either of these factors influence light leaf spot development.

Variations in *Pyrenopeziza brassicae* pathogen populations

Differences in light leaf spot disease severity on the same cultivars between sites observed in field experiments suggest that there may be regional differences in *P. brassicae* pathogen races. Cultivars Poh Bolko, Castille and Yudal showed greater disease severity in Norwich, Norfolk compared to Harpenden, Hertfordshire, suggesting *P. brassicae* pathogen races in Norwich were more virulent than those in Harpenden. Cultivars Campus, Catana, Kielder and especially Acacia, conversely, remained symptomless across all sites tested, suggesting stable underlying resistances. Similarly, disease severity was greater in Hereford, Herefordshire compared to Huntingdon, Cambridgeshire for both cultivars Aquila and Flamingo, but disease severity varied between cropping seasons. Hereford historically experiences more rainfall and is more humid compared to Huntingdon (Climate Data, 2024) and certain weather factors, particularly increased rainfall, are known to promote light leaf spot disease development. Rainfall may splash spores onto new leaves and pod and is an important contributing factor that makes the disease polycyclic in spring (Fitt *et al*., 1998a,b; Gilles *et al*., 2001b,c; Welham *et al*.,

2004). Similarly, greater disease incidence in Herefordshire compared to Cambridgeshire correlated with increased rainfall in Herefordshire in a previous study (Welham *et al*., 2004). Limited studies exist comparing pathogenic *P. brassicae* races between sites and seasons (Evans *et al*., 2017; Klöppel, 2015) despite evidence suggesting high levels of genetic diversity between regions (Majer *et al*., 1998) with the potential of causing increasingly severe epidemics.

Studies of specific host-pathogen interactions in the *B. napus-P. brassicae* pathosystem showed significant differences between cultivar/lines, between isolates and significant cultivar/lines-isolate interactions. Cultivar Imola, breeding line Q02 and line NPZ 06/22 showed non-specific stable resistance against most isolates tested. The observed resistant phenotypes of these lines were absent or limited *P. brassicae* sporulation (often confined to the leaf midrib) and presence of a necrotic response (black flecking) on the leaf petiole, midrib or surface. The materials with genotypes that prevent or limit asexual sporulation are valuable resources for resistance breeding, , because reduced asexual sporulation can reduce the secondary infection initiated by splash-dispersed conidia (Boys *et al*., 2007; Fitt *et al*., 1998a). Cultivars Charger, Bristol, Yudal and Barbados were susceptible to most of the isolates tested. However, cv. Yudal showed a resistant response to *P. brassicae* populations from England and Scotland (Chapter 5); this suggests that a cultivar that showed a susceptible response against a single-spore isolate of *P. brassicae* may not be susceptible to a population (i.e. a mixture of different isolates). A population may consist of isolates that are both virulent or avirulent against a certain cultivar, but avirulent isolates causing an incompatible (resistance) response within the host may mask the virulence of other isolates in the same population, causing the overall host response to be resistant. Overall, light leaf spot disease development on a certain cultivar can vary depending on the inoculum used and the experimental conditions. Host resistance is most valuable when it is stable across different environments.

Single-spore isolates of *P. brassicae* from six European countries, including the UK, were tested and showed significant variations in light leaf spot disease scores and % leaf area with sporulation between countries, suggesting

differences in aggressiveness between the isolates from these countries. Single-spore isolates obtained from England were less virulent compared to EU isolates, which may be due to the cultivars/lines tested being less resistant to non-UK isolates. Considering that light leaf spot severity in northern Europe is increasing (Karandeni Dewage *et al*., 2018), it is important to monitor *P. brassicae* populations in non-UK areas as well, although it should be noted that only a limited number of representative isolates were used here and follow-up studies using more isolates per country are recommended. Unfortunately, phenotypic results obtained in this study could not be correlated with molecular marker analyses to establish patterns between disease phenotype and pathogen genotype. The overall knowledge of differences in virulence among *P. brassicae* pathogen races remains limited.

Results of this study confirmed that *P. brassicae* can cross-infect between different *Brassica* hosts. Both oilseed rape and kale (*B. oleracea*) were affected by *P. brassicae* populations derived either from oilseed rape or kale, although the oilseed rape-derived inoculum generally caused more severe disease. *P. brassicae* has a wide *Brassica* host range (Cheah *et al*., 1980; Karolewski, 2010; Maddock *et al*., 1981) and is known to be able to cross-infect between *Brassicas* (isolates can infect a host that is different from the pathogen's initial *Brassica* host) (Maddock *et al*., 1981; Simons and Skidmore, 1998). It is therefore important to separate *Brassica* crops spatially and temporally. In this study, the kale-derived inoculum caused less severe disease on both oilseed rape and kale; however, even if certain pathogen populations affect a host less severely, the affected hosts can still act as reservoirs of pathogens and exacerbate epidemics.

Results of this study showed that *P. brassicae* can continuously re-sporulate every time after removing the previous sporulation until the leaf tissue fully senesced. The *P. brassicae* sporulation was observed at four separate times, each time after leaf washing to remove the previous sporulation (Chapter 3, experiment 4). Sporulation remained sustainable if there was sufficient available green leaf tissue for the pathogen to colonize for both oilseed rape and kale, which was up to 60 days after inoculation, including up to 30 days

after harvest if plant material was kept at 4°C. In field conditions, the secondary disease infections in spring result from fungal hyphae underneath the leaf surface producing acervuli, leading to splash-dispersal of asexual conidia (Boys *et al*., 2007; Fitt *et al*., 1998a). The re-sporulation on infected leaves was observed in controlled-environment in this study; testing the pathogenicity of *P. brassicae* conidial spores from each re-sporulation cycle could help provide further insight into impact of secondary infection on disease severity and disease epidemics.

Identification of quantitative resistance against *Pyrenopeziza brassicae*

Phenotypic analysis from two glasshouse experiments (GH-E, which used a *P. brassicae* population from England, and GH-S, which used a *P. brassicae* population from Scotland) suggested segregation of resistance against *P. brassicae* within the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population with distinct amounts and patterns of sporulation between susceptibility and resistance lines. There was good correlation between light leaf spot disease score and % leaf area covered with *P. brassicae* asexual sporulation, while correlation for the other leaf symptoms (dead, deformed and symptomatic leaves) remained modest. The parental lines, cv. Darmor-*bzh* and cv. Yudal, also showed differences in disease phenotype, with cv. Darmor-*bzh* appearing susceptible to *P. brassicae*, whereas cv. Yudal showed a resistant response. However, in field experiments in France, cv. Darmor-*bzh* showed resistant while cv. Yudal showed susceptible to *P. brassicae* (Pilet *et al*., 1998b) highlighting how disease phenotypes can be drastically influenced by environment and pathogen populations used.

Composite interval mapping analysis for eight quantitative traits from two glasshouse experiments (GH-E and GH-S) identified 21 QTL distributed across nine linkage groups using the transformed data. Of these, there was one overlapping QTL found in GH-S for two different quantitative traits (disease score and % leaf area covered in *P. brassicae* asexual sporulation). Comparisons between QTL found here with those found in previous studies (Pilet *et al.,* 1998a,b; Huang *et al*., 2016; 2019) identified four common QTL for different traits or for two different diseases (phoma stem canker and light leaf

spot). Especially interesting was the QTL on linkage group A06 which co-localised with both previous studies (Pilet *et al*., 1998a,b) and another QTL on linkage group A02 that co-localised with a QTL previously described as environmentally stable for control of phoma stem canker (Huang *et al*., 2016). Despite most of these QTL not exceeding a LOD threshold of 3 and only accounting for less than 10% of genotypic variation, their stability across different environments makes them valuable for further genetic studies of resistance for pre-breeding material. Quantitative resistance remains poorly understood (Poland *et al.,* 2008) compared to *R*-gene mediated qualitative resistance (Brun *et al*., 2010).

Black necrotic flecking may appear in association with severe disease and may be isolate-specific, but its underlying mechanisms remain largely unknown. Although the black flecking phenotype could not be mapped to a genomic locus within the DY DH mapping population, it was assessed in several other experiments reported in this thesis with varying levels of consistency. Presence of a necrotic response remained site- and/or isolate-specific in field experiments, even when separate sites had similar disease severities, as the case for cv. Aquila in the 2021/22 season, where the disease severity was the same in both Huntingdon and Hereford, but flecking appeared only in leaves sampled in Hereford. When investigating host-pathogen interactions, necrotic flecking appeared on cv. Imola for 13 out of 24 single-spore *P. brassicae* isolates but flecking also appeared indiscriminately in both resistant and susceptible responses. Boys *et al.* (2012) characterized an *R* gene for necrotic flecking at the bottom of chr A1 within a DH mapping population derived from cv. Imola, which remains the only cultivar that consistently shows necrotic flecking under any experimental conditions. Necrotic flecking also appeared in both parental lines of the DY DH mapping population, but cv. Darmor-*bzh* (which had a susceptible response) only showed flecking in GH-S which used a *P. brassicae* population from Scotland (more virulent, as was also suggested in results from Chapter 4) and had overall greater disease severity, while cv. Yudal (which had a resistant response), showed flecking in both glasshouse experiments. Interestingly, when cv. Yudal showed a susceptible response in a different glasshouse experiment (Chapter 4), flecking appeared against only

one out of 24 single-spore *P. brassicae* isolates. These results suggested that the same cultivar can show a necrotic response depending on the environmental conditions and pathogen populations or isolates, while it is considered as a phenotype of resistance, it can also be triggered within susceptible cultivars when there is great enough disease severity. Black flecking was also assessed on different areas of the leaf (petiole, lamina and midrib) in several experiments, but no patterns between flecking location and disease severity could be established. A study by Karandeni Dewage *et al.* (2022) that investigated resistance QTL within a different DH mapping population concluded that this phenotype may be attributed to a network of host responses, which could account for the differences in intensity and location of flecking observed in different lines. Qualitative assessment of necrotic flecking may be insufficient; development of a quantitative assessment in relation to flecking amount and location may provide more accurate information to understand this phenotype.

Conclusion

Light leaf spot remains the most economically damaging disease of oilseed rape in the UK and current control measures (including chemical resistance) are proving increasingly insufficient at managing the disease. Despite this, there is limited information available on host resistance against *P. brassicae* and limited information about pathogenic *P. brassicae* races. This project improved understanding of host resistance against *P. brassicae* within oilseed rape by confirming existing knowledge and presenting novel findings. Host, pathogen and environmental factors contributing to disease development were investigated; specific host-pathogen interactions, including cross-infectivity to non-oilseed rape hosts, were identified; quantitative trait loci (QTL) related to host genetic resistance, including environmentally stable resistance, were found. The hope is that this knowledge can be applied for light leaf spot disease management and improve resistance againt *P. brassicae* in oilseed rape.

References

Abbadi, A. and Leckband, G. (2011) Rapeseed breeding for oil content, quality, and sustainability, European Journal of Lipid Science and Technology. 113:1198-1206

Agriculture in the United Kingdom (2022) National Statistics. Department of environment, food and rural affairs. Available online at https://assets.publishing.service.gov.uk/media/6548e4bc59b9f5000d85a2cc/auk-2022-13jul23ii.pdf [Accessed 18/01/2024]

AHDB (2024a). Recommended List Archive | AHDB. https://ahdb.org.uk/knowledge-library/recommended-lists-archive [Accessed 24/01/2024]

AHDB (2024b) Oilseeds market outlook (February 2024). Available online at https://ahdb.org.uk/oilseeds-market-outlook [Accessed 16/04/2024]

AHDB (2024c) Historic light leaf spot forecasts (harvest years 2015–21). Available online at https://ahdb.org.uk/historic-light-leaf-spot-forecasts-harvest-years-2015-21 [Accessed 18/04/2024]

AHDB (2024d) The yield plateau of oilseed rape. Available online at https://ahdb.org.uk/knowledge-library/the-yield-plateau-in-oilseed-rape [Accessed 23/04/2024]

All, J. (2004) Cultural control of insect pests. In: Encyclopedia of Entomology. Springer, Dordrecht. 649. DOI: https://doi.org/10.1007/0-306-48380-7_1108

Ashby, A. M. (1997) A molecular view through the looking glass: The *Pyrenopeziza brassicae-Brassica* Interaction. Advances in Botanical Research. 24:31-70

Balesdent, M. H., Attard, A., Ansan-Melayah, D., Delourme, R., Renard, M., Rouxel, T. (2000) Genetic control and host range of avirulence toward *Brassica napus* cultivars Quinta and Jet Neuf in *Leptosphaeria maculans*. Phytopathology. 91:70-76

Ball, A. M., Ashby, A. M., Daniels, M. J., Ingram, D. S., Johnstone, K. (1991) Evidence for the requirement of extracellular protease in the pathogenic interaction of *Pyrenopeziza brassicae* with oilseed rape. Physiological and Molecular Plant Pathology. 38:147–161

Ballaré, C. L. (2014) Light regulation of plant defense. Annual Review of Plant Pathology. 65:335-363

Barna, B. and Györgyi, B. (1992) Resistance of young versus old tobacco leaves to necrotrophs, fusaric acid, cell wall-degrading enzymes and autolysis of membrane lipids. Physiological and Molecular Plant Pathology. 40:247-257

Barna, B. (2022) Manipulation of senescence of plants to improve biotic stress resistance. Life. 12:1496

Batish, S., Hunter, A., Ashby, A. M., & Johnstone, K. (2003) Purification and biochemical characterisation of Psp1, an extracellular protease produced by the oilseed rape pathogen *Pyrenopeziza brassicae*. Physiological and Molecular Plant Pathology. 62:13–20

Bayer Crop Science UK (2012). Bayer expert guide oilseed rape. Available online at https://cropscience.bayer.co.uk/mediafile/86018/bcs-expert-guide-osr-v3.pdf [Accessed 10/09/2020]

Booth, E. J., Bingham, I., Sutherland, K. G., Allcroft, D., Roberts, A., Elcock, S., Turner, J. (2005) Evaluation of factors affecting yield improvement in oilseed rape. HGCA Research Review No 53. HGCA, London, UK

Boys, E. F., Roques, S. E., Ashby, A. M., Evans, N., Latunde-Dada, A. O., Thomas, J. E., West, J. S., Fitt, B. D. L. (2007) Resistance to infection by stealth: *Brassica napus* (winter oilseed rape) and *Pyrenopeziza brassicae* (light leaf spot). European Journal of Plant Pathology. 118:307-321

Boys, E. F. (2009). Resistance to *Pyrenopeziza brassicae* (light leaf spot) in *Brassica napus* (oilseed rape). PhD Thesis, University of Nottingham, Nottingham, UK

Boys, E. F. Roques, S. E., West, J. S., Werner, C. P., King, G. J., Dyer, P. S., Fitt, B. D. L. (2012) Effects of *R* gene-mediated resistance in *Brassica napus* (oilseed rape) on asexual and sexual sporulation of *Pyrenopeziza brassicae* (light leaf spot). Plant Pathology. 61:543-554

Bradburne, R., Majer, D., Magrath, R., Werner, C. P., Lewis, B., Mithen, R. (1999) Winter oilseed rape with high levels of resistance to *Pyrenopeziza brassicae* derived from wild *Brassica* species. Plant Pathology. 48:550-558

Brent, K. J. and Hollomon, D. W. (2007a) Fungicide resistance: The assessment of risk. FRAC - CropLife International. 53

Brent, K. J. and Hollomon, D. W. (2007b) Fungicide resistance management in crop pathogens: how can it be managed? FRAC - CropLife International. 60

Brown, J. (2005). EDGAR: Experimental design generator and randomiser. John Innes Centre. Available at http://www.edgarweb.org.uk [accessed 13 November 2023]

Brun, H., Chèvre, A.-M., Fitt, B. D. L., Powers, S., Besnard, A.-L., Ermel, M., Huteau, V., Marquer, B., Eber, F., Renard, M., Andrivon, D. (2010) Quantitative resistance increases the durability of qualitative resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytologist. 185:285-299

Bucur, D. E., Huang, Y. J., Fitt, B. D. L., Kildea, S. (2024) Azole fungicide sensitivity and molecular mechanisms of reduced sensitivity in Irish *Pyrenopeziza brassicae* populations. Pest Management Science. 80:2393-2404. DOI: 10.1002/ps.7219

Calderon, C., Ward, E., Freeman, J., Foster, S. J., McCartney, H. A. (2002) Detection of airborne inoculum of *Leptosphaeria maculans* and *Pyrenopeziza brassicae* in oilseed rape crops by polymerase chain reaction (PCR) assays. Plant Pathology. 51:303-310

CanolaCouncil (2024). Canola Council of Canada. Available at https://www.canolacouncil.org/ [Accessed 27/06/2024]

Carmody, S. M., King, K. M., Ocamb, C. M., Fraaije, B. A., West, J. S., du Toit, L. J. (2020). A phylogenetically distinct lineage of *Pyrenopeziza brassicae* associated with chlorotic leaf spot of *Brassicaceae* in North America. Plant Pathology. 69:518-537

Carolan, K., Helps, J., van den Berg, F., Bain, R., Paveley, N., van den Bosch, F. (2017) Extending the durability of cultivar resistance by limiting epidemic growth rates. Proceedings of the Royal Society B. 284:20170828. http://doi.org/10.1098/rspb.2017.0828

Carré, P. and Pouzet, A. (2014) Rapeseed market, worldwide and in Europe. OCL. 21:D102

Carter, H. E., Cools, H. J., West, J. S., Shaw, M. W., Fraaije, B. A. (2013) Detection and molecular characterisation of *Pyrenopeziza brassicae* isolates resistant to methyl benzimidazole carbamates. Pest Management Science. 69:1040–1048

Carter, H. E., Fraaije, B. A., West, J. S., Kelly, S. L., Mehl, A., Shaw, M. W., Cools, H. J. (2014) Alterations in the predicted regulatory and coding regions of the sterol 14A-demethylase gene (*CYP51*) confer decreased azole sensitivity in the oilseed rape pathogen *Pyrenopeziza brassicae*. Molecular Plant pathology. 15:513-522

Cerón-Bustamante, M., Balducci, E., Beccari, G., Nicholson, P., Covarelli, L., Benincasa, P. (2023) Effect of light spectra on cereal fungal pathogens, a review. Fungal Biology Reviews. 43:100291

Chalhoub, B., Denoeud, F., Liu, S., Parkin, IAP, Tang, H., Wang, X. et al. (2014) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science. 345:950-953

Cheah, L. H., Hartill, W. F. T., Corbin, J. B. (1980) First report of the natural occurrence of *Pyrenopeziza brassicae* Sutton et Rawlinson, the apothecial state of *Cylindrosporium concentricum* Greville, in brassica crops in New Zealand. New Zealand Journal of Botany. 18:197–202

Cheah, L. H. and Hartill, W. F. T. (1985) Disease cycle in New Zealand of light leaf spot of brassicas, induced by *Pyrenopeziza brassicae*. New Zealand Journal of Agricultural Research. 28:567-573

Chen, R., Aono, M., Nakajima, N., Ohsawa, R., Yoshioka, T. (2020) Genetic diversity and population structure of feral rapeseed (*Brassica napus* L.) in Japan. PLoS One. 15:e0227990

Climate Data (2024). Climate Data in England. Available at: https://en.climate-data.org/europe/united-kingdom/england-256/ [Accessed 11/09/2024]

Collard B. C. Y., Jahufer M. Z. Z., Brouwer J. B. and Pang E. C. K. (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica. 142:169–196

Cooke, L. R., Carlisle, D. J., Donaghy, C., Quinn, M., Perez, F. M., Deahl, K. L. (2006) The Northern Ireland *Phytophthora infestans* population 1998-2002 characterized by genotypic and phenotypic markers. Plant Pathology. 55:320-330

Courtice, G. R. M., Ingram, D. S. (1987) Isolation of auxotrophic mutants of the hemibiotrophic ascomycete pathogen of brassicas, *Pyrenopeziza brassicae*. Transactions of the British Mycological Society. 89:301–306

CropMonitor (2018) Survey of commercially grown winter oilseed rape. Department for Environment, Food and Rural Affairs. Available at: https://www.cropmonitor.co.uk/

Davies, K. A. (1997) Early events in pathogenesis of *Pyrenopeziza brassicae* on Brassica napus. PhD Thesis, UK: University of Cambridge.

Davies, K. A., De Lorono, I, Foster, S. J., Li, D., Johnstone, K., Ashby, A. M. (2000) Evidence for a role of cutinase in pathogenicity of *Pyrenopeziza brassicae* on brassicas. Physiological and Molecular Plant Pathology. 57:63–75

De Mezenes, H., Massola Jr, N. S., Flint, S. D., Silva Jr, G. J., Bachmann, L., Rangel, D. E. N., Braga, G. U. L. (2015) Growth under visible light increases conidia and mucilage production and tolerance to UV-B radiation in the plant pathogenic fungus *Colletotrichum acutatum*. Photochemistry and Photobiology. 91:397–402

DEFRA (2024) Agriculture in the United Kingdom data sets. Available online at https://www.gov.uk/government/statistical-data-sets/agriculture-in-the-united-kingdom [Accessed 16/04/2024]

Delourme, R., Chèvre, A.-M., Brun, H., Rouxel, T., Balesdent, M. H., Dias, J. S., Salisbury, P. (2006) Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). European Journal of Plant Pathology 114: 41–52

Diers, B. W. and Osborn, T. C. (1994) Genetic diversity of oilseed *Brassica napus* germplasm based on restriction fragment length polymorphisms. Theoretical and Applied Genetics. 88:662-668

Dong, O. X. and Ronald, P. C. (2019). Genetic engineering for disease resistance in plants: recent progress and future perspectives. Plant Physiology. 180:26-38

Doughari, J. H. (2015) An overview of plant immunity. Plant Pathology & Microbiology. 6:322. http://dx.doi.org/10.4172/2157-7471.1000322

EC (2020) EU pesticides database – European Commission. Available online at https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN [Accessed 24/08/2021]

Elling, B. Neuffer, B. Bleeker, W. (2009) Sources of genetic diversity in feral oilseed rape (*Brassica napus*) populations. Basic and Applied Ecology. 10:544-553

Evans, N., Fitt, B. D. L., McCartney, H. A., Welham, S. J., McRoberts, N. (1999) Sampling winter oilseed rape crops to assess incidence of light leaf spot (*Pyrenopeziza brassicae*). Protection and Production of Combinable Break Crops. Aspects of Applied Biology. 56:123-128

Evans, N., Baierl, A., Brain, P., Welham, S. J., Fitt, B. D. L. (2003) Spatial aspects of light leaf spot (*Pyrenopeziza brassicae*) epidemic development on oilseed rape (*Brassica napus*) in the United Kingdom. Phytopathology. 93:657-665

Evans, N., Ritchie, F., West, J., Havis, N., Matthewman, C., Maguire, K. (2017) Investigating components of the oilseed rape light leaf spot epidemic

responsible for increased yield loss to the UK Arable Industry. AHDB. Report number. PR587. 33

FAO (2024) FAOSTAT statistical database. Food and Agriculture Organization of the United Nations (FAO). Available online at http://www.fao.org/faostat/en/compare [Accessed 16/04/2024]

Farber, D. H. and Mundt, C. C (2017) Effect of plant age and leaf position on susceptibility to wheat stripe rust. Phytopathology. 107:412-417

Fell, H., Ali, A. M., Wells, R., Mitrousia, G. K., Woolfenden, H., Schoonbeek, H. J., Fitt, B. D. L., Ridout, C. J., Stotz, H. (2023) Novel gene loci associated with susceptibility or cryptic quantitative resistance to *Pyrenopeziza brassicae* in *Brassica napus*. Theoretical and Applied Genetics. 136:71

FERA (2022) Arable Crops in the United Kingdom 2022. Available online at file:///C:/Users/laura/Downloads/Arable%20crops%20in%20the%20UK.pdf [Accessed 19/04/2024]

Figueroa L., Shaw, M. W., Fitt, B. D. L., McCartney, H. A., Welham, S. J. (1994) Effects of previous cropping and fungicide timing on the development of light leaf spot (*Pyrenopeziza brassicae*), seed yield and quality of winter oilseed rape (*Brassica napus*). Annals of Applied Biology. 124:221–239

Figueroa, L., Fitt, B. D. L., Welham, S. J., Shaw, M. W., McCartney, H. A. (1995) Early development of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in relation to temperature and leaf wetness. Plant Pathology. 44:641-654

Fitt, B. D. L., Doughty, K. J., Gladders, P., Steed, J. M., Sutherland, K. G. (1998a) Diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. Annals of Applied Biology. 133:155-166

Fitt, B. D. L., Doughty, K. J., Gilles, T., Gladders, P., Steed, J. M, Su, H., Sutherland, K. G. (1998b) Methods for assessment of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. Annals of Applied Biology. 133:329-341

Flaherty, J. E. and Dunkle, L. D. (2005) Identification and expression analysis of regulatory genes induced during conidiation in *Exserohilum turcicum*. Fungal Genetics and Biology. 42:471-481

Foisset, N., Delourme, R., Barret, P., Hubert, N. (1996) Molecular-mapping analysis in *Brassica napus* using isozyme, RAPD and RFLP markers on a doubled-haploid progeny. Theoretical and Applied Genetics. 93:1017-1025

Fortune, J. A., Gi, A., Ritchie, F., Karandeni Dewage, C. S., Fitt, B. D. L., Huang, Y. J. (2021) Effects of cultivar resistance and fungicide application on stem

canker of oilseed rape (*Brassica napus*) and potential interseasonal transmission of *Leptosphaeria* spp. Inoculum. Plant Pathology. 70:2115-2124

Fortune, J. A. (2022) Understanding the interactions between phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) and light leaf spot (*Pyrenopeziza brassicae*) pathogens of oilseed rape (*Brassica napus*). PhD thesis, University of Hertfordshire, Hatfield, UK

Foster, S. J., Ashby, A. M., Fitt, B. D. L. (2002) Improved PCR-based assays for pre-symptomatic diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape. European Journal of Plant Pathology. 108: 379-383

FRAG-UK (2017) Fungicide Resistance management in oilseed rape. Available online at https://ahdb.org.uk/frag [Accessed 22/09/2020]

French, E., Kim, B. S. and Iyer-Pascuzzi, A. S. (2016) Mechanisms of quantitative disease resistance in plants. Seminars in Cell & Developmental Biology. 56:201-208

Gilles, T., Evans, N., Fitt, B. D. L., Jeger, M. J. (2000a). Epidemiology in relation to methods for forecasting light leaf spot (*Pyrenopeziza brassicae*) severity on winter oilseed rape (*Brassica napus*) in the UK. European Journal of Plant Pathology. 106:593-605

Gilles, T., Fitt, B. D. L., Kennedy, R., Welham, S. J., Jeger M. J. (2000b) Effects of temperature and wetness duration on conidial infection, latent period and asexual sporulation of *Pyrenopeziza brassicae* on leaves of oilseed rape. Plant Pathology. 49:498-508

Gilles, T., Ashby, A. M., Fitt, B. D. L, Cole, T. (2001a) Development of *Pyrenopeziza brassicae* apothecia on agar and oilseed rape debris. Mycological Research. 105:705-714

Gilles, T., Fitt, B. D. L., McCartney, H. A., Papastamati, K., Steed, J. M (2001b). The roles of ascospores and conidia of *Pyrenopeziza brassicae* in light leaf spot epidemics on winter oilseed rape (*Brassica napus*) in the UK. Annals of Applied Biology. 138:141-152

Gilles, T., Fitt, B. D. L., Jeger, M. J. (2001c). Effects of environmental factors on development of *Pyrenopeziza brassicae* (light leaf spot) apothecia on oilseed rape debris. Phytopathology. 91:392-398

Gilles, T., Fitt, B. D. L., Welham, S. J., Evans, N., Steed, J. M., Jeger, M. J. (2001d). Modelling the effects of temperature and wetness duration on development of light leaf spot on oilseed rape leaves inoculated with *Pyrenopeziza brassicae* conidia. Plant Pathology. 50:42-52

Gongora-Canul, C. C. and Leandro, L. F. S. (2011) Plant age affects root infection and development of foliar symptoms of soybean sudden death syndrome. Plant Disease. 95:242-247

Goodwin, S. B. (2002) The barley scald pathogen *Rhynchosporium secalis* is closely related to the discomycetes *Tapesia* and *Pyrenopeziza*. Mycological Research. 106:645–654

Graham, G. C., Mayers, P., Henry, R. J. (1994). A simplified method for the preparation of fungal genomic DNA for PCR and RAPD analysis. Biotechniques. 16: 48–50

Greville, R. K. (1823) *Cylindrosporium concentricum*. Scottish Cryptogamic Flora 1, No. 27

Gururani, M. A., Venkatesh, J., Upadhyaya, C. P., Nookaraju, A., Pandey, S. K., Park, S. W. (2012) Plant disease resistance genes: Current status and future directions. Physiological and Molecular Plant Pathology. 78:51-65

Haddadi, P., Larkan, N. J., Borhan, M. H. (2019) Dissecting *R* gene and host genetic background effect on the *Brassica napus* defense response to *Leptosphaeria maculans*. Scientific Reports. 9:6947

Hartill, W. F. T. and Cheah, L. H. (1984) Some effects of climate and plant growth on the development of light leaf spot in cauliflowers. New Zealand Journal of Agricultural Research. 27:441-9

Hickman, C. J., Schofield, E. R., Taylor, R. E. (1955) Light leaf spot of *Brassicae*. Plant Pathology. 4:129–131

Hillocks, R.J. (2012) Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. Crop Protection. 31:85–93

HSE (2018) Refinzar. Available online at https://secure.pesticides.gov.uk/pestreg/getfullproduct.asp?productid=30067&pageno=1&origin=prodsearch [Accessed 24/08/2021]

Huang, Y.J., Evans, N., Li, Z.-Q., Eckert, M., Chèvre, A.-M., Renard, M., Fitt, B. D. L. (2006) Temperature and leaf wetness duration affect phenotypic expression of *Rlm6*-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytologist. 170:129-141

Huang, Y.J., Pirie, E. J., Evans, N., Delourme, R., King, G. J., Fitt, B. D. L. (2009) Quantitative resistance to symptomless growth of *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape). Plant Pathology. 58:314-323

Huang, Y. J., Hood, J. R., Eckert, M. R., Stonard, J. F., Cools, H. J., King, et al. (2011). Effects of fungicide on growth of *Leptosphaeria maculans* and *L. biglobosa* in relation to development of phoma stem canker on oilseed rape (*Brassica napus*). Plant Pathology. 60:607-620

Huang, Y. J., Jestin, C., Welham, S. J., King, G. J., Manzanares-Dauleux, M. J., Fitt, B. D. L., Delourme, R. (2016) Identification of environmentally stable QTL for resistance against *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). Theoretical and Applied Genetics. 129:169-180

Huang, Y. J., Mitrousia, G. K., Sidique, S. N. M., Qi, A., Fitt, B. D. L. (2018) Combining *R* gene and quantitative resistance increases effectiveness of cultivar resistance against *Leptosphaeria maculans* in *Brassica napus* in different environments. PLoS One. 13: e0197752

Huang, Y. J., Paillard, S., Kumar, V., King, G. J., Fitt, B. D. L., Delourme, R. (2019) Oilseed rape (*Brassica napus*) resistance to growth of *Leptosphaeria maculans* in leaves of young plants contributes to quantitative resistance in stems of adult plants. PLoS One. 14: e0222540

Huang, Y. J., Karandeni Dewage, C. S., Fitt, B. D. L. (2021) Current understanding of phoma stem canker and light leaf spot on oilseed rape in the UK. Management of Diseases and Pests of Oilseed Rape: Papers from a forum held at the University of Hertfordshire. Jellis, G. J. & Fitt, B. D. L. (eds.). United Kingdom: University of Hertfordshire Press. 1-12

Ilott, T. W., Ingram, D. S., Rawlinson, C. J. (1984) Heterothallism in *Pyrenopeziza brassicae*, cause of light leaf spot of brassicas. Transactions of the British Mycological Society. 82:477–483

Inman, A. J., Fitt, B. D. L., Evans, R. L. (1992) A species of *Unguicularia* on oilseed rape, and its importance in studies of the epidemiology of light leaf spot (*Pyrenopeziza brassicae*). Plant Pathology. 41:646-652

Jacott, C. N., Schoonbeek, H. J., Sidhu, G. S., Steuernagel, B., Kirby, R., Zheng X. *et al.* (2024) Pathogen lifestyle determines host genetic signature of quantitative disease resistance loci in oilseed rape (*Brassica napus*). Theoretical and Applied Genetics. 137:65

Jankovska-Bortkevič, E., Jurkonienė, S., Gavelienė, V., Prakas, P. (2022) Oilseed rape: biology, use, current cultivation and agronomic management. Oilseed Crops - Uses, Biology and Production. IntechOpen. DOI:10.5772/intechopen.109180

Jursík, M., Kolářová, M., Soukup. J. (2023) Control of conventional and Clearfield® volunteer oilseed rape in spring barley. Crop Protection. 163:106110

Kaczmarek, J., Jêdryczka, M., Fitt, B. D. L., Lucas, J. A. Latunde-Dada, A. O. (2009) Analyses of air samples for ascospores of *Leptosphaeria maculans* and *L.biglobosa* by light microscopy and molecular techniques. Journal of Applied Genetics. 50:411-419

Kaczmarek, J., West, J. S., King, K. M., Canning, G. G. M., Latunde-Dada, A. O., Huang, Y. J., Fitt, B. D. L., Jedryczka, M. (2024) Efficient qPCR estimation and discrimination of airborne inoculum of *Leptosphaeria maculans* and *L. biglobosa*, the causal organisms of phoma leaf spotting and stem canker of oilseed rape. Pest Management Science. 80:2453-2460. DOI: 10.1002/ps.7800

Karandeni Dewage, C. S., Klöppel, C. A, Stotz, H. U., Fitt, B. D. L. (2018) Host-pathogen interactions in relation to management of light leaf spot disease (caused by *Pyrenopeziza brassicae*) on *Brassica* species. Crop and Pasture Science. 69:9-19

Karandeni Dewage, C.S. (2019) Understanding oilseed rape (*Brassica napus*) resistance against *Pyrenopeziza brassicae* for control of light leaf spot. PhD thesis, University of Hertfordshire, Hatfield

Karandeni Dewage, C. S., Qi, A., Stotz, H. U., Huang, Y.J., Fittt, B. D. L. (2021) Interactions in the *Brassica napus–Pyrenopeziza brassicae* pathosystem and sources of resistance to *P. brassicae* (light leaf spot). Plant Pathology. 70:2104-21114. DOI: https://doi.org/10.1111/ppa.13455

Karandeni Dewage, C. S., Cools, K., Stotz, H. U., Qi, A., Huang, Y. J., Wells, R., Fitt, B. D. L. (2022) Quantitative trait locus mapping for resistance against *Pyrenopeziza brassicae* derived from a *Brassica napus* secondary gene pool. Frontiers in Plant Science. 13. DOI: https://doi.org/10.3389/fpls.2022.786189

Karolewski, Z. (1999) The occurrence of light leaf spot on winter oilseed rape in Western Poland in 1991–1996 and the characteristics of *Pyrenopeziza brassicae* isolates. Phytopatologia Polonica. 18:113–121

Karolewski, Z., Fitt, B. D. L., Latunde-Dada, A.O., Foster, S. J., Todd, A. D., Downes, K., Evans, N. (2006) Visual and PCR assessment of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) cultivars. Plant Pathology 55: 387-400

Karolewski, Z. (2010) Development of light leaf spot (*Pyrenopeziza brassicae*) on brassicas. Phytopathologia. 55:13–20

Karolewski, Z., Kaczmarek, J., Jedryczka, M., Cools, H. J., Fraaije, B. A., Lucas, J. A., Latunde-Dada, A. O. (2012) Detection and quantification of airborne inoculum of *Pyrenopeziza brassicae* in Polish and UK winter oilseed rape crops by real-time PCR assays. Grana. 51:270-279

Karpinski, S., Gabrys, H., Mateo, A., Karpinska, B., Mullineaux, P. M. (2003). Light perception in plant disease defence signalling. Current Opinion in Plant Biology. 6:390-396

Keane, P. J. and Kerr, A. (1997) Factors affecting disease development. In Plant Pathogens and Plant Diseases. Edted by J. F. Brown and H. J. Ogle. Australia: Rockwale Publications. 287-298

Kimber, D. and Mcgregor, D.I. (1995) *Brassica* oilseeds: Production and utilization. Oxon: CAB international. 416

King, K. M., West, J. S., Brunner, P. C., Dyer, P. S., Fitt, B. D. L. (2013) Evolutionary relationships between *Rhynchosporium lolii* sp. nov. and other *Rhynchosporium* species on grasses. PLoS One. 8:e72536

King, K. M., West, J. S., Fitt, B. D. L., Dyer, P. S. (2015) Differences in *MAT* gene distribution and expression between *Rhynchosporium* species on grasses. Plant Pathology. 64:344–354

King, K. M., Bucur, D. E., Ritchie, F., Hawkins, N. J., Kaczmarek, A. M., Duan, Y., Kildea, S., West, J. S., Fraaije, B. A (2021) Fungicide resistance status and chemical control options for the *brassica* pathogen *Pyrenopeziza brassicae*. Plant Pathology. 70:2086-2103

Kistner, M. B., Romero, A. M., Iglesias, J. (2022) Unravelling the complexity of maize resistance to bacterial and fungal diseases: an integrative perspective. Tropical Plant Pathology. 47:332–352

Klöppel, C. (2015) Improved management of light leaf spot in brassicas by exploiting resistance and understanding pathogen variation. Annual report. AHDB Project number FV422

Koch, K., Hartmann, K. D., Schreiber, L., Barthlott, W., Neinhuis, N. (2005) Influences of air humidity during the cultivation of plants on wax chemical composition, morphology and leaf surface wettability. Environmental and Experimental Botany. 56:1–9

Koch, K. and Ensikat, H. J. (2008) The hydrophobic coatings of plant surfaces: Epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. Micron. 39:759-772

Kogan, M. (1998) Integrated pest management: historical perspectives and contemporary developments. Annual Review of Entomology. 43:243-270

Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D., Turkington, T. K. (2002) Managing plant disease risk in diversified cropping systems. Agronomy Journal. 94:198-209

Kumar N. S., Gurusubramanian, G. (2011) Random amplified polymorphic DNA (RAPD) markers and its applications. Science Vision. 11:116-124

Kumar, V., Paillard, S., Fopa-Fomeju, B., Falentin, C., Deniot, G., Baron, C., Vallée, P., Manzanares-Dauleux, M. J., Delourme, R. (2018) Multi-year linkage and association mapping confirm the high number of genomic regions involved in oilseed rape quantitative resistance to blackleg. Theoretical and Applied Genetics. 131:1627-1643. DOI: https://doi.org/10.1007/s00122-018-3103-9

Kwok, I. M. Y. and Loeffler, R. T. (1993) The biochemical mode of action of some newer azole fungicides. Pest Management Science, 39:1-11

Lacey, M. E. and West, J. S. (2006) The Air Spora. Netherlands: Springer. 172

Larkan, N. J., Lydiate, D. J., Parkin, I. A. P., Nelson, M. N., Epp. D. J., Cowling, W. A., Rimmer, S. R. (2013) The *Brassica napus* blackleg resistance gene *LepR3* encodes a receptor-like protein triggered by the *Leptosphaeria maculans* effector *AVRLM1*. New Phytologist. 197:595-605

Larkan, N. J., Ma, L., Borhan, M. H. (2015) The *Brassica napus* receptor-like protein RLM2 is encoded by a second allele of the *LepR3/Rlm2* blackleg resistance locus. Plant Biotechnology Journal. 13:983-992

Larkan, N. J., Ma, L., Habbadi, P., Buchwaldt, M., Parkin, I. A. P., Djavaheri, M., Borhan, M. H. (2020) The *Brassica napus* wall-associated kinase-like (WAKL) gene *Rlm9* provides race-specific blackleg resistance. The Plant Journal. 104:892-900

Lauter, F. R. and Russo, V. E. A. (1991) Blue light induction of conidiation-specific genes in *Neurospora crassa*. Nucleic Acids Research. 19:6883-6886

Leadbeater, A. (2015) Recent developments and challenges in chemical disease control – a review. Plant Protection Science. 51:163-169

Lee, K., Singh, P., Chung, W. C., Ash, J., Kim., T. S., Hang, L., Park., S. (2006) Light regulation of asexual development in the rice blast fungus, *Magnaporthe oryzae*. Fungal Genetics and Biology. 43:694–706

Li, D., Ashby, A. M., Johnstone, K. (2003) Molecular evidence that the extracellular cutinase Pbc1 is required for pathogenicity of *Pyrenopeziza brassicae* on oilseed rape. Molecular Plant-Microbe Interactions. 16:545–552

Liu, S., Liu, Y., Yang, X., Tong, C., Edwards, D., Parkin, I. A. et al. (2014). The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genomes. Nature Communications. 5:3930

Lob, S., Jaspers, M. V., Ridgway, H. J., Jones. E. E. (2013). *Leptosphaeria maculans/L. biglobosa* disease progression in oilseed rape and timing of

ascospore release under New Zealand conditions. New Zealand Plant Protection. 66: 214-222

Lu, K., Wei, L., Li, X., Wang, Y., Wu, J. et al. (2019) Whole-genome resequencing reveals *Brassica napus* origin and genetic loci involved in its improvement. Nature Communications. 10:1154

Maddock, S. E., Ingram, D. S., Gilligan, C. A. (1981) Resistance of cultivated brassicas to *Pyrenopeziza brassicae*. Transactions of the British Mycological Society. 76:371–382

Mahfooz., S., Singh, S. P., Mishra, N., Mishra, A. (2017) A comparison of microsatellites in phytopathogenic *Aspergillus* species in order to develop markers for the assessment of genetic diversity among its isolates. Frontiers in Microbiology. 8. DOI: https://doi.org/10.3389/fmicb.2017.01774

Majer, D., Lewis, B. G., Mithen, R. (1998). Genetic variation among field isolates of *Pyrenopeziza brassicae*. Plant Pathology. 47:22-28

Marcroft, S. J., van de Wouw, A. P., Salisbury, P. A., Potter, T. D., Howlett, B. J. (2012) Effect of rotation of canola (*Brassica napus*) cultivars with different complements of blackleg resistance genes on disease severity. Plant Pathology. 61:934-944

McCartney, H. A. and Lacey, M. E. (1990). The production and release of ascospores of *Pyrenopeziza brassicae* on oilseed rape. Plant Pathology. 39:17–32

McDonald, B. A., McDermott, J. M (1993) Population genetics of plant pathogenic fungi. BioScience. 43:311-319

McDonald, B. A. and Linde, C. (2002) Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology. 40:349–379

McDonald, M., McDonald, B. A., Solomon, P. S. (2015) Recent advances in the *Zymoseptoria trinici*-wheat interaction: insights from pathogenomics. Frontiers in Plant Science. 6:102

McDonald, B. A., Suffert, F., Bernasconi, A., Mikaberidze, A. (2022) How large and diverse are field populations of fungal plant pathogens? The case of *Zymoseptoria tritici*. Evolutionary Applications. 15:1360-1373

Meng, L., Li, H., Zhang, L., Wang, J. (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in bi-parental populations. The Crop Journal. 3:269-283

Mitrousia, G. K., Huang, Y. J., Qi, A., Sidique, S. N. M., Fitt, B. D. L. (2018) Effectiveness of *Rlm7* resistance against *Leptosphaeria maculans* (phoma stem canker) in UK winter oilseed rape cultivars. Plant Pathology. 67:1339-1353

Murphy, A. M., Pryce-Jones, E., Johnstone, K., Ashby, A. M. (1997) Comparison of cytokinin production *in vitro* by *Pyrenopeziza brassicae* with other plant pathogens. Physiological and Molecular Plant Pathology. 50:53–65

Nagaharu, U. (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. Napus* and peculiar mode of fertilization. Japanese Journal of Botany. 7:389-452

Nath, R. K., Begum, K. H., Choudhury, M. R. (2020) Plant disease forecasting models. Indian Journal of Pure & Applied Biosciences. 8:454-461

Newlands, N. K. (2018) Model-based forecasting of agricultural crop disease risk at the regional scale, integrating airborne inoculum, environmental, and satellite-based monitoring data. Frontiers in Environmental Science. 6:63

Noel. K., Wolf, I. R., Hughes, D., Valente, G. T., Qi, A., Huang, Y. J., Fitt, B. D. L., Stotz, H. U. (2024) Transcriptomics of temperature-sensitive *R* gene-mediated resistance identifies a *WAKL10* protein interaction network. Scientific Reports. 14:5023

Obermeier, C., Mason, A. S., Meiners, T., Petschenka, G., Rostas, M., Will, T., Wittkop, B., Austel, N. (2022) Perspectives for integrated insect pest protection in oilseed rape breeding. Theoretical and Applied Genetics. 135:3917-3946

Oliver, R. P. and Ipcho, S. V. S. (2004) *Arabidopsis* pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. Molecular Plant Pathology. 5:347-352

Orlovius, K. and Kirkby, E. A. (2003) Oilseed rape: Fertilizing for high yield and quality. International Potash Institute. Basel, Switzerland. 125

Papastamati, K., van den Bosch, F., Welham, S. J., Fitt, B. D. L., Evans, N. Steed, J. M. (2002) Modelling the daily progress of light leaf spot epidemics on winter oilseed rape (*Brassica napus*), in relation to *Pyrenopeziza brassicae* inoculum concentrations and weather factors. Ecological Modelling. 148:169-189

Parcell, J., Kojima, Y., Roach, A., Cain, W. (2018) Global edible vegetable oil market trends. Biomedical Journal of Scientific and Technical Research. 2:2282-2291

Paritosh, K., Pradhan, A.K., Pental, D. A. (2020) highly contiguous genome assembly of *Brassica nigra* (BB) and revised nomenclature for the pseudochromosomes. BMC Genomics 21:887

Payne, R. W., Lane, P. W., Baird, D. B., Gilmour, A. R., Harding, S. A., Morgan, G. W., Murray, D. A., Thompson, R., Todd, A. D., Tunnicliffe Wilson, G., Webster, R., Welham, S. J. (1993) Genstat 5, Release 3, Reference Manual. Oxford, UK: Clarendon Press. 812

Payne, R. W., Harding, S. A., Murray, D. A., Soutar, D. M., Baird, D. B., Glaser, A. I. *et al*. (2011) The guide to Genstat release 14, part 2: statistics. Hemel Hempstead: VSN International

Penselin, D., Münsterkötter, M., Kirsten, S., Felder, M., Taudien, S., Platzer, M., et al. (2016) Comparative genomics to explore phylogenetic relationship, cryptic sexual potential and host specificity of *Rhynchosporium* species on grasses. BMC Genomics. 17:953

Peries, O. S. (1971) Environmental factors affecting plant diseases. Environment and Agriculture. 42:188-195

Pilet, M. L., Delourme, R., Foisset, N., Renard, M. (1998a) Identification of loci contributing to quantitative field resistance to blackleg disease, causal agent *Leptosphaeria maculans* (Desm.) Ces. et de Not., in winter rapeseed (*Brassica napus* L.). Theoretical and Applied Genetics. 96:23-30

Pilet, M. L., Delourme, R., Foisset, N., Renard, M. (1998b) Identification of QTL involved in field resistance to light leaf spot (*Pyrenopeziza brassicae*) and blackleg resistance (*Leptosphaeria maculans*) in winter rapeseed (*Brassica napus* L.). Theoretical and Applied Genetics. 97: 398-406

Pilet-Nayel, M. L., Moury, B., Caffier, V., Montarry, J., Kerlan, M. C., Fournet, S., Durel, C. E., Delourme, R. (2017) Quantitative resistance to plant pathogens in pyramiding strategies for durable crop protection. Frontiers in Plant Science. 8:1838. DOI: https://doi.org/10.3389/fpls.2017.01838

Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C., Nelson, R. J. (2008) Shades of gray: the world of quantitative disease resistance. Trends in Plant Science. 14:21–29

Price, C. L., Parker, J. E., Warrilow, A. G. S., Kelly, D. E., Kelly, S. L. (2015) Azole fungicides - understanding resistance mechanisms in agricultural fungal pathogens. Pest Management Science 71:1054–1058

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at https://www.R-project.org/

Rajarammohan, S. (2021) Redefining plant-necrotroph interactions: the hin Lline between hemibiotrophs and necrotrophs. Frontiers in Microbiology. 12: 673518

Raman, H., Raman, R., Diffey, S., Qiu, Y., McVittie, B., Barbulescu, D. M. *et al.* (2018) Stable wuantitative resistance loci to blackleg disease in canola (*Brassica napus* L.) over continents. Frontiers in Plant Science. 9. DOI: https://doi.org/10.3389/fpls.2018.01622

Rapeseed Digest (1974). Rapeseed Digest. Rapeseed Association of Canada. 8:3

Rawlinson, C. J., Sutton, B. C., Muthyalu, G. (1978) Taxonomy and biology of *Pyrenopeziza brassicae* sp. nov. (*Cylindrosporium concentricum*), a pathogen of winter oilseed rape (*Brassica napus* ssp. *oleifera*). Transactions of the British Mycological Society. 71:425–439

Razdan, V. and Sabitha, M. (2009) Integrated Pest Management: Innovation-Development Process. Springer. 704

Roden, L. C. and Ingle R. A. (2009) Lights, rhythms, infection: the role of light and the circadian clock in determining the outcome of plant–pathogen interactions. The Plant Cell. 21:2546-2552

Rouxel, T., Penaud, A., Picochet, X., Brun, H., Gout, L., Delourme, R., Schmit J., Balesdent, M.-H. (2003) A 10-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape. European Journal of Plant Pathology. 109:871-881

SAC (2009) Winter oilseed rape pests and diseases. Available online at www.sruc.a.uk [accessed 22/09/2022]

Schepers, H. T. A. M (1984) Persistence of resistance to fungicides in *Sphaerotheca fuliginea.* Netherlands Journal of Plant Pathology. 90:165–171

Seimandi-Corda, G., Winkler, J., Jenkins, T., Kirschner, S.M., Cook, S. M. (2024) Companion plants and straw mulch reduce cabbage stem flea beetle (*Psylliodes chrysocephala*) damage on oilseed rape. Pest Management Science. 80:2333-2341

Sharabani, G., Shtienberg, D., Borenstein, M., Shulhani, R., Lofthouse, M., Sofer, M., Chalupowicz, L., Barel, V., Manulis-Sasson, S. (2012) Effects of plant age on disease development and virulence of *Clavibacter michiganensis* subsp. *michiganensis* on tomato. Plant Pathology. 62:1114-1122

Simons, A. J. and Skidmore, D. I. (1998) Race-specific resistance to light leaf spot in *Brassica oleracea*. Transactions of the British Mycological Society. 90:431-435

Singh, G. and Ashby, A. M. (1998) Cloning of the mating type loci from *Pyrenopeziza brassicae* reveals the presence of a novel mating type gene within a discomycete *MAT 1-2* locus encoding a putative metallothionein-like protein. Molecular Microbiology. 30:799-806

Singh G and Ashby A. M. (1999) Erratum to Singh and Ashby (1998) Cloning of the mating type loci from *Pyrenopeziza brassicae* reveals the presence of a novel mating type gene within a discomycete *MAT 1-2* locus encoding a putative metallothionein-like protein. Molecular Microbiology. 32:1115

Smooker, A. M., Wells, R., Morgan, C., Beaudoin, F., Cho, K., Fraser, F., & Bancroft, I. (2011) The identification and mapping of candidate genes and QTL involved in the fatty acid desaturation pathway in *Brassica napus*. Theoretical and Applied Genetics. 122:1075-1090

Snowdon, R. J. and Iniguez Luy, F. L. (2012) Potential to improve oilseed rape and canola breeding in the genomics era. Plant Breeding. 131:351-360

Sprague, S. J., Balesdent, M.-H., Brun, H., Hayden, H. L., Marcroft, S. J., Pinochet, X., Rouxel, T., Howlett, B. J. (2006) Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. European Journal of Plant Pathology. 114:33-40

Stachowiak, A., Olechnowicz, J., Jedryczka, M., Rouxel, T., Balesdent, M.-H., Happstadius, I., Gladders, P., Latunde-Dada, A., Evans, N. (2006) Frequency of avirulence alleles in field populations of *Leptosphaeria maculans* in Europe. European Journal of Plant Pathology.114:67–75

Starosta, E., Szwarc, J., Niemann, J., Szewczyk, K., Weigt, D. (2023) *Brassica napus* and double haploid production and its latest applications. Current Issues in Molecular Biology. 45:4431-4450

Staub, T. (1991) Fungicide resistance: practical experience with antiresistance strategies and the role of integrated use. Annual Review of Phytopathology. 29:421-442

Staunton, W., Kavanagh, T. (1966) Natural occurrence of the perfect stage of *Gloeosporium concentricum* (Grev.) Berk. and Br. Irish Journal of Agricultural Research. 5:140–141

Staunton, W. P. (1967) Studies on light leaf spot (*Gloeosporium concentricum*) of *Brassica*. Irish Journal of Agricultural Research. 6:203–211

Stotz, H. U., Mitrousia, G. K., de Wit, P. J. G. M., Fitt, B. D. L. (2014) Effector-triggered defence against apoplastic fungal pathogens. Trends in Plant Science. 19:491-500

Stuthman, D. D., Leonard, K. J., Miller-Garvin, J. (2007) Breeding crops for durable resistance to disease. Advances in Agronomy. 95:319-367

Sutton, B. C. and Gibson, I. A. S. (1977) *Cylindrosporium concentricum*. CMI Descriptions of Pathogenic Fungi and Bacteria No. 536. Commonwealth Mycological Institute, Wallingford.

Sylvester-Bradley, R. (1985) A revised code for stages of development in oilseed rape (*Brassica napus* L.). Field trials methods and data handling. Aspects of Applied Biology, 10:395–400

Thakur, R. P. (2007) Host plant resistance to diseases: potential and limitations. Indian Journal of Plant Protection. 35:17-21

The *Brassica rapa* Genome Sequencing Project Consortium, Wang, X., Wang, H., Wang, J., Sun, R., Wu, J., Liu, S. *et al.* (2011) The genome of the mesopolyploid crop species *Brassica rapa*. Nature Genetics. 43:1035-1039

Thomas, W. J., Serdani, M., Claassen, B., Schneider, M., Hinds-Cook, A. M., Mallory-Smith, C., Ocamb, C. M. (2019) A real-time PCR assay for detection of light leaf spot on *Brassica* crops in the Pacific Northwest of the USA. Canadian Journal of Plant Pathology. 41:566-575

Torriani, S. F. F., Brunner, P. C., McDonald, B. A., Sierotzki, H. (2008). QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola*. Pest Management Science. 65:155-162

Travadon, R. Marquer, B., Ribulé, A., Sache, I., Masson, J. P., Brun, H., Delourme, R., Bousset, L. (2009) Systemic growth of *Leptosphaeria maculans* from cotyledons to hypocotyls in oilseed rape: influence of number of infection sites, competitive growth and host polygenic resistance. Plant Pathology. 58:461-469

USDA (2022) United Kingdom: Oilseeds and Products Annual. Available online at https://fas.usda.gov/data/united-kingdom-oilseeds-and-products-annual-0 [Accessed 16/04/2024]

Van de Wouw, A. P., Stonard, J. F., Howlett, B. J., West, J. S., Fitt, B. D. L. Atkins, S. D. (2010) Determining frequencies of avirulent alleles in airborne *Leptosphaeria maculans* inoculum using quantitative PCR. Plant Pathology. 59:809-818

Van der Plank, J. E. (1963). Plant Diseases: Epidemics and Control. New York, USA: Academic Press. 349

van der Spiegel, M., Noordam, M. Y., van der Fels-Klerx, H. J. (2013) Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Comprehensive Reviews in Food Science and Food Safety. 12:662-678

Velásquez, A. C., Castroverde, C. D. M., He, S. Y. (2018) Plant-pathogen warfare under changing climate conditions. Current Biology. 28:619-634

Veys, C., Chatziavgerinos. F., Al Suwaidi, A., Hibbert, J., Hansen, M., Bernotas, G., Smith, M., Yin, H., Rolfe, S., Grieve, B. (2019) Multispectral imaging for presymptomatic analysis of light leaf spot in oilseed rape. Plant Methods. 15:4

Voorrips, R. E. (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. Journal of Heredity. 93:77–78

Walker, P., Ritchie, F., Burnett., F., Knight, S., Gosling, P. (2022) Fungicides for light leaf spot control in winter oilseed rape. Annual Report. AHDB. 21120013

Walters, D. R., McRoberts, N., Fitt, B. D. L. (2008) Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. Biological Reviews. 83:79-102

Wang, Y., Bao, Z., Zhu, Y. Hua, J. (2009) Analysis of temperature modulation on plant defense against biotrophic microbes. Molecular Plant-Microbe Interactions. 22:498-506

Welham, S. J., Turner, J. A., Gladders, P., Fitt, B. D. L., Evans, N., Baierl, A. (2004) Predicting light leaf spot (*Pyrenopeziza brassicae*) risk on winter oilseed rape (*Brassica napus*) in England and Wales, using survey, weather and crop information. Plant Pathology. 53:713-724

West, J. S., Townsend, J. A., Stevens, M., Fitt, B. D. L. (2012) Comparative biology of different plant pathogens to estimate effects of climate change on crop diseases in Europe. European Journal of Plant Pathology. 133:315-331

Willis, C. E., Foster, S. P., Zimmer, C. T., Elias, J., Chang, X., Field, L. M., Williamson, M. S. and Emyr Davis, T. G. (2020) Investigating the status of pyrethroid resistance in UK populations of the cabbage stem flea beetle (*Psylliodes chrysocephala*). Crop Protection. 138:105316

Wood, T. J. and Goulson, D. (2017) The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. Environmental Science and Pollution Research. 24:17285–17325

Yang, J., Liu, D., Wang, X., Ji, C., Cheng, F., Liu, B., Hu, Z., Chen, S., Pental, D., Ju, Y. (2016) The genome sequence of allopolyploid *Brassica juncea* and

analysis of differential homoeolog gene expression influencing selection. Nature Genetics. 48:1225–1232

Yoder, O. C., Valent, B., Chumley, F. (1986) Genetic nomenclature and practice for plant pathogenic fungi. Phytopathology. 76:383–385

Young, N. D. (1996) QTL mapping and quantitative disease resistance in plants. Annual Review of Phytopathology. 34:479-501

Zhang, X., White, R. P., Demir, E., Jedryczka, M., Lange, R. M., Islam, M. et al. (2014) *Leptosphaeria* spp., phoma stem canker and potential spread of *L. maculans* on oilseed rape crops in China. Plant Pathology. 63:598-612

Zhu, Y., Elkins-Arce, H., Wheeler, T. A., Dever, J., Whitelock, D., Hake, K., Wedegaertner, T., Zhang, J. (2022) Effect of growth stage, cultivar, and root wounding on disease development in cotton caused by Fusarium wilt race 4 (*Fusarium oxysporum* f. sp. *vasinfectum*). Crop Science. 63:101-114

Appendix A: Publications

Conference paper - Agri-Food Charities Partnership conference 2021, online, June 2021

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

By LAURA SAPELLI[1], CHINTHANI KARANDENI DEWAGE[1], FAYE RITCHIE[2], BRUCE D L FITT[1] and YONGJU HUANG[1]

[1]*School of Life and Medical Sciences, University of Hertfordshire, UK*

[2]*ADAS Boxworth, Cambridge, UK*

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Summary

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

Introduction

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is the most economically damaging disease of winter oilseed rape (*Brassica napus*) in the UK, with annual yield losses of > £100M (CropMonitor, 2021, Karandeni Dewage *et al*., 2017). Control of light leaf spot is challenging because it is a polycyclic disease, with epidemics started in autumn by ascospores released from apothecia on infected crop debris from the previous season. Subsequently, conidia produced by asexual sporulation on infected leaves cause secondary infections on all parts of the plant (Boys *et al.*, 2007, Evans *et al*., 2003, Fitt *et al*, 1998). Current control relies on fungicides;

however, insensitivity development in *P. brassicae* isolates highlights the need for non-chemical controls like host resistance (Carter *et al.*, 2014, Huang *et al.*, 2006). There is currently little information about pathogenic *P. brassicae* populations and host resistance mechanisms, highlighting a need for new research. There is a need to improve our current knowledge about host resistance of winter oilseed rape against *P. brassicae* by studying virulent races in pathogen populations, identifying candidate resistance genes, and investigating mechanisms of host resistance.

Materials and Methods

Results of a preliminary experiment to produce *P. brassicae* conidial inoculum were expanded to investigate the light leaf spot disease development on the *B. napus* susceptible cultivar Charger in different treatment conditions. Plants of cultivar Charger were grown in a controlled-environment cabinet and inoculated with *P. brassicae* conidial suspensions with 10^4 or 10^5 spores/ml when the plants were 4, 5, 6 or 7 weeks old. Severity of light leaf spot and plant height on inoculated plants were assessed at 23 days post-inoculation (dpi) and compared between different treatments to identify the effects of inoculum concentration and growth stage of plants on light leaf spot severity. To establish a collection of *P. brassicae* isolates, leaves with light leaf spot symptoms were sampled from winter oilseed rape crops and fungal isolates were obtained through single-spore isolation and subculturing on malt extract agar plates for further study (Fig 1).

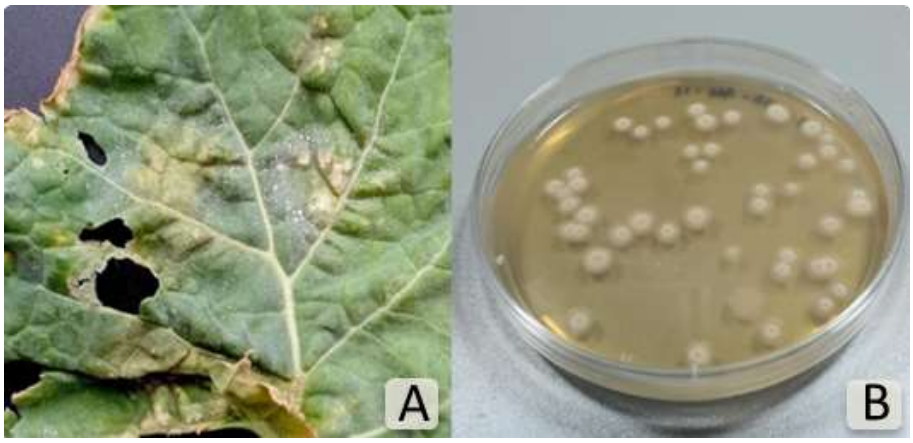

Fig 1: Leaf of oilseed rape cv Charger with light leaf spots (white *P. brassicae* acervuli containing conidia) (A). Single-conidial colonies derived from the same acervulus grown on a malt extract agar plate (B).

Results

Older plants of cv. Charger (7 weeks at time of inoculation) grown in a controlled-environment cabinet that received the higher inoculum concentration (10^5 spores/ml) produced the most severe light leaf spot symptoms. Plants inoculated with the higher

inoculum concentration were significantly shorter (by up to 5 cm) than those inoculated with the lower inoculum concentration (10^4 spores/ml) (Figs 2 & 3).

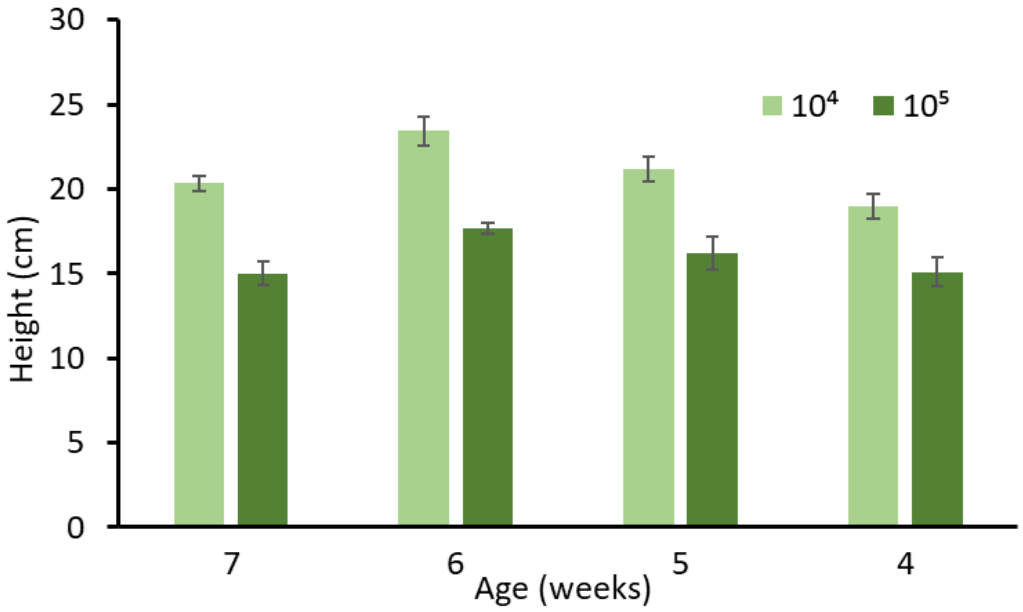

Fig 2: Height of plants inoculated with different conidial concentrations at 23 days post-inoculation (dpi). Error bars are standard error of the mean (DF = 3).

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

Discussion

Higher fungal inoculum concentration (10^5 spores/ml) produced a greater disease severity and reduced height of plants, suggesting a possible correlation between inoculum concentration and plant growth. Observations of plant growth stunting on light leaf spot-infected plants have been reported previously in field trials (Karandeni Dewage *et al*., 2017), with no prior reports in cabinet-grown plants. These results will be investigated further in future experiments.

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

Acknowledgements

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

References

Boys, E F, Roques, S E, Ashby, A M, Evans, N, Latunde-Dada, A O, Thomas, J E, West, J S, Fitt, B D L. 2007. Resistance to infection by stealth: *Brassica napus* (winter oilseed rape) and *Pyrenopeziza brassicae* (light leaf spot). *European Journal of Plant Pathology* **118**:307-321.

Carter, H E, Fraaije, B A, West, J S, Kelly, S L, Mehl, A, Shaw, M W, Cools, H J. 2014. Alterations in the predicted regulatory and coding regions of the sterol 14A-demethylase gene (CYP51) confer decreased azole sensitivity in the oilseed rape pathogen *Pyrenopeziza brassicae. Molecular Plant Pathology* **15**:513-522.

CropMonitor. 2021. Survey of commercially grown winter oilseed rape. Department for Environment, Food and Rural Affairs. Available at: www.cropmonitor.co.uk/wosr/surveys/wosr.cfm (accessed 29 June 2021).

Evans, N, Baierl, A, Brain, P, Welham, S J, Fitt, B D L. 2003. Spatial aspects of light leaf spot (*Pyrenopeziza brassicae*) epidemic development on oilseed rape (*Brassica napus*) in the United Kingdom. *Phytopathology* **93**:657-665.

Fitt, B D L, Doughty, K J, Gladders, P, Steed, J M, Sutherland, K G. 1998. Diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. *Annals of Applied Biology* **133**:155-166.

Huang, Y J, Evans, N, Li, Z-Q, Eckert, M, Chèvre, A-M, Renard, M, Fitt, B D L. 2006.Temperature and leaf wetness duration affect phenotypic expression of *Rlm6*-mediated resistance to *Leptosphaeria maculans* in *Brassica napus. New Phytologist* **170**:129-141.

Karandeni-Dewage, C S, Klöppel, C A, Stotz, H U, Fitt, B D L. 2017. Host-pathogen interactions in relation to management of light leaf spot disease (caused by *Pyrenopeziza brassicae*) on *Brassica* species. *Crop and Pasture Science* **69**:9-19.

Appendix B: Presentations

1. Poster - British Society of Plant Pathology conference "Our Plants, Our Future" 2021, Birmingham, UK, December 2021

University of Hertfordshire UH

School of Life and Medical Sciences

Understanding host resistance to improve control of light leaf spot on winter oilseed rape in the UK

Sapelli, L.[1], Karandeni Dewage, C. S.[1], Ritchie, F.[2], Fitt, B. D. L.[1], Huang, Y. J.[1]

[1] School of Life and Medical Sciences, University of Hertfordshire, UK [2] ADAS Boxworth, Cambridge, UK

Introduction

Oilseed rape (*Brassica napus*) is the second most produced oilseed in the world and the third most important arable crop in the UK. Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is the most economically damaging disease of winter oilseed rape in the UK. Disease control is challenging because it is a polycyclic disease, with epidemics starting in autumn by ascospores. Subsequently, conidia produced through asexual sporulation on infected leaves cause secondary infections on all aerial parts of the plant (Fig. 1).

Figure 1: *Pyrenopeziza brassicae* (light leaf spot) life cycle.

Current control relies on fungicides; however, insensitivity development highlights the need for non-chemical controls like host resistance. However, host resistance against *P. brassicae* is poorly understood. The aim of this project is to improve our current knowledge by researching virulent races in pathogen populations, identifying candidate resistance genes and investigating mechanisms of host resistance.

Figure 2: Leaf of oilseed rape cv Charger with light leaf spots (white *P. brassicae* acervuli containing conidia spores) (A). Single conidial colonies derived from the same acervulus grown on a malt extract agar plate (B).

Results

- Plants that received the higher inoculum concentration (10^5 spores/ml) developed more severe disease (Fig. 3) and were significantly shorter by up to 5 cm than those with the lower inoculum concentration (10^4 spores/ml) (Fig. 4).

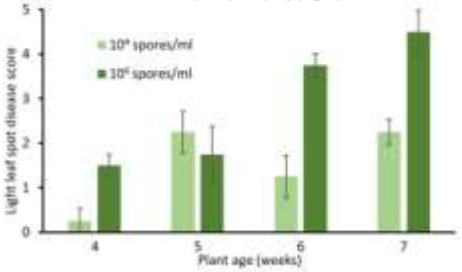

Figure 3: Disease score on plants inoculated with different conidia concentrations at different growth stages. Error bars are standard error of the mean.

Figure 4: Height of plants that received 10^4 spores/ml (1) or 10^5 spores/ml (2) inoculation at 21 dpi.

- Over 25 *P. brassicae* isolates from the 2021 season have been obtained from oilseed rape and kale cultivars across England.

Materials and Methods

- Plants of cultivar Charger were inoculated with different concentrations (10^4 and 10^5 spores/ml) of *P. brassicae* conidia at different ages (1, 2, 3, 4 weeks old) in controlled environment. Severity of light leaf spot and plant height were assessed at 21 days post-inoculation (i.e. when plants were 4, 5, 6, 7 weeks old).
- Leaves with light leaf spot symptoms were sampled from field and *P. brassicae* isolates were obtained by single-spore isolation (Fig. 2).

Conclusions and Discussion

- Higher fungal inoculum concentration produced higher disease severity but reduced the height of plants, suggesting a possible correlation between inoculum concentration and plant growth. This is the first observation in controlled conditions. There is a need of further investigation to test this hypothesis.
- The isolates obtained from different cultivars and different regions in England will be further screened for virulence.

Acknowledgements

This work is part of a four-year HKEP (Hertfordshire Knowledge Exchange Partnership) funded PhD project in collaboration with the Perry Foundation and the industrial partner ADAS.

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2. Poster - Agri-Food Charities Partnership student forum 2022, Cranfield, UK, March 2022

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

Sapelli, L.[1], Karandeni Dewage, C. S.[1], Ritchie, F.[2], Fitt, B. D. L.[1], Huang, Y. J.[1]
[1] School of Life and Medical Sciences, University of Hertfordshire, UK [2] ADAS Boxworth, Cambridge, UK

Introduction

Oilseed rape (*Brassica napus*) is the second most produced oilseed in the world and the third most important arable crop in the UK.

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is the most economically damaging disease of winter oilseed rape in the UK. Disease control is challenging because it is a polycyclic disease with epidemics starting in autumn by ascospores. Subsequently, conidia produced through asexual sporulation on infected leaves cause secondary infections on all aerial parts of the plant (Fig 1).

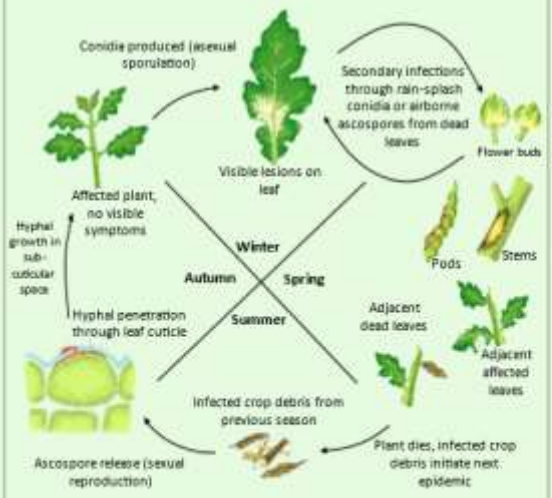

Figure 1: *Pyrenopeziza brassicae* (light leaf spot) life cycle.

Current disease control mainly relies on fungicides; however, insensitivity development highlights the need for non-chemical controls like host resistance. However, host resistance against *P. brassicae* is poorly understood. The aim of this project is to improve our current knowledge by researching virulent races in pathogen populations, identifying candidate resistance genes and investigating mechanisms of host resistance.

Figure 2: Leaf of oilseed rape cv Charger with light leaf spot (white *P. brassicae* acervuli containing conidia) (A). Single conidial colonies derived from the same acervulus grown on a malt extract agar plate (B).

Results

- Plants that received higher inoculum concentration (10^5 spores/ml) developed more severe disease (Fig 3) and were significantly shorter by up to 5 cm than those with the lower inoculum concentration (10^4 spores/ml) (Fig 4).

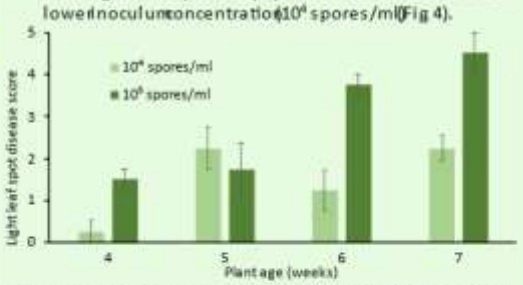

Figure 3: Disease scores on plants inoculated with different inoculum concentrations at different growth stages. Error bars are standard error of the mean.

Figure 4: Comparison of the height of plants that received 10^4 spores/ml (1) or 10^5 spores/ml (2) inoculation at 21 dpi.

- Over 25 *P. brassicae* isolates from the 2021 season were obtained from oilseed rape and kale cultivars across England. Another 60 isolates from continental Europe were obtained through Rothamsted Research.

Materials and Methods

- Plants of cultivar Charger at different stages (1, 2, 3, 4 weeks old) were inoculated with different concentrations (10^4 and 10^5 spores/ml) of *P. brassicae* conidia in controlled environment. Severity of light leaf spot and plant height were assessed at 21 days post inoculation (i.e. when plants were 4, 5, 6, 7 weeks old).
- Leaves with light leaf spot symptoms were sampled from oilseed rape crops and *P. brassicae* isolates were obtained by single spore isolation (Fig 2).

Conclusions and Discussion

- Higher inoculum concentration produced higher disease severity and significantly reduced the height of plants, suggesting a possible correlation between inoculum concentration and plant growth. This is the first record of growth stunting observed in controlled conditions. There is a need of further investigation to test this hypothesis.
- The isolates obtained from different cultivars and different regions in England and Europe will be tested for virulence to understand *P. brassicae* population structure.

Acknowledgements
This work is part of a four-year HKEP (Hertfordshire Knowledge Exchange Partnership) funded PhD project at the University of Hertfordshire in collaboration with the Perry Foundation and the industrial partner ADAS.
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3. Abstract - International Organization for Biological and Integrated Control – Integrated Control in Oilseed Crops, online, May 2022

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

Laura Sapelli[1], Chinthani Karandeni Dewage[1], Faye Ritchie[2], Bruce D L Fitt[1], Yongju Huang[1]

[1] Centre for Agriculture, Food and Environmental Research, School of Life and Medical Sciences, University of Hertfordshire, Hatfield AL10 9AB, UK;

[2] Disease and Pest Management, ADAS Boxworth, Cambridge, CB23 4NN, UK

Abstract: Light leaf spot is caused by the fungal pathogen *Pyrenopeziza brassicae* and is the most economically damaging disease of oilseed rape (*Brassica napus*) in the UK. Current control relies on repeated fungicide applications; however, pathogen fungicide-insensitivity development highlights the need for non-chemical controls like host resistance. A study was done to assess light leaf spot disease phenotype on the susceptible *B. napus* cultivar Charger in different treatment conditions; factors studied included plant age and inoculum concentration. Results showed that older plants grown in a controlled-environment cabinet produced the most visible symptoms. Plants that received higher inoculum concentration (10^5 spores/ml) were significantly shorter by 5 cm than those inoculated with lower inoculum concentration (10^4 spores/ml), suggesting possible correlations between fungal inoculum concentration and plant growth. Additionally, > 25 *P. brassicae* field isolates were collected from leaf samples across England through single-spore isolation and will be screened for virulence.

Key words: oilseed rape, light leaf spot, plant growth stunting, *P. brassicae* pathogen populations

Introduction

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is the most economically damaging disease of winter oilseed rape (*Brassica napus*) in the UK, with annual yield losses of > £100M (CropMonitor, 2021; Karandeni Dewage *et al*., 2017). Control of light leaf spot is challenging because it is a polycyclic disease, with epidemics started in autumn by ascospores released from apothecia on infected crop debris from the previous season. Subsequently, conidia produced by asexual sporulation on infected leaves cause secondary infections on all aerial parts of the plant (Boys *et al*., 2007; Evans *et al*., 2003; Fitt *et al*, 1998). Current control relies on fungicides; however, insensitivity development in *P. brassicae* isolates highlights the need for non-chemical controls like host resistance (Carter *et al*., 2014; Huang *et al*., 2006; Karandeni Dewage *et al*., 2022). There is currently little information about pathogenic *P. brassicae* populations and host resistance mechanisms, highlighting a need for new research. There is a need to improve our current knowledge about host resistance of winter oilseed rape against *P. brassicae* by studying virulent races in pathogen populations, identifying candidate resistance genes, and investigating mechanisms of host resistance.

Material and methods

Light leaf spot disease phenotype in different treatment conditions

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

Collection of P. brassicae field isolates

Leaves with light leaf spot symptoms were sampled from winter oilseed rape crops and fungal isolates were obtained through single-spore isolation and subculturing on malt extract agar plates for further study.

Results and discussion

Disease assessment and plant growth stunting results

Older plants of cv. Charger (7 weeks at the time of inoculation) grown in a controlled-environment cabinet that received the higher inoculum concentration (10^5 spores/ml) produced the most severe light leaf spot symptoms. Plants inoculated with the higher inoculum concentration were significantly shorter (by up to 5 cm) than those inoculated with the lower inoculum concentration (10^4 spores/ml), as shown in Figure 1 and Figure 2.

Higher fungal inoculum concentration (10^5 spores/ml) produced a greater disease severity and reduced height of plants, suggesting a possible correlation between inoculum concentration and plant growth. Observations of plant growth stunting on light leaf spot-infected plants have been reported previously in field experiments (Karandeni Dewage *et al*., 2017), with no prior reports in cabinet-grown plants. These results will be investigated further in future experiments.

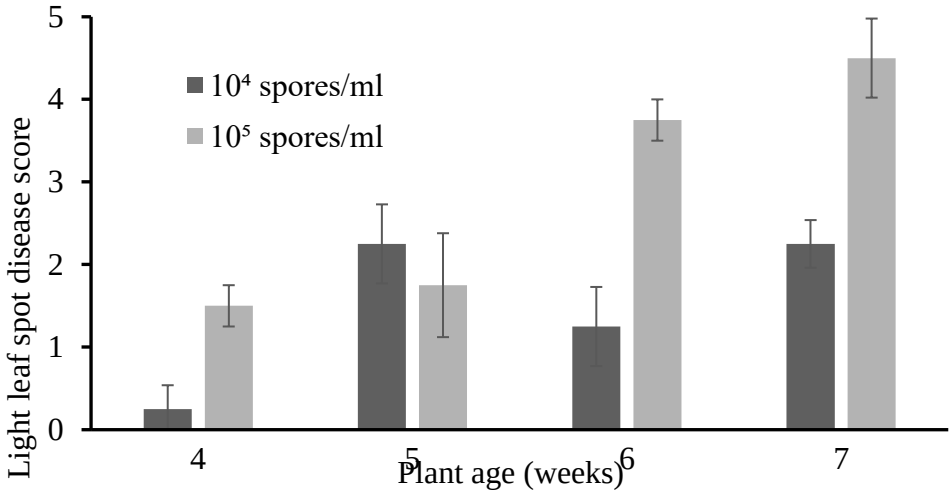

Figure 1. Disease scores on plants inoculated with different inoculum concentrations at different growth stages. Error bars are standard error of the mean.

Figure 2. Comparison of the height of plants that received 10^4 spores/ml (1) or 10^5 spores/ml (2) inoculation at 21 dpi.

Establishment of P. brassicae field isolates collection

Over 25 *P. brassicae* field isolates have been collected from different cultivars of oilseed rape and kale across England. These isolates will be further screened for virulence to understand pathogen population structures, through inoculation of a differential set of cultivars/lines, as well as molecular analyses using 'neutral' markers.

Acknowledgements

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

References

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CropMonitor. 2021: Survey of commercially grown winter oilseed rape. Department for Environment, Food and Rural Affairs. [WWW document] URL www.cropmonitor.co.uk/wosr/surveys/wosr.cfm. Cited 8 June 2022.

Evans, N., Baierl, A., Brain, P., Welham, S. J. & Fitt, B. D. L. 2003: Spatial aspects of light leaf spot (*Pyrenopeziza brassicae*) epidemic development on oilseed rape (*Brassica napus*) in the United Kingdom. Phytopathology. 93: 657-665.

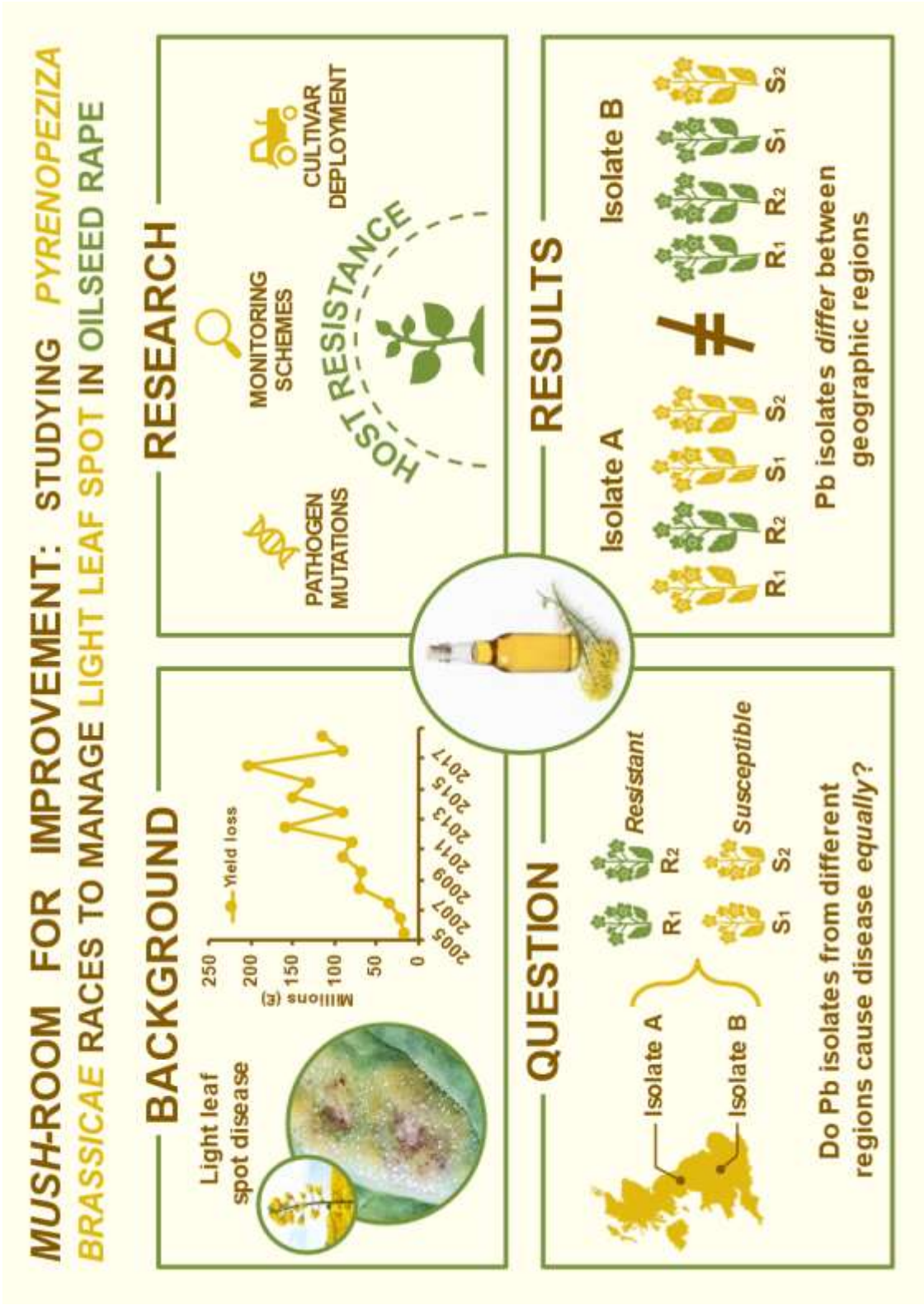
Fitt, B. D. L., Doughty, K. J., Gladders, P., Steed, J. M. & Sutherland, K. G. 1998: Diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. Annals of Applied Biology. 133: 155-166.

Huang, Y. J., Evans, N., Li, Z.-Q., Eckert, M., Chèvre, A.-M., Renard, M. & Fitt, B. D. L. 2006: Temperature and leaf wetness duration affect phenotypic expression of *Rlm6*-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytologist. 170: 129-141.

Karandeni Dewage, C. S., Klöppel, C. A., Stotz, H. U. & Fitt, B. D. L. 2017: Host-pathogen interactions in relation to management of light leaf spot disease (caused *by Pyrenopeziza brassicae*) on Brassica species. Crop and Pasture Science. 69: 9-19.

Karandeni Dewage, C. S., Cools, K., Stotz, H. U., Qi, A., Huang, Y. J., Wells, R. & Fitt, B. D. L. 2022: Quantitative trait locus mapping for resistance against *Pyrenopeziza brassicae* derived from a *Brassica napus* secondary gene pool. Frontiers in Plant Science doi: 10.3389/fpls.2022.786189

4. Flash-talk – Three-Minute-Thesis finals at the Postgraduate Research Student conference 2023, Hertfordshire, UK, June 2023



5. Abstract – 12th International Congress of Plant Pathology 2023, Lyon, France, August 2023

Understanding *Pyrenopeziza brassicae* populations for effective control of light leaf spot in winter oilseed rape

Laura Sapelli[1], Chinthani Karandeni Dewage[1], Faye Ritchie[2], Bruce D L Fitt[1], Yongju Huang[1]

[1] Centre for Agriculture, Food and Environmental Research, School of Life and Medical Sciences, University of Hertfordshire, Hatfield AL10 9AB, UK;

[2] Disease and Pest Management, ADAS Boxworth, Cambridge, CB23 4NN, UK

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae,* is the most economically damaging disease of oilseed rape (*Brassica napus*) in the UK. Fungicide insensitivity development highlights the need for non-chemical controls like host resistance. Currently, there is limited information on virulence of UK *P. brassicae* populations, which is crucial for effective use of host resistance. To study pathogen populations, isolates from different regions and different hosts were tested in glasshouse experiments. *P. brassicae* isolates were obtained from oilseed rape and kale cultivars across England, and other European isolates were acquired through Rothamsted Research. A total of 24 *P. brassicae* isolates were tested on a differential set of nine oilseed rape cultivars/lines. In addition, field experiments were done in England for the 2021/2022 cropping season at Hereford and Huntingdon with cultivars Aquila and Flamingo. Disease severity was assessed by measuring disease score (scale 1-8, with 1 being resistant), percentage area with sporulation on leaves and presence of necrotic flecking (collapsed epidermal cells). Results from glasshouse experiments showed differences in disease severity between both cultivars and isolates. Results from field experiments differed between locations and cultivars, suggesting variations in pathogen populations between locations. Genotypic differences between *P. brassicae* isolates will be studied using molecular techniques.

6. Poster – 12th International Congress of Plant Pathology 2023, Lyon, France, August 2023

Understanding *Pyrenopeziza brassicae* populations for effective control of light leaf spot in winter oilseed rape

Sapelli, L.[1], Qi, A.[1], Karandeni Dewage, C. S.[1], Ritchie, F.[2], Fitt, B. D. L.[1], Huang, Y. J.[1]

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Introduction

Oilseed rape (*Brassica napus*) is the second most produced oilseed in the world and the third most important arable crop in the UK.

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae* (Pb), is the most economically damaging disease of oilseed rape in the UK. Current disease control relies on fungicides; however, insensitivity development highlights the need for non-chemical controls like host resistance. Information on virulent races of *P. brassicae* populations in different UK areas is critical for effective use of host resistance.

This study aimed to investigate the disease phenotype of specific host-isolate interactions to help identify underlying mechanisms of resistance.

Materials and Methods

Figure 1: Outline of experimental work.

- Glasshouse experiments: 24 *P. brassicae* field isolates from the UK and four other EU countries were tested on nine *B. napus* cultivars/lines with different levels of resistance (Fig. 1)
- Data analysis was done using analysis of variance (ANOVA)

Results

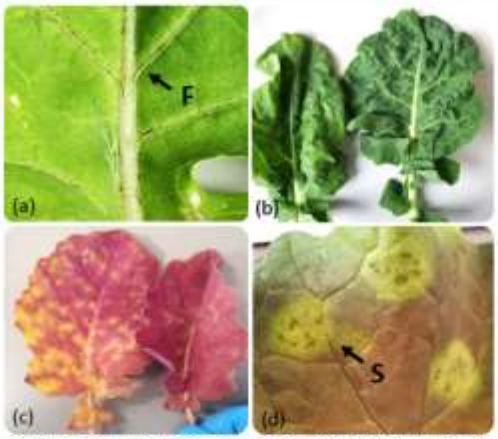


Figure 2: Disease phenotypes: (a) black necrotic flecking (F), (b) leaf distortion, (c) leaf discoloration, (d) green islands formation with *P. brassicae* sporulation (S).

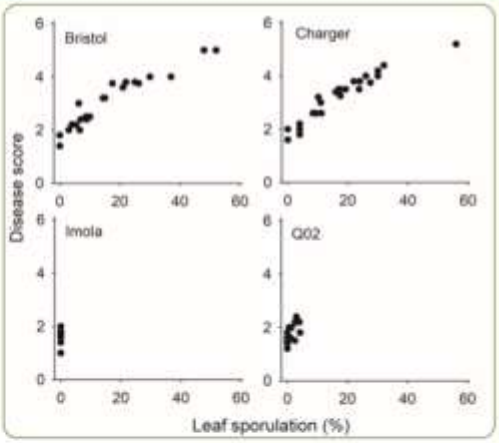


Figure 3: Relationship between light leaf spot disease score and percent leaf area with sporulation on representative cultivars/lines.

- Different disease phenotypes were observed, including sporulation on leaf area, leaf discoloration, leaf distortion, green islands formation and black necrotic flecking (Fig. 2)
- Analysis of variance showed significant difference between isolates ($p<0.01$) and between cultivars ($p<0.01$) in light leaf spot disease scores
- Strong correlations were observed between disease score and leaf area with sporulation for most cultivars (Fig. 3)

Discussion

- Light leaf spot disease score of a given cultivar varied between isolates, implying there are differences in virulence/aggressiveness between isolates
- Disease score and percentage leaf area with sporulation are good measurements of light leaf spot resistance
- Cultivars/lines Imola, NPZ 05/22, NPZ 06/22 and Q02 were resistant to those *P. brassicae* isolates, while cultivars Bristol and Charger were susceptible to those *P. brassicae* isolates. Their resistance needs to be further tested with more isolates

7. Abstract – 16th International Rapeseed Congress 2023, Sydney, Australia, September 2023

Investigating *Pyrenopeziza brassicae* pathogen races to combat light leaf spot in winter oilseed rape

Laura Sapelli[1], Chinthani Karandeni Dewage[1], Faye Ritchie[2], Bruce D L Fitt[1], Yongju Huang[1]
[1] Centre for Agriculture, Food and Environmental Research, School of Life and Medical Sciences, University of Hertfordshire, Hatfield AL10 9AB, UK;
[2] Disease and Pest Management, ADAS Boxworth, Cambridge, CB23 4NN, UK

Background:

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae,* is the most economically damaging disease of oilseed rape (*Brassica napus*) in the UK. Fungicide insensitivity development highlights the need for non-chemical controls like host resistance. Currently, there is limited information on virulence of UK *P. brassicae* populations, which is crucial for effective use of host resistance.

Objective:

The aim of this work was to investigate differences in virulence between different *P. brassicae* populations by testing different isolates in glasshouse experiments, and testing the same cultivars in field experiments at different geographic regions.

Methods:

Isolates from different regions and different hosts were tested in glasshouse experiments. *P. brassicae* isolates were obtained from oilseed rape and kale cultivars across England, and other European isolates were acquired through Rothamsted Research. A total of 24 *P. brassicae* isolates were tested on a differential set of nine oilseed rape cultivars/lines. In addition, field experiments were done in England for the 2021/2022 cropping season at Hereford and Huntingdon with cultivars Aquila and Flamingo. Disease severity was assessed by measuring disease score (scale 1-8, with 1 being resistant), percentage area with sporulation on leaves and presence of necrotic flecking (collapsed epidermal cells).

Results:

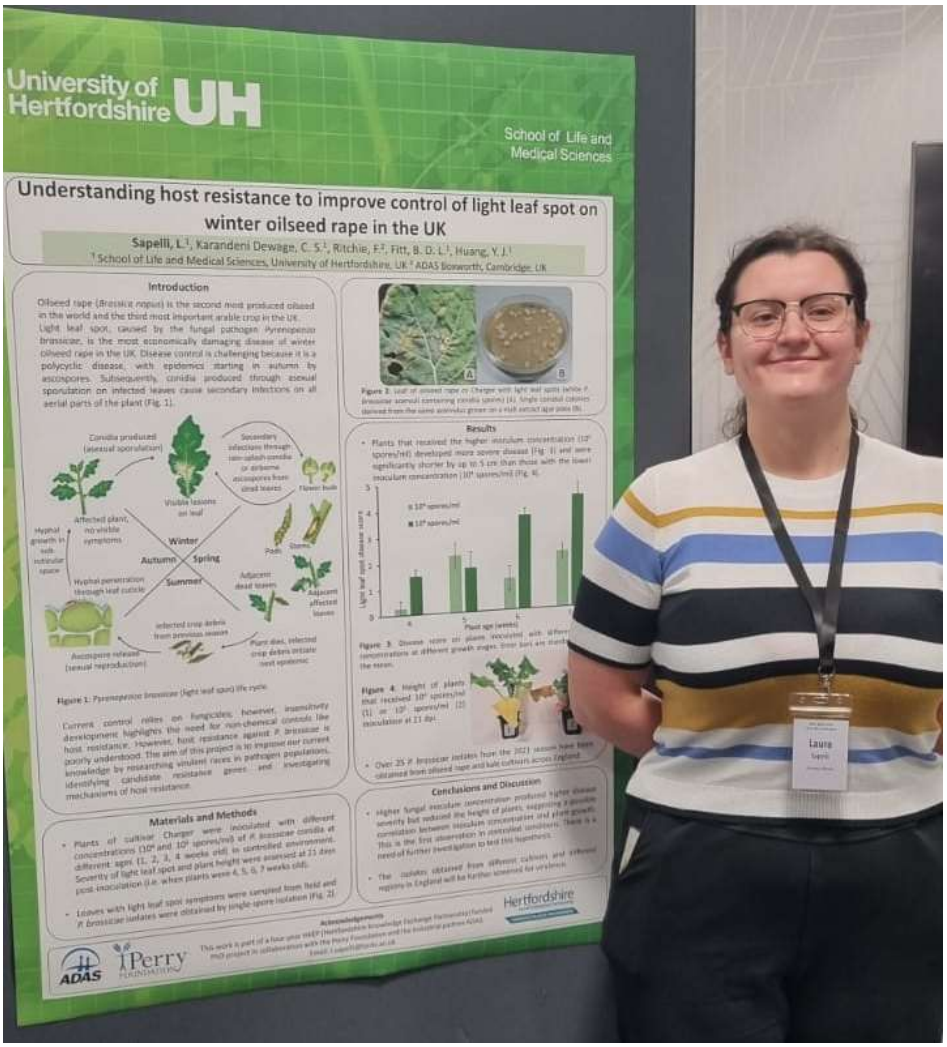
Glasshouse experiments results showed differences in disease severity, in terms of disease score, percentage leaf area with sporulation, and presence of necrotic flecking in three locations (leaf petiole, midrib, and lamina) between both cultivars and isolates. Susceptible cultivars overall developed more severe disease than resistant cultivars. Some isolates caused moderate disease severity in resistant cultivars, while other isolates showed low or no disease development across all cultivars tested. For the field experiments, there were differences in disease severity between cultivars and between locations, particularly for cultivar Flamingo, with the presence of necrotic flecking on most leaves at Hereford, but not at Huntingdon.

Conclusions:

There were distinct disease phenotypes between various isolate-cultivar combinations, with some isolates causing disease on resistant cultivars, while others did not produce disease even on susceptible cultivars. These results suggested that there were differences between the *P. brassicae* isolates in virulent races. Results from field experiments showed differences between the two locations Hereford and Huntingdon in disease severity. Considering that the same cultivars were used in both locations, results indicate differences in the *P. brassicae* populations between the two geographic regions. Overall, those results reinforce the hypothesis of different pathogen races existing in different areas of the UK.

Appendix C: Events

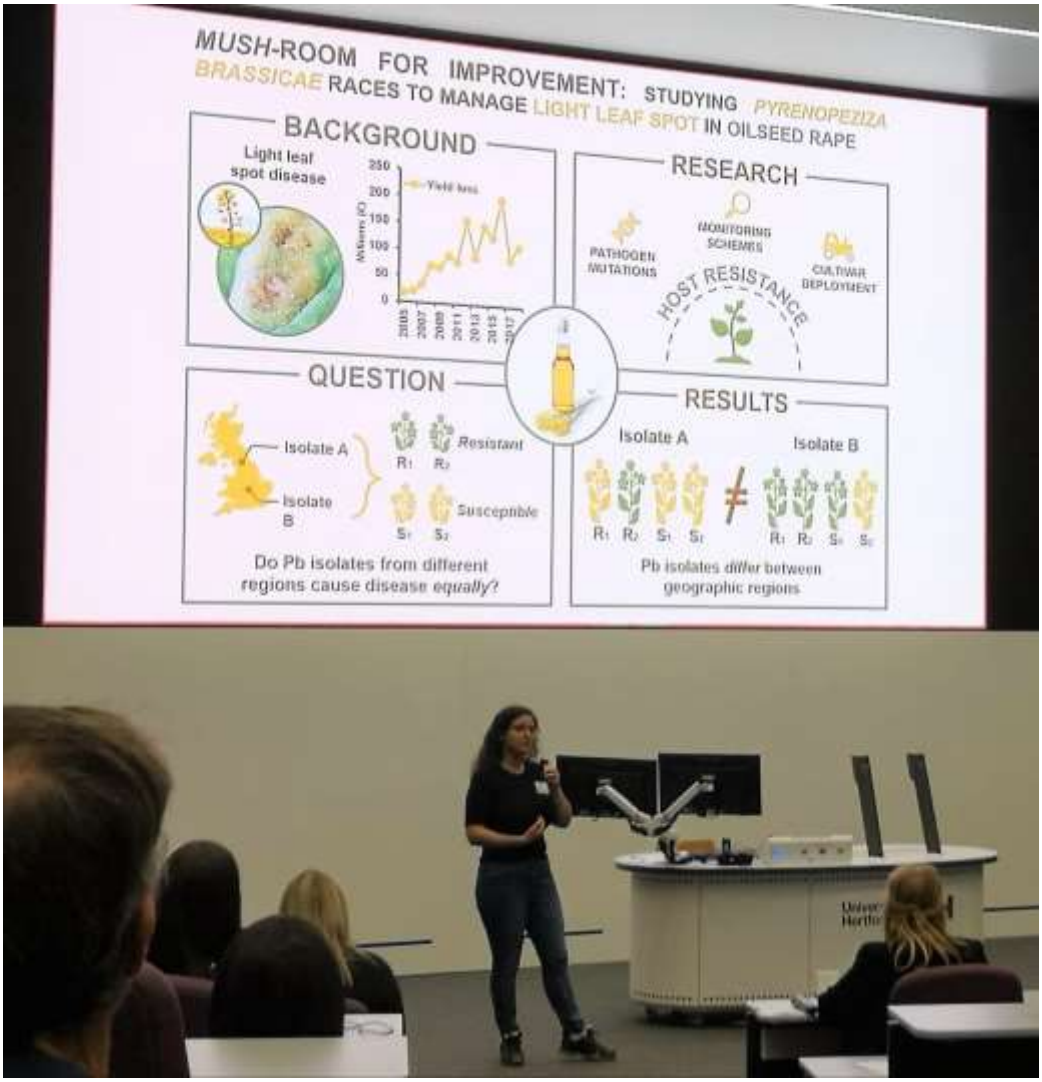
1. Poster - British Society of Plant Pathology conference "Our Plants, Our Future" 2021, Birmingham, UK, December 2021



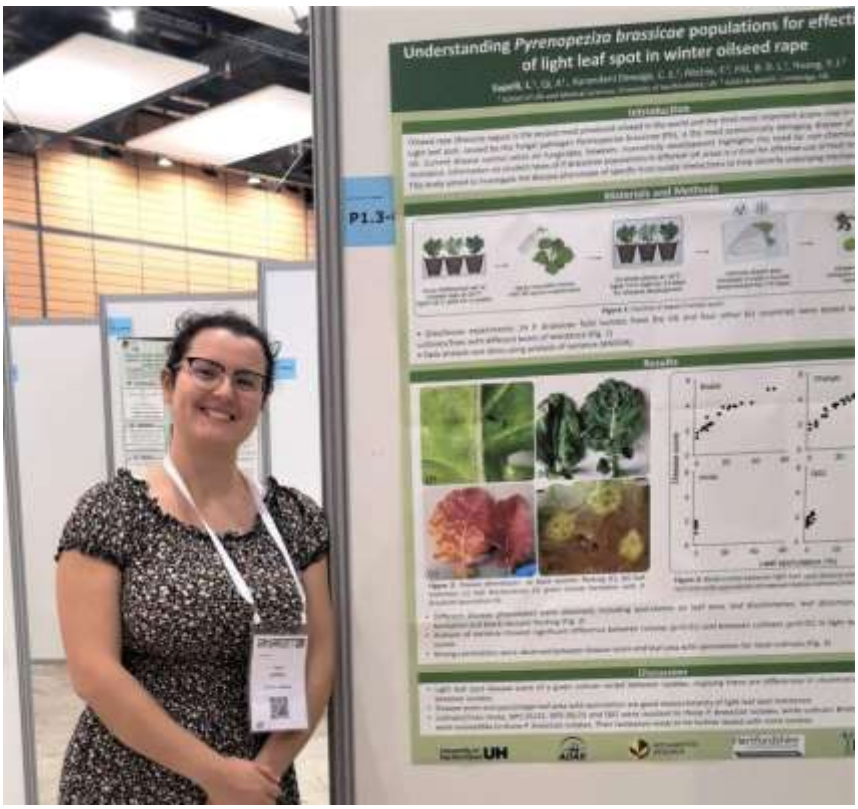
2. **Talk - Life and Medical Sciences research conference 2022, Hertfordshire, UK, June 2022**

3. **Talk - UK Brassica Research Community / Oilseed Rape Genetic Improvement Network Stakeholders Meeting 2022, Norwich, UK, October 2022**

4. **Talk - Postgraduate Research Student conference 2023, Hertfordshire, UK, June 2023**



5. **Poster – 12th International Congress of Plant Pathology 2023, Lyon, France, August 2023**



6. **Talk – 16th International Rapeseed Congress 2023, Sydney, Australia, September 2023**

7. **Talk - British Crop Production Council Disease Review 2023, Cambridge, October 2023**

8. **Talk - Agri-Food Charities Partnership student forum 2024, Cambridge, UK, March 2024**

Appendix D: Oilseed rape growth stage key (Sylvester-Bradley, 1985).

Description	Growth stage
GERMINATION AND EMERGENCE	0,0
LEAF PRODUCTION	
Both cotyledons unfolded and green	1,0
First true leaf	1,1
Second true leaf	1,2
Third true leaf	1,3
Fourth true leaf	1,4
Xth true leaf	1,X
STEM EXTENSION	
No internodes (rosette)	2,0
About five internodes	2,5
FLOWER BUD DEVELOPMENT	
Only leaf buds present	3,0
Flower buds present but enclosed by leaves	3,1
Flower buds visible from above ('green bud')	3,3
Flower buds level with leaves	3,4
Flower buds raised above leaves	3,5
First flower stalks extending	3,6
First flower buds yellow ('yellow bud')	3,7
FLOWERING	
First flower opened	4,0
10% of all buds opened	4,1
30% of all buds opened	4,3
50% of all buds opened	4,5
POD DEVELOPMENT	
30% potential pods	5,3
50% potential pods	5,5
70% potential pods	5,7
All potential pods	5,9
SEED DEVELOPMENT	
Seeds expanding	6,1
Most seeds translucent but full size	6,2
Most seeds green	6,3
Most seeds green-brown mottled	6,4
Most seeds brown	6,5
Most seeds dark brown	6,6
Most seeds dark but soft	6,7
Most seeds black and hard	6,8
All seeds black and hard	6,9
Leaf senescence	7,0
STEM SENESCENCE	
Most stem green	8,1
Half stem green	8,5
Little stem green	8,9
POD SENESCENCE	
Most pods green	9,1
Half pods green	9,5
Few pods green	9,9

Appendix E: Layout of field experiment in Harpenden, Hertfordshire in the 2023/24 cropping season. Plots 21-50 were assessed for light leaf spot disease severity.

Appendix F: Layout of field experiment in Norwich, Norfolk in the 2023/24 cropping season. Plots 21-50 were assessed for light leaf spot disease severity.

Appendix G: Dates of fungicide applications on field experiments in Harpenden, Hertfordshire and Norwich, Norfolk in the 2023/24 cropping season.

Site	Date	Fungicide (active ingredient)
Harpenden	06 December 2023	Aurelia (prothioconazole)
	03 April 2024	Tectura (azoxystrobin)
Norwich	16 October 2023	Filan (boscalid)
	27 February 2024	Proline 275 (prothioconazole)
	08 April 2024	Pictor (boscalid + dimoxystrobin)

Appendix H: Split-plot design for glasshouse experiment assessing differential interactions between 24 single-spore *Pyrenopeziza brassicae* isolates and 9 oilseed rape cultivars and breeding lines across six batches/experiments.

Batch 1

Isolate	Cultivar								
3	NPZ6	Yu	Im	Bar	NPZ5	Ch	Bri	Q60	Q2
2	Ch	Im	Bar	NPZ6	Bri	Yu	NPZ5	Q60	Q2
1	NPZ6	Bri	Im	Q60	Yu	NPZ5	Bar	Q2	Ch
4	Bri	NPZ6	Ch	Bar	Im	NPZ5	Q60	Q2	Yu

Isolate	Cultivar								
1	NPZ6	Yu	Ch	Bar	Im	NPZ5	Q2	Q60	Bri
3	Bar	Q60	NPZ6	Yu	Q2	NPZ5	Im	Bri	Ch
2	Ch	Bri	Q2	Q60	Yu	NPZ6	NPZ5	Im	Bar
4	Q60	Ch	NPZ5	Im	Q2	Bar	Bri	Yu	NPZ6

Isolate	Cultivar								
3	Bar	Yu	Q2	Bri	Ch	NPZ6	Im	NPZ5	Q60
1	Yu	NPZ6	Ch	Bri	NPZ5	Q60	Im	Bar	Q2
4	NPZ5	Bri	Yu	Q2	NPZ6	Ch	Im	Bar	Q60
2	Bar	Bri	Q2	NPZ5	Yu	Ch	NPZ6	Im	Q60

Isolate	Cultivar								
2	Ch	Im	Q60	NPZ5	Bar	NPZ6	Yu	Q2	Bri
3	Im	Q2	Bri	Q60	NPZ6	Bar	Yu	Ch	NPZ5
1	Q2	Bri	Ch	Bar	Q60	NPZ6	Im	Yu	NPZ5
4	Ch	NPZ6	Q60	Bar	Im	Q2	NPZ5	Bri	Yu

Isolate	Cultivar								
3	Im	Q2	Ch	NPZ5	NPZ6	Bri	Q60	Bar	Yu
4	NPZ6	Im	Bar	NPZ5	Yu	Ch	Q60	Q2	Bri
1	Bri	Yu	Bar	NPZ5	Im	Q2	Q60	Ch	NPZ6
2	Ch	Bar	Bri	Im	NPZ6	Q2	Q60	Yu	NPZ5

Batch 2

Isolate	Cultivar								
4	Bri	NPZ5	Bar	Im	Ch	Q60	NPZ6	Q2	Yu
3	NPZ5	NPZ6	Yu	Bar	Q60	Ch	Q2	Bri	Im
1	Q2	Bar	NPZ5	Bri	NPZ6	Ch	Im	Q60	Yu
2	Bar	Q2	Q60	Im	Yu	Ch	Bri	NPZ6	NPZ5

Isolate	Cultivar								
1	Im	Q2	Ch	NPZ5	Q60	Bar	NPZ6	Yu	Bri
2	NPZ6	Bar	Q2	Yu	Im	Bri	Ch	Q60	NPZ5
3	NPZ5	Q2	NPZ6	Bri	Ch	Im	Bar	Q60	Yu
4	NPZ5	Q2	Yu	NPZ6	Im	Ch	Bar	Bri	Q60

Isolate	Cultivar								
2	Q60	Bri	Bar	NPZ5	Q2	Yu	Im	Ch	NPZ6
1	Q60	Q2	NPZ6	Bri	Im	Bar	NPZ5	Yu	Ch
3	Bri	Im	Q2	Q60	Bar	Yu	NPZ6	NPZ5	Ch
4	Bri	NPZ6	Ch	Q2	Q60	Bar	Yu	Im	NPZ5

Isolate	Cultivar								
3	Bar	Bri	Q60	Yu	Ch	NPZ6	Q2	Im	NPZ5
4	Yu	Bar	Q60	NPZ6	Q2	Ch	NPZ5	Im	Bri
1	Bar	Yu	Bri	Q2	NPZ6	Ch	Im	NPZ5	Q60
2	Ch	Bar	NPZ5	Im	Yu	Q60	NPZ6	Q2	Bri

Isolate	Cultivar								
1	Bri	NPZ5	Bar	Ch	Q60	Q2	Im	NPZ6	Yu
4	Q60	Bar	Ch	NPZ5	Im	Yu	Bri	Q2	NPZ6
3	Bar	Yu	NPZ5	Im	Bri	Q60	NPZ6	Q2	Ch
2	Yu	Q2	Bri	Im	Bar	NPZ6	Q60	Ch	NPZ5

Batch 3

Isolate	Cultivar								
3	Q2	Bri	Q60	Im	Yu	Ch	NPZ6	NPZ5	Bar
4	Bri	Q2	Q60	Bar	NPZ5	NPZ6	Ch	Im	Yu
2	Im	Ch	Q60	Yu	Bri	NPZ6	Bar	Q2	NPZ5
1	Im	Q2	Yu	NPZ5	NPZ6	Bri	Ch	Q60	Bar

Isolate	Cultivar								
4	Ch	Yu	Bar	Q60	Im	NPZ6	Bri	NPZ5	Q2
1	Bri	Bar	NPZ5	Q2	Q60	Ch	NPZ6	Yu	Im
3	NPZ6	Q2	Im	Ch	Q60	Yu	Bri	NPZ5	Bar
2	Yu	NPZ6	Im	Q60	NPZ5	Bar	Q2	Bri	Ch

Isolate	Cultivar								
4	Yu	Q2	Bar	NPZ5	Im	Q60	NPZ6	Bri	Ch
3	Ch	NPZ5	Im	NPZ6	Yu	Bri	Q2	Bar	Q60
2	Yu	Ch	NPZ6	Q2	Bri	Bar	Im	NPZ5	Q60
1	Q2	Ch	Q60	NPZ6	Yu	Bri	NPZ5	Im	Bar

Isolate	Cultivar								
4	Yu	Bri	NPZ6	Ch	Bar	NPZ5	Q2	Im	Q60
3	NPZ5	Yu	Bar	Q60	Im	Q2	Bri	Ch	NPZ6
2	NPZ6	Ch	Bri	Yu	Bar	Q2	Im	Q60	NPZ5
1	Yu	Im	Ch	Bar	NPZ5	Q2	NPZ6	Q60	Bri

Isolate	Cultivar								
2	Q2	Bar	Ch	Bri	NPZ5	Q60	NPZ6	Yu	Im
4	Bar	Im	Q60	Bri	Yu	Q2	Ch	NPZ5	NPZ6
3	Bar	Ch	Yu	Q60	NPZ5	Q2	Bri	NPZ6	Im
1	Q60	Ch	Bar	Im	NPZ6	Bri	NPZ5	Q2	Yu

Batch 4

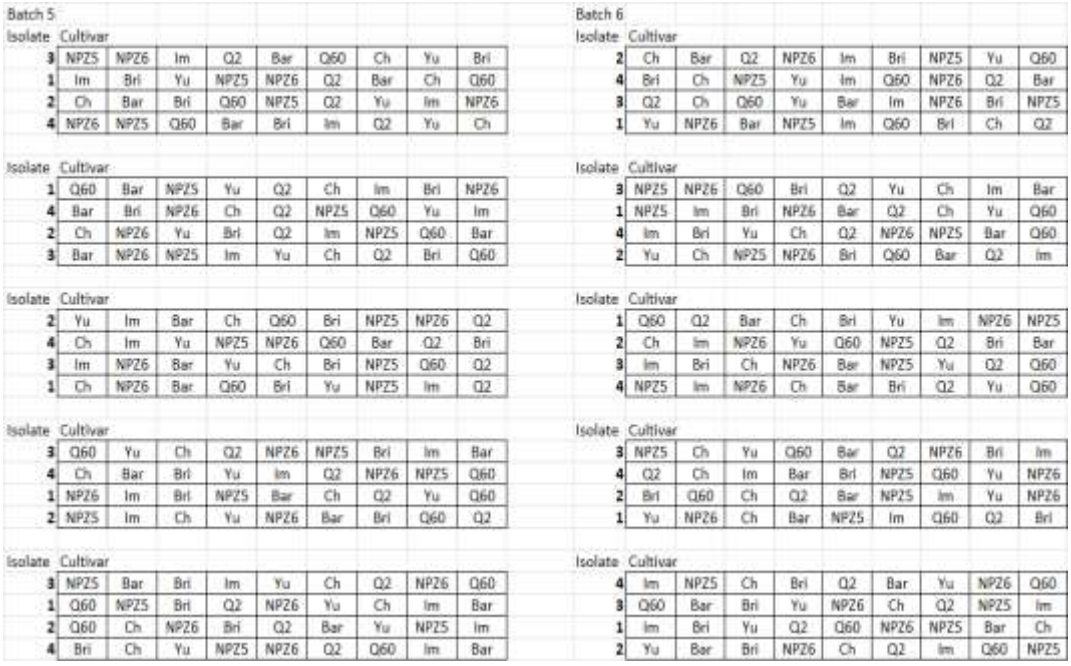
Isolate	Cultivar								
2	Q2	NPZ6	Yu	Im	Q60	Bar	Ch	Bri	NPZ5
3	Q2	NPZ5	Yu	Q60	Ch	Bar	NPZ6	Im	Bri
4	Q2	Bar	Ch	Q60	NPZ5	Bri	Im	Yu	NPZ6
1	Bri	NPZ5	Im	Yu	Ch	NPZ6	Q60	Q2	Bar

Isolate	Cultivar								
1	Yu	Q2	Bri	Im	Bar	Ch	Q60	NPZ6	NPZ5
4	Q60	Yu	Q2	Bri	Im	NPZ6	Ch	NPZ5	Bar
2	Bar	Q60	Im	Q2	NPZ5	NPZ6	Ch	Yu	Bri
3	NPZ6	Bar	Q60	Bri	Yu	Q2	Im	NPZ5	Ch

Isolate	Cultivar								
4	Q2	NPZ5	Q60	Ch	Bri	Bar	NPZ6	Im	Yu
1	Ch	Yu	Bar	Im	NPZ5	Bri	Q2	Q60	NPZ6
2	NPZ5	Bar	Bri	Im	Q60	NPZ6	Yu	Q2	Ch
3	Yu	Im	Bar	Ch	Q2	NPZ6	Bri	Q60	NPZ5

Isolate	Cultivar								
4	Q60	Q2	NPZ6	Ch	Bri	NPZ5	Bar	Yu	Im
1	Yu	NPZ5	Im	Bar	Q60	NPZ6	Bri	Q2	Ch
2	Yu	Im	NPZ6	Ch	Q2	Bri	NPZ5	Bar	Q60
3	Yu	NPZ5	Ch	NPZ6	Im	Bar	Q60	Q2	Bri

Isolate	Cultivar								
4	Bar	Ch	Im	Q60	Bri	Q2	Yu	NPZ5	NPZ6
3	Ch	Yu	Im	NPZ5	Bar	Bri	Q60	Q2	NPZ6
1	Im	Yu	Q60	Bar	Ch	NPZ6	Bri	Q2	NPZ5
2	Q60	Bar	NPZ6	Im	NPZ5	Q2	Yu	Ch	Bri



Batch 5

Isolate	Cultivar								
3	NPZ5	NPZ6	Im	Q2	Bar	Q60	Ch	Yu	Bri
1	Im	Bri	Yu	NPZ5	NPZ6	Q2	Bar	Ch	Q60
2	Ch	Bar	Bri	Q60	NPZ5	Q2	Yu	Im	NPZ6
4	NPZ6	NPZ5	Q60	Bar	Bri	Im	Q2	Yu	Ch

Isolate	Cultivar								
1	Q60	Bar	NPZ5	Yu	Q2	Ch	Im	Bri	NPZ6
4	Bar	Bri	NPZ6	Ch	Q2	NPZ5	Q60	Yu	Im
2	Ch	NPZ6	Yu	Bri	Q2	Im	NPZ5	Q60	Bar
3	Bar	NPZ6	NPZ5	Im	Yu	Ch	Q2	Bri	Q60

Isolate	Cultivar								
2	Yu	Im	Bar	Ch	Q60	Bri	NPZ5	NPZ6	Q2
4	Ch	Im	Yu	NPZ5	NPZ6	Q60	Bar	Q2	Bri
3	Im	NPZ6	Bar	Yu	Ch	Bri	NPZ5	Q60	Q2
1	Ch	NPZ6	Bar	Q60	Bri	Yu	NPZ5	Im	Q2

Isolate	Cultivar								
3	Q60	Yu	Ch	Q2	NPZ6	NPZ5	Bri	Im	Bar
4	Ch	Bar	Bri	Yu	Im	Q2	NPZ6	NPZ5	Q60
1	NPZ6	Im	Bri	NPZ5	Bar	Ch	Q2	Yu	Q60
2	NPZ5	Im	Ch	Yu	NPZ6	Bar	Bri	Q60	Q2

Isolate	Cultivar								
3	NPZ5	Bar	Bri	Im	Yu	Ch	Q2	NPZ6	Q60
1	Q60	NPZ5	Bri	Q2	NPZ6	Yu	Ch	Im	Bar
2	Q60	Ch	NPZ6	Bri	Q2	Bar	Yu	NPZ5	Im
4	Bri	Ch	Yu	NPZ5	NPZ6	Q2	Q60	Im	Bar

Batch 6

Isolate	Cultivar								
2	Ch	Bar	Q2	NPZ6	Im	Bri	NPZ5	Yu	Q60
4	Bri	Ch	NPZ5	Yu	Im	Q60	NPZ6	Q2	Bar
3	Q2	Ch	Q60	Yu	Bar	Im	NPZ6	Bri	NPZ5
1	Yu	NPZ6	Bar	NPZ5	Im	Q60	Bri	Ch	Q2

Isolate	Cultivar								
3	NPZ5	NPZ6	Q60	Bri	Q2	Yu	Ch	Im	Bar
1	NPZ5	Im	Bri	NPZ6	Bar	Q2	Ch	Yu	Q60
4	Im	Bri	Yu	Ch	Q2	NPZ6	NPZ5	Bar	Q60
2	Yu	Ch	NPZ5	NPZ6	Bri	Q60	Bar	Q2	Im

Isolate	Cultivar								
1	Q60	Q2	Bar	Ch	Bri	Yu	Im	NPZ6	NPZ5
2	Ch	Im	NPZ6	Yu	Q60	NPZ5	Q2	Bri	Bar
3	Im	Bri	Ch	NPZ6	Bar	NPZ5	Yu	Q2	Q60
4	NPZ5	Im	NPZ6	Ch	Bar	Bri	Q2	Yu	Q60

Isolate	Cultivar								
3	NPZ5	Ch	Yu	Q60	Bar	Q2	NPZ6	Bri	Im
4	Q2	Ch	Im	Bar	Bri	NPZ5	Q60	Yu	NPZ6
2	Bri	Q60	Ch	Q2	Bar	NPZ5	Im	Yu	NPZ6
1	Yu	NPZ6	Ch	Bar	NPZ5	Im	Q60	Q2	Bri

Isolate	Cultivar								
4	Im	NPZ5	Ch	Bri	Q2	Bar	Yu	NPZ6	Q60
3	Q60	Bar	Bri	Yu	NPZ6	Ch	Q2	NPZ5	Im
1	Im	Bri	Yu	Q2	Q60	NPZ6	NPZ5	Bar	Ch
2	Yu	Bar	Bri	NPZ6	Ch	Q2	Im	Q60	NPZ5

Cultivar/line name	ID
Charger	Ch
Imola	Im
Yudal	Yu
Bristol	Bri
Barbados	Bar
Q02	Q2
Q60	Q60
NPZ 05/22	NPZ5
NPZ 06/22	NPZ6

Batch 1

Isolate name	Number
Aqu-Here22-1	1
Aqu-Hunt22-1	2
Kale-H-6	3
Kale-H-7	4

Batch 2

Isolate name	Number
Aqu-Here-1	1
Charger-H-1	2
Charger-H-2	3
Tenor-L-4	4

Batch 3

Isolate name	Number
A7	1
Parkside-L-1	2
Park-L-3	3
19DEN03	4

Batch 4

Isolate name	Number
19GERA1	1
19GERA3	2
19GERC2	3
19DEN19	4

Batch 5

Isolate name	Number
19DEN33	1
20POL01	2
20POL04	3
20POL14	4

Batch 6

Isolate name	Number
18CAR02	1
18CAR04	2
18CAR05	3
A11	4

Appendix I: Complete collection of single-spore *Pyrenopeziza brassicae* isolates.

Isolate number	Isolate name	Crop	Cultivar	Location	Isolated by	Year	Mating type
1	Campari-L-1	Oilseed rape	Campari	Lincolnshire, England	Laura Sapelli	2021	MAT-1
2	Campari-L-2	Oilseed rape	Campari	Lincolnshire, England	Laura Sapelli	2021	MAT-1
3	Tenor-L-1	Oilseed rape	Tenor	Lincolnshire, England	Laura Sapelli	2021	MAT-1
4	Tenor-L-2	Oilseed rape	Tenor	Lincolnshire, England	Laura Sapelli	2021	MAT-1
5	Tenor-L-3	Oilseed rape	Tenor	Lincolnshire, England	Laura Sapelli	2021	Untested
6	Tenor-L-4	Oilseed rape	Tenor	Lincolnshire, England	Laura Sapelli	2021	MAT-2
7	Tenor-L-5	Oilseed rape	Tenor	Lincolnshire, England	Laura Sapelli	2021	MAT-1
8	Parkside-L-1	Oilseed rape	Parkside	Lincolnshire, England	Laura Sapelli	2021	MAT-2
9	Parkside-L-2	Oilseed rape	Parkside	Lincolnshire, England	Laura Sapelli	2021	MAT-1
10	Parkside-L-3	Oilseed rape	Parkside	Lincolnshire, England	Laura Sapelli	2021	MAT-2
11	Charger-H-1	Oilseed rape	Charger	Hertfordshire, England	Laura Sapelli	2021	MAT-2
12	Charger-H-2	Oilseed rape	Charger	Hertfordshire, England	Laura Sapelli	2021	Untested
13	Charger-H-3	Oilseed rape	Charger	Hertfordshire, England	Laura Sapelli	2021	MAT-2
14	Charger-H-4	Oilseed rape	Charger	Hertfordshire, England	Laura Sapelli	2021	MAT-2
15	Kale-H-1	Kale	Nero di Toscana	Hertfordshire, England	Laura Sapelli	2021	MAT-2
16	Kale-H-2	Kale	Nero di Toscana	Hertfordshire, England	Laura Sapelli	2021	Untested
17	Kale-H-3	Kale	Nero di Toscana	Hertfordshire, England	Laura Sapelli	2021	Untested
18	Kale-H-4	Kale	Nero di Toscana	Hertfordshire, England	Laura Sapelli	2021	MAT-2
19	Kale-H-5	Kale	Nero di Toscana	Hertfordshire, England	Laura Sapelli	2021	MAT-2
20	Kale-H-6	Kale	Nero di Toscana	Hertfordshire, England	Laura Sapelli	2021	MAT-2
21	Kale-H-7	Kale	Nero di Toscana	Hertfordshire, England	Laura Sapelli	2021	Untested
22	DK-Imp-Y-1	Oilseed rape	DK-Imprint	Yorkshire, England	Laura Sapelli	2021	Untested
23	DK-Imp-Y-2	Oilseed rape	DK-Imprint	Yorkshire, England	Laura Sapelli	2021	MAT-2
24	DK-Imp-Y-3	Oilseed rape	DK-Imprint	Yorkshire, England	Laura Sapelli	2021	MAT-2
25	Aqu-Here-1	Oilseed rape	Aquila	Herefordshire, England	Laura Sapelli	2021	MAT-2
26	Aqu-Here-2	Oilseed rape	Aquila	Herefordshire, England	Laura Sapelli	2021	MAT-2
27	Aqu-Here-3	Oilseed rape	Aquila	Herefordshire, England	Laura Sapelli	2021	Untested
28	Aqu-Here-4	Oilseed rape	Aquila	Herefordshire, England	Laura Sapelli	2021	Untested
29	Fla-Hunt22-1	Oilseed rape	Flamingo	Cambridgeshire, England	Laura Sapelli	2022	MAT-1
30	Fla-Hunt22-2	Oilseed rape	Flamingo	Cambridgeshire, England	Laura Sapelli	2022	MAT-1
31	Aqu-Hunt22-1	Oilseed rape	Aquila	Cambridgeshire, England	Laura Sapelli	2022	MAT-1
32	Aqu-Hunt22-2	Oilseed rape	Aquila	Cambridgeshire, England	Laura Sapelli	2022	Untested
33	Aqu-Hunt22-3	Oilseed rape	Aquila	Cambridgeshire, England	Laura Sapelli	2022	MAT-1
34	Fla-Here22-1	Oilseed rape	Flamingo	Herefordshire, England	Laura Sapelli	2022	MAT-1

35	Fla-Here22-2	Oilseed rape	Flamingo	Herefordshire, England	Laura Sapelli	2022	MAT-1
36	Fla-Here22-3	Oilseed rape	Flamingo	Herefordshire, England	Laura Sapelli	2022	MAT-1
37	Aqu-Here22-1	Oilseed rape	Aquila	Herefordshire, England	Laura Sapelli	2022	MAT-1
38	Aqu-Here22-2	Oilseed rape	Aquila	Herefordshire, England	Laura Sapelli	2022	Untested
39	Aqu-Here22-3	Oilseed rape	Aquila	Herefordshire, England	Laura Sapelli	2022	MAT-2
40	Ch-Herts23-1	Oilseed rape	Charger	Hertfordshire, England	Laura Sapelli	2023	MAT-1
41	Ch-Herts23-2	Oilseed rape	Charger	Hertfordshire, England	Laura Sapelli	2023	MAT-1
42	Fla-Here23-1	Oilseed rape	Flamingo	Herefordshire, England	Laura Sapelli	2023	MAT-1
43	Fla-Here23-2	Oilseed rape	Flamingo	Herefordshire, England	Laura Sapelli	2023	MAT-1
44	Fla-Hunt23-1	Oilseed rape	Flamingo	Cambridgeshire, England	Laura Sapelli	2023	MAT-1
45	Fla-Hunt23-2	Oilseed rape	Flamingo	Cambridgeshire, England	Laura Sapelli	2023	MAT-1
46	NR-Linc23-1	Oilseed rape	Unknown	Lincolnshire, England	Laura Sapelli	2023	MAT-2
47	NR-Linc23-2	Oilseed rape	Unknown	Lincolnshire, England	Laura Sapelli	2023	MAT-2
48	Lisa-Linc23-1	Oilseed rape	Lisabeth	Lincolnshire, England	Laura Sapelli	2023	MAT-2
49	Lisa-Linc23-2	Oilseed rape	Lisabeth	Lincolnshire, England	Laura Sapelli	2023	MAT-2
50	Man-Linc23-1	Oilseed rape	Manshold	Lincolnshire, England	Laura Sapelli	2023	MAT-2
51	Man-Linc23-2	Oilseed rape	Manshold	Lincolnshire, England	Laura Sapelli	2023	Untested
52	A1	Oilseed rape	Anastasia	Aberdeenshire, Scotland	Coretta Klöppel	2015	Untested
53	A2	Oilseed rape	Marathon	Aberdeenshire, Scotland	Coretta Klöppel	2015	Untested
54	A4	Brussels sprouts	NZ 16-616	Angus, Scotland	Coretta Klöppel	2013	Untested
55	A6	Brussels sprouts	NZ16-653	Berwickshire, Scotland	Coretta Klöppel	2013	Untested
56	A7	Brussels sprouts	Clodius	Fife, Scotland	Coretta Klöppel	2013	Untested
57	A9	Oilseed rape	Recital	Aberdeenshire, Scotland	Coretta Klöppel	2015	Untested
58	A11	Brussels sprouts	Braemer	Berwickshire, Scotland	Coretta Klöppel	2013	MAT-1
59	A14	Brussels sprouts	Clodius	Fife, Scotland	Coretta Klöppel	2013	Untested
60	A26	Oilseed rape	Cuillin	Aberdeenshire, Scotland	Coretta Klöppel	2015	Untested
61	A29	Oilseed rape	Cuillin	Aberdeenshire, Scotland	Coretta Klöppel	2013	Untested
62	A36	Oilseed rape	Cracker	Kincardineshire, Scotland	Coretta Klöppel	2013	Untested
63	A44	Oilseed rape	Alizze	Fife, Scotland	Coretta Klöppel	2014	Untested
64	A46	Oilseed rape	Rivalda	Aberdeenshire, Scotland	Coretta Klöppel	2013	Untested
65	A49	Oilseed rape	Catana	Aberdeenshire, Scotland	Coretta Klöppel	2013	Untested
66	A50	Oilseed rape	Unknown	Kinkardineshire, Scotland	Coretta Klöppel	2013	Untested
67	A51	Oilseed rape	Imola/Bristol	Aberdeenshire, Scotland	Coretta Klöppel	2016	Untested
68	19GERA1	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
69	19GERA3	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
70	19GERB1	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
71	19GERB2	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
72	19GERC1	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
73	19GERC2	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested

74	19GERC3	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
75	19GERC5	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
76	19GERD1	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
77	19GERD5	Oilseed rape	Unknown	Bleckendorf, Germany	Dr Kevin King	2019	Untested
78	19DEN03	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
79	19DEN04	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
80	19DEN05	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
81	19DEN09	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
82	19DEN16	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
83	19DEN19	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
84	19DEN31	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
85	19DEN33	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
86	19DEN35	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
87	19DEN39	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
88	19DEN41	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
89	19DEN47	Oilseed rape	Unknown	Skandeborg/Kerteminde, Denmark	Dr Kevin King	2019	Untested
90	20POL01	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
91	20POL02	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
92	20POL03	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
93	20POL04	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
94	20POL05	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
95	20POL07	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
96	20POL08	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
97	20POL09	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
98	20POL12	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
99	20POL14	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
100	20POL16	Oilseed rape	Unknown	West Pomerania, Poland	Dr Kevin King	2019	Untested
101	18CAR01	Oilseed rape	Unknown	Carlow, Ireland	Dr Kevin King	2018	Untested
102	18CAR02	Oilseed rape	Unknown	Carlow, Ireland	Dr Kevin King	2018	Untested
103	18CAR04	Oilseed rape	Unknown	Carlow, Ireland	Dr Kevin King	2018	Untested
104	18CAR05	Oilseed rape	Unknown	Carlow, Ireland	Dr Kevin King	2018	Untested
105	18CAR06	Oilseed rape	Unknown	Carlow, Ireland	Dr Kevin King	2018	Untested
106	T18P18	Oilseed rape	Unknown	Carlow, Ireland	Dr Nichola Hawkins	2018	Untested
107	T18U17	Oilseed rape	Unknown	Carlow, Ireland	Dr Nichola Hawkins	2018	Untested
108	T18U22	Oilseed rape	Unknown	Carlow, Ireland	Dr Nichola Hawkins	2018	Untested
109	T18U9	Oilseed rape	Unknown	Carlow, Ireland	Dr Nichola Hawkins	2018	Untested

Appendix J: List of 190 DY DH lines and their parental lines, and which glasshouse experiment (GH-E and/or GH-S) they were used in.

Line	GH-E	GH-S
DY001	x	x
DY002	x	x
DY004	x	x
DY006	x	x
DY007	x	x
DY008	x	x
DY009	x	x
DY016		x
DY017	x	
DY018	x	x
DY019		x
DY021	x	x
DY025		x
DY028	x	x
DY029	x	x
DY030	x	x
DY031	x	x
DY032		x
DY033	x	x
DY036	x	x
DY037	x	x
DY039	x	x
DY042	x	x
DY043		x
DY044	x	x
DY045		x
DY047	x	x
DY048	x	x
DY049		x
DY050	x	x
DY051	x	x
DY052	x	x
DY055	x	x
DY057	x	x
DY059	x	x
DY060		x
DY061	x	x
DY063	x	x
DY065	x	x
DY067	x	x
DY069	x	x
DY070	x	x
DY071	x	x
DY074		x
DY075	x	x
DY080	x	x
DY081		x
DY084	x	x

Line	GH-E	GH-S
DY085	x	x
DY086	x	x
DY087	x	x
DY088		
DY089	x	x
DY090	x	x
DY092	x	x
DY093	x	x
DY095	x	x
DY096	x	x
DY097	x	x
DY099	x	x
DY100	x	x
DY101	x	x
DY104	x	x
DY106	x	x
DY108	x	x
DY110	x	x
DY115		x
DY116	x	x
DY117	x	x
DY118	x	x
DY119		
DY120		x
DY121	x	x
DY122	x	x
DY123	x	x
DY124	x	x
DY125	x	x
DY126	x	x
DY127	x	x
DY128	x	x
DY129	x	x
DY130		x
DY132	x	x
DY133		x
DY134	x	x
DY135		x
DY138	x	x
DY139	x	x
DY141	x	x
DY143	x	
DY146	x	x
DY147	x	x
DY151	x	x
DY152	x	x
DY158	x	x
DY160	x	x

Line	GH-E	GH-S
DY161	x	x
DY162	x	x
DY164	x	x
DY169	x	x
DY170	x	x
DY172	x	x
DY176	x	x
DY180	x	x
DY181	x	x
DY182	x	x
DY185	x	x
DY186	x	x
DY190	x	x
DY192	x	
DY193	x	x
DY195	x	x
DY196	x	x
DY197	x	x
DY199	x	x
DY204	x	x
DY206	x	x
DY207	x	x
DY208	x	x
DY209	x	x
DY210	x	x
DY222	x	x
DY225	x	
DY226		
DY228	x	x
DY229		x
DY230	x	x
DY237	x	x
DY239	x	x
DY242	x	x
DY247	x	x
DY248	x	x
DY250	x	x
DY253		x
DY257	x	x
DY259	x	x
DY260	x	x
DY269	x	x
DY270	x	x
DY271	x	x
DY272	x	x
DY275		x
DY277	x	X
DY280	x	x

Line	GH-E	GH-S
DY283	x	x
DY284	x	x
DY285	x	x
DY288	x	x
DY292	x	x
DY298	x	x
DY303	x	x
DY308		x
DY309	x	x
DY310	x	x
DY314	x	x
DY315	x	x
DY317		
DY320	x	x
DY321	x	x
DY329	x	x
DY330	x	x
DY341	x	x
DY349	x	x
DY351	x	x
DY354	x	x
DY355	x	
DY357	x	x
DY363	x	x
DY365	x	x
DY366	x	x
DY371	x	x
DY372	x	x
DY373	x	x
DY377	x	x
DY383	x	x
DY390	x	x
DY391	x	x
DY393		x
DY394		
DY400	x	x
DY402	x	x
DY408	x	x
DY413	x	x
DY414	x	x
DY419		x
DY422	x	x
DY432	x	
DY433	x	x
DY436	x	x
DY445	x	x
Darmor-*bzh*	x	x
Yudal	x	x

Appendix K: DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population glasshouse experiment alpha design. Experiments were done in six batches and five replicates were used for each line.

Replicate 1	Blocks								
Plot	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	21	11	8	27	17	12	5	22	13
2	34	29	36	7	18	6	28	2	32
3	25	15	4	14	30	23	24	3	10
4	35	33	20	9	1	19	31	16	26

Replicate 2	Blocks								
Plot	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	7	29	36	5	4	10	35	3	21
2	2	32	19	9	6	24	28	26	31
3	33	20	34	15	17	12	16	11	13
4	25	1	14	18	22	27	30	23	8

Replicate 3	Blocks								
Plot	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	4	27	36	20	11	2	32	1	15
2	5	29	10	9	31	8	7	34	16
3	26	22	30	35	14	18	28	24	19
4	25	21	33	23	17	12	6	3	13

Replicate 4	Blocks								
Plot	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	27	29	23	9	2	12	28	8	13
2	1	30	5	31	17	35	11	6	24
3	16	26	21	33	10	14	34	3	7
4	25	19	36	22	20	32	18	15	4

Replicate 5	Blocks								
Plot	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	8	2	27	25	24	26	21	18	16
2	11	34	33	5	32	14	1	13	31
3	9	36	29	3	17	28	12	30	6
4	15	35	4	22	23	10	20	7	19

Batch 1		Batch 2		Batch 3		Batch 4		Batch 5		Batch 6	
ID	Number	ID	Number	ID	Number	ID	Number	ID	Number	ID	Number
DY043	1	Excel	1	DY182	1	Excel	1	DY164	1	DY127	1
DY209	2	DY037	2	DY002	2	DY018	2	DY146	2	DY349	2
Imola	3	Charger	3	Charger	3	DY033	3	DY104	3	DY186	3
Charger	4	DY141	4	DY086	4	DY028	4	DY237	4	DY075	4
DY045	5	DY135	5	DY292	5	Imola	5	DY108	5	DY436	5
DY114	6	DY259	6	DY250	6	DY044	6	Imola	6	Excel	6
DY351	7	DY272	7	DY195	7	DY422	7	Darmor	7	DY383	7
DY176	8	Imola	8	Yudal	8	DY106	8	Excel	8	DY025	8
DY130	9	DY115	9	DY357	9	DY193	9	DY181	9	DY190	9
DY315	10	DY310	10	DY133	10	DY093	10	DY119	10	DY123	10
DY321	11	DY172	11	DY197	11	DY257	11	DY092	11	DY225	11
DY052	12	DY099	12	DY413	12	DY303	12	DY129	12	Q02	12
DY088	13	DY096	13	DY152	13	DY004	13	DY132	13	DY081	13
Q02	14	DY147	14	DY074	14	DY126	14	DY260	14	DY354	14
DY402	15	DY097	15	Excel	15	DY355	15	Q02	15	DY288	15
DY047	16	DY001	16	DY226	16	DY277	16	DY228	16	DY377	16
DY061	17	DY160	17	DY373	17	DY196	17	DY050	17	Charger	17
DY283	18	DY065	18	DY118	18	DY128	18	DY007	18	DY036	18
DY055	19	DY320	19	DY419	19	DY394	19	DY208	19	DY269	19
DY390	20	DY089	20	DY009	20	DY125	20	DY408	20	DY365	20
DY048	21	DY124	21	DY139	21	DY117	21	DY222	21	DY121	21
DY180	22	DY371	22	DY158	22	DY248	22	DY049	22	DY366	22
DY341	23	DY239	23	DY090	23	DY057	23	DY210	23	DY059	23
DY229	24	DY314	24	DY063	24	DY161	24	DY204	24	DY192	24
DY008	25	DY085	25	DY100	25	Charger	25	DY298	25	DY116	25
DY242	26	DY060	26	DY042	26	DY230	26	DY030	26	DY329	26
DY122	27	DY006	27	Q02	27	DY330	27	DY414	27	DY070	27
DY169	28	Q02	28	DY032	28	DY270	28	DY391	28	DY080	28
DY393	29	DY087	29	DY253	29	DY284	29	Charger	29	DY206	29
DY120	30	DY372	30	DY138	30	DY363	30	DY101	30	DY143	30
Excel	31	DY271	31	Imola	31	Q02	31	DY207	31	Imola	31
DY247	32	DY445	32	DY051	32	DY067	32	DY275	32	DY199	32
DY031	33	DY134	33	DY151	33	DY170	33	DY084	33	DY162	33
DY400	34	DY280	34	DY071	34	DY021	34	DY019	34	DY432	34
DY433	35	DY309	35	DY285	35	DY317	35	DY095	35	DY017	35
DY069	36	DY016	36	DY185	36	DY039	36	DY308	36	DY029	36

Appendix L: List of DY (Darmor-*bzh* x Yudal) doubled haploid (DH) lines and their parental lines that showed a necrotic response (black flecking) in two glasshouse experiments (GH-E and GH-S).

Line	GH-E	GH-S
DY001	x	
DY002		
DY004		
DY006		
DY007	x	
DY008	x	x
DY009		x
DY018		x
DY021		
DY028	x	
DY029		x
DY030		x
DY031	x	x
DY033		x
DY036	x	x
DY037	x	x
DY039		x
DY042		x
DY044		
DY047	x	x
DY048	x	x
DY050		x
DY051		x
DY052	x	x
DY055	x	
DY057		x
DY059	x	x
DY061	x	x
DY063		x
DY065		x
DY067	x	
DY069	x	x
DY070	x	
DY071		x
DY075		
DY080	x	x
DY084	x	x
DY085	x	
DY086	x	
DY087	x	
DY089	x	x
DY090		x
DY092	x	x

Line	GH-E	GH-S
DY093		
DY095	x	x
DY096		x
DY097		x
DY099	x	x
DY100	x	x
DY101		x
DY104		x
DY106		x
DY108		x
DY110		
DY116	x	x
DY117		x
DY118		x
DY121	x	
DY122	x	x
DY123	x	x
DY124	x	x
DY125	x	
DY126		
DY127	x	x
DY128		
DY129		x
DY132		x
DY134	x	
DY138		
DY139	x	x
DY141		x
DY146		x
DY147		x
DY151		
DY152	x	x
DY158		x
DY160		
DY161	x	x
DY162		x
DY164	x	
DY169	x	x
DY170		
DY172		x
DY176	x	x
DY180	x	x
DY181		

Line	GH-E	GH-S
DY182		x
DY185	x	x
DY186	x	x
DY190		x
DY193		x
DY195		x
DY196	x	x
DY197		x
DY199		
DY204	x	x
DY206		
DY207		x
DY208	x	x
DY209	x	x
DY210	x	x
DY222		
DY228	x	x
DY230		x
DY237	x	x
DY239		
DY242	x	x
DY247	x	x
DY248	x	
DY250		x
DY257		x
DY259	x	x
DY260	x	x
DY269		x
DY270		x
DY271		
DY272	x	
DY277	x	x
DY280	x	x
DY283	x	x
DY284		x
DY285	x	x
DY288	x	x
DY292	x	x
DY298	x	x
DY303		x
DY308		x
DY309		
DY310	x	x

Line	GH-E	GH-S
DY314	x	x
DY315	x	x
DY320	x	x
DY321	x	x
DY329		x
DY330		x
DY341	x	x
DY349	x	x
DY351	x	x
DY354	x	x
DY357		x
DY363		x
DY365	x	x
DY366		
DY371		
DY372		
DY373		x
DY377		x
DY383		
DY390	x	
DY391		x
DY400	x	x
DY402	x	
DY408		x
DY413		x
DY414	x	x
DY422		
DY433	x	
DY436		x
DY445		x
Darmor-*bzh*		x
Yudal	x	x

Appendix M: Correlation graphs and frequency distribution graphs for light leaf spot symptoms in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in two glasshouse experiments (GH-E and GH-S) using the original (un-transformed) data.

Frequency distribution graphs were done by Dr Hao Wang at the Huazhong Agricultural University, China.

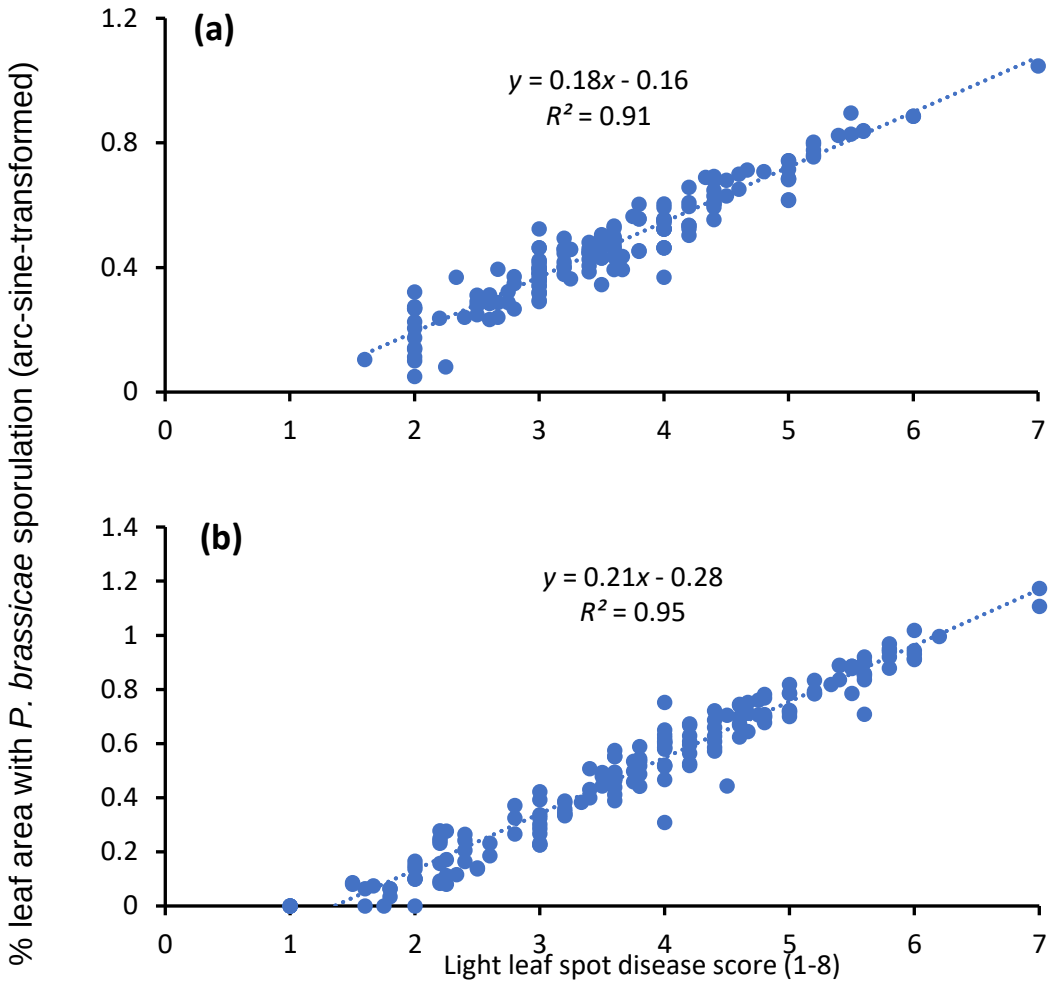


Figure M.1: Correlation between light leaf spot disease score (1-8) and percentage leaf area covered with *Pyrenopeziza brassicae* sporulation in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in two glasshouse experiments.

Lines of the DY DH mapping population were inoculated with two different *P. brassicae* populations in two separate glasshouse experiments (GH-E and GH-S). Plants were assessed for light leaf spot severity using a disease score on a 1-8 scale (where 1 is no disease) and by visual estimation of the % leaf area covered with *P. brassicae* sporulation after samples were incubated at 4°C for 7-9 days. Correlations are shown for the two assessment methods for (a) GH-E (sample correlation coefficient = 0.95, $P < 0.001$, $n = 165$) and (b) GH-S (sample correlation coefficient = 0.97, $P < 0.001$, $n = 179$). Percentage leaf area with sporulation values are presented on an arc-sine-transformed scale.

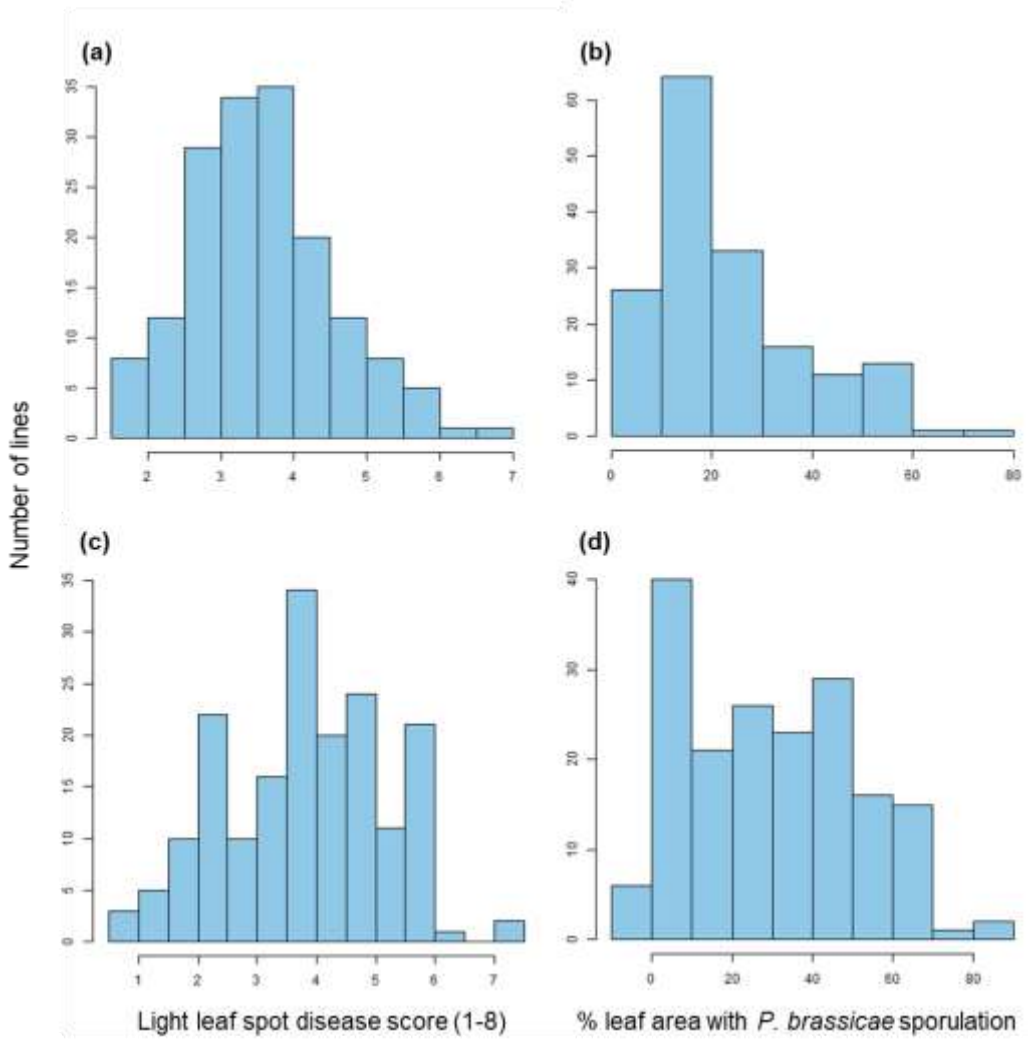


Figure M.2: Frequency distribution of light leaf spot disease score (1-8) and percentage leaf area covered with *Pyrenopeziza brassicae* sporulation in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in two glasshouse experiments.

Lines of the DY DH mapping population were inoculated with two different *P. brassicae* populations in two separate glasshouse experiments (GH-E and GH-S). Plants were assessed for light leaf spot severity using a disease score on a 1-8 scale (where 1 is no disease) and by visual estimation of the % leaf area covered with *P. brassicae* sporulation after samples were incubated at 4°C for 7-9 days. All traits (a & b) for GH-E and all traits (c & d) for GH-S conformed to a normal distribution.

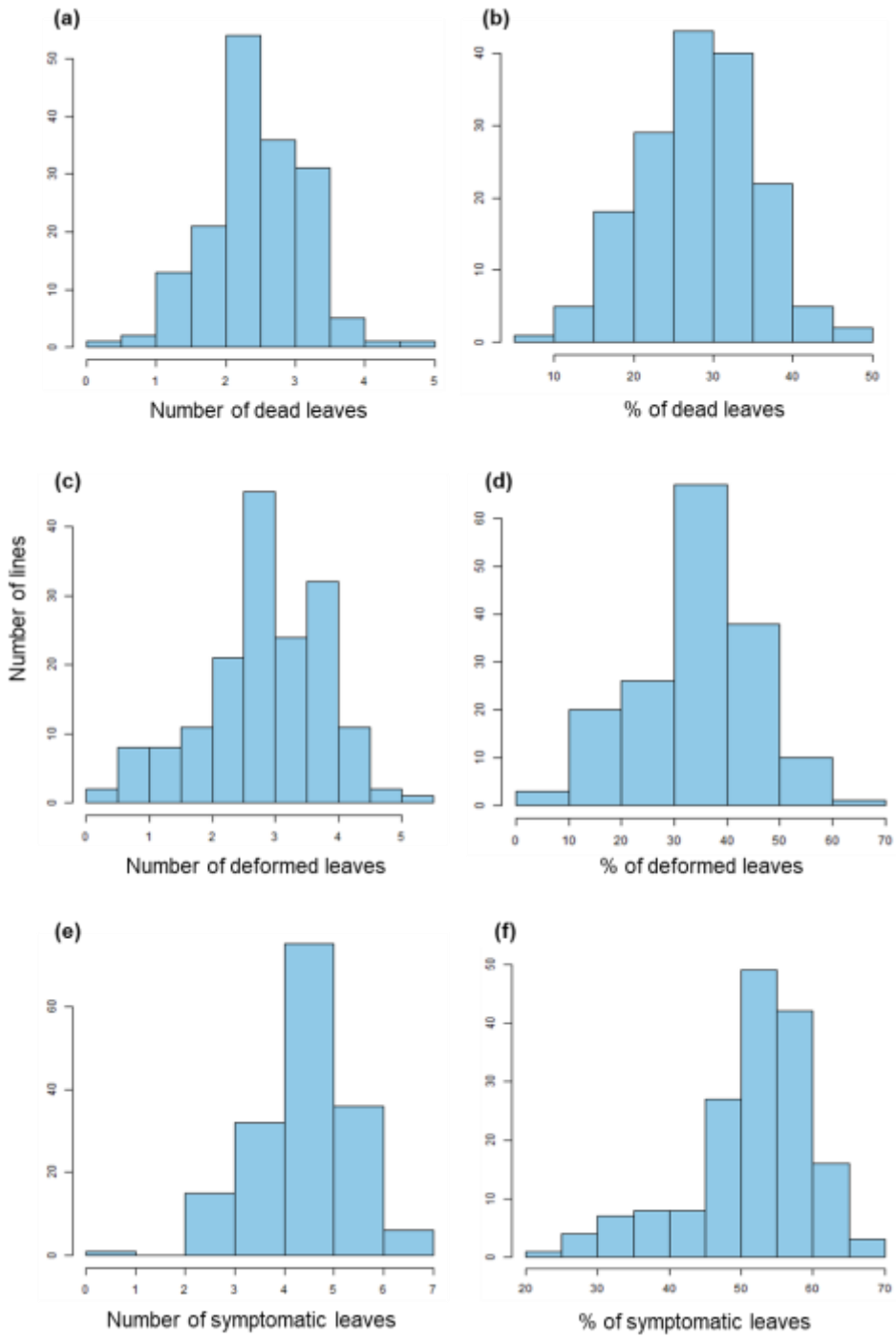


Figure M.3: Frequency distribution of light leaf spot (*Pyrenopeziza brassicae*) foliar symptoms in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in a glasshouse experiment (GH-E).

Lines of the DY DH mapping population were inoculated with a *P. brassicae* population from England (England-Pb, Table 2.1) in a glasshouse experiment (GH-E). Light leaf spot foliar symptoms were assessed. Measurements of (a & b) dead leaves, (c & d) deformed leaves and (e & f) symptomatic leaves conformed to a normal distribution.

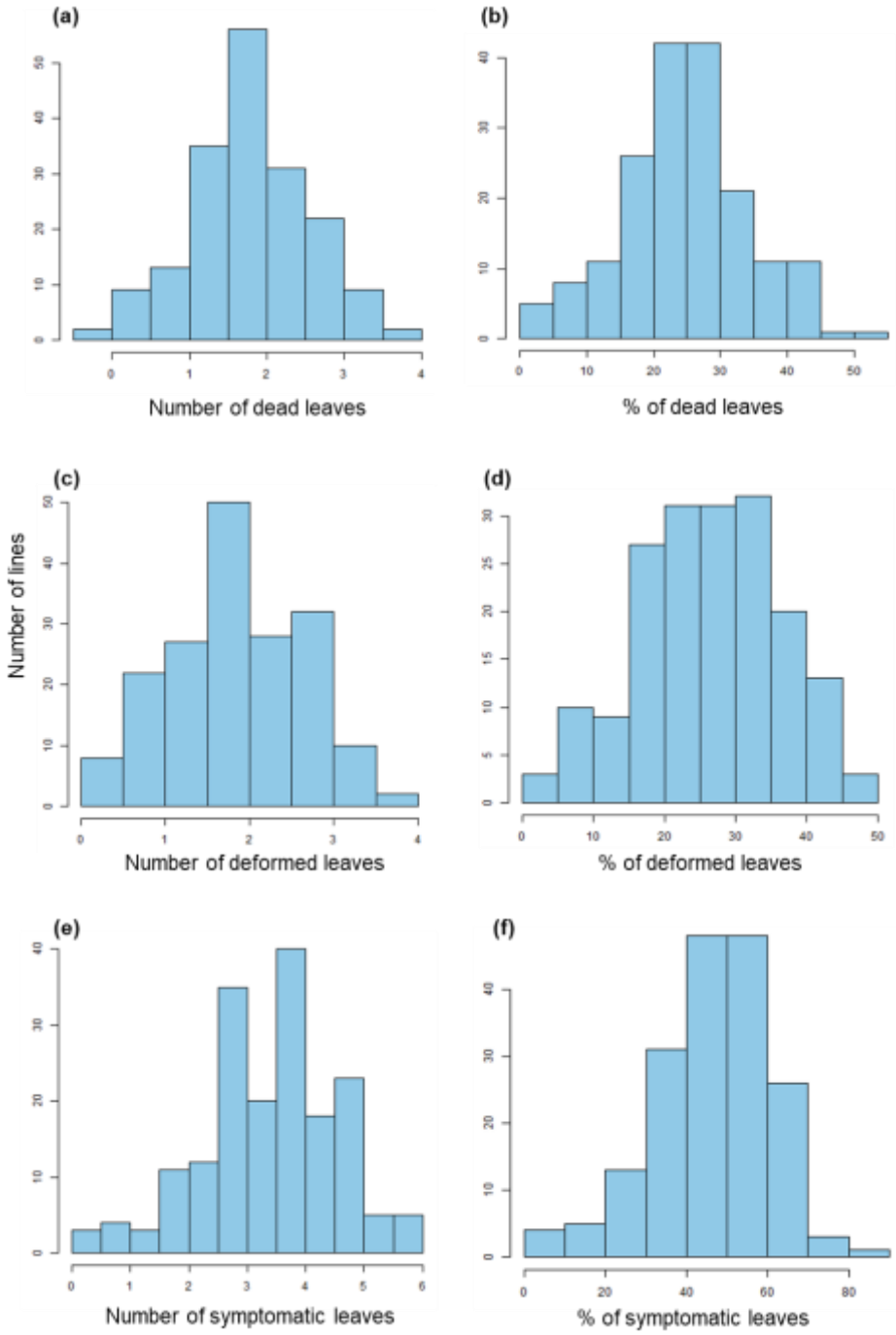


Figure M.4: Frequency distribution of light leaf spot (*Pyrenopeziza brassicae*) foliar symptoms in the DY (Darmor-*bzh* x Yudal) doubled haploid (DH) mapping population in a glasshouse experiment (GH-S).

Lines of the DY DH mapping population were inoculated with a *P. brassicae* population from Scotland (Scotland-Pb, Table 2.1) in a glasshouse experiment (GH-S). Light leaf spot foliar symptoms were assessed. Measurements of (a & b) dead leaves, (c & d) deformed leaves and (e & f) symptomatic leaves conformed to a normal distribution.

Appendix N: QTL for resistance against *Pyrenopeziza brassicae* (light leaf spot) detected in glasshouse experiments within the oilseed rape DY DH mapping population determined by composite interval mapping analysis using the original (un-transformed) data.

Experiment*	Quantitative trait	Linkage group†	QTL position (cM)‡	Support interval (cM)	Peak LOD	R^2 (%)§	Additive effect
GH-E	Nr dead leaves	A03	125.5	125.3-125.7	2.6	7.1	0.18
		C02	42.0	41.4-42.7	2.8	7.9	0.19
	Nr deformed leaves	A04	61.3	60.8-61.8	4.1	10.7	-0.29
		C01	43.6	43.3-43.9	3.6	9.4	0.27
		C02	36.3	35.7-37	3.0	7.8	0.25
	Nr symptomatic leaves	C02	42.8	42.7-43	2.5	6.7	0.25
	% dead leaves	C02	42.8	42.7-43	2.7	7.5	1.98
	% deformed leaves	C01	53.6	53.5-53.8	3.4	9.8	3.29
		C02	19.5	19.4-19.7	2.9	8.3	3.05
	% symptomatic leaves	C01	53.3	53.2-53.5	2.5	6.8	2.26
	Light leaf spot disease score	A09	122.5	122.3-122.8	2.6	7.1	0.27
GH-S	Nr dead leaves	A03	126.6	126.3-126.9	3.0	7.9	0.22
		C01	53.6	53.5-53.8	2.8	7.4	-0.21
	Nr deformed leaves	A06	113.7	113.5-113.9	6.6	16.9	0.31
		C06	52.3	51.9-52.7	2.8	6.9	0.22
		C08	14.4	12.8-16.1	3.2	7.7	-0.21
	% deformed leaves	A06	113.7	113.5-113.9	4.5	10.9	3.32
		C08	3.5	3.4-3.7	3.8	9.2	-3.05

* Phenotypic data from the DY DH population were collected from two separate glasshouse experiments: GH-E, where plants were inoculated with a *P. brassicae* population from England and GH-S, where plants were inoculated with a *P. brassicae* population from Scotland

† Linkage groups are labelled according to the standard *Brassica napus* chromosome/linkage group A1–A10 and C1–C9 designations agreed by the Multinational *Brassica* Genome Project (MBGP) Steering Committee (http://www.brassica.info/resource/maps/lg-assignments.php)

‡ The marker closest to the position of the maximum effect of the QTL

§ Proportion (%) of the phenotypic variance explained by the QTL