ORIGINAL ARTICLE

Interactions in the *Brassica napus–Pyrenopeziza brassicae* pathosystem and sources of resistance to *P. brassicae* (light leaf spot)

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Abstract

Pyrenopeziza brassicae, cause of light leaf spot (LLS), is an important pathogen of oilseed rape and vegetable brassicas and has a wide geographic distribution. Exploitation of host resistance remains the most sustainable and economically viable solution for disease management. This study evaluated 18 oilseed rape cultivars or breeding lines for host resistance against *P. brassicae* in glasshouse experiments. Selected cultivars/lines were inoculated with eight single-spore isolates of the pathogen obtained from three different regions in England. Analysis of *P. brassicae* infection-related changes on host plants identified leaf deformation as a characteristic feature associated with *P. brassicae* infection, this showed poor correlation to LLS severity measured as the amount of pathogen sporulation on infected plants. Resistant host phenotypes were identified by limitation of *P. brassicae* sporulation, with or without the presence of a necrotic response (black flecking phenotype). Investigation of this pathosystem revealed significant differences between cultivars/lines, between isolates, and significant cultivar/line-by-isolate interactions. In total, 37 resistant and 16 moderately resistant interactions were identified from 144 cultivar/line-by-isolate interactions using statistical methods. Most of the resistant/moderately resistant interactions identified in this study appeared to be nonspecific towards the isolates tested. Our results suggested the presence of isolate-specific resistant interactions for some cultivars. Several sources of resistance have been identified that are valuable for oilseed rape breeding programmes.

KEYWORDS
crop losses, differential interactions, fungal pathogens, isolate-specific resistance, oilseed rape

1 | INTRODUCTION

Pyrenopeziza brassicae (anamorph *Cylindrosporium concentricum*), causing light leaf spot (LLS), is an economically important fungal pathogen of oilseed rape (*Brassica napus*) and other *Brassica* species. It thrives in areas with cool temperate climates (Figueroa et al., 1995) and occurs in many regions of the world, from the UK and continental Europe to Japan, New Zealand (Boys et al., 2007; Karandeni Dewage et al., 2018; Karolewski et al., 2012) and North America (Carmody et al., 2020). Isolates of *P. brassicae* in North America are considered as a different

lineage from the isolates found in other geographic regions (Carmody et al., 2020). *P. brassicae* is a polycyclic pathogen and can be present throughout the growing season, affecting crops at all plant growth stages (Gilles et al., 2001). Severe LLS can cause up to 30% yield reduction of winter oilseed rape (Agriculture and Horticulture Development Board, 2021). In vegetable brassicas, apart from yield losses, quality of the fresh produce can also be severely affected, reducing their market value (Karandeni Dewage et al., 2018).

Currently, LLS is considered as the most economically damaging foliar disease of oilseed rape in the UK. Severity of LLS epidemics has increased in recent years and it has replaced phoma stem canker (*Leptosphaeria maculans*) as the main disease of winter oilseed rape in the UK (Karandeni Dewage et al., 2018). Therefore, LLS has become one of the main targets for fungicide applications and for oilseed rape breeding programmes. However, various constraints associated with fungicide applications, such as poor timing (Gilles et al., 2000), development of fungicide insensitivity within *P. brassicae* populations (Carter et al., 2013) and legislative and environmental concerns (Hillocks, 2012) make chemical control of LLS unreliable. Therefore, the use of resistant cultivars, which is cost-effective and environmentally safe, remains an important alternative to fungicide application. However, limited understanding of the mechanism of host resistance against *P. brassicae* hinders the potential success of the deployment of cultivar resistance to control LLS. Often, oilseed rape breeders have to rely on sources of host resistance without knowledge of their genetic background when breeding for resistant cultivars.

Cultivation of resistant cultivars without knowledge about their genetic background can lead to growing of cultivars with the same resistance gene/s over a long period of time. This can ultimately exert selection on local pathogen populations, leading to the build-up of virulent pathogen races that reduces the efficacy of corresponding resistance genes over time (McDonald & Linde, 2002). For example, a sudden change in cultivar Bristol from resistance to susceptibility has been reported following its extensive cultivation across the UK (Boys et al., 2007). Additionally, there have been recent records of the breakdown of resistance in some cultivars with good resistance ratings (based on UK Agriculture and Horticulture Development Board (AHDB) recommended list [RL] ratings) for the north region of the UK (Karandeni Dewage et al., 2018). Therefore, it is important to identify and differentiate cultivar resistance against *P. brassicae* and to monitor the frequency of virulent pathogen races. This enables the spatial and temporal deployment of different sources of cultivar resistance to increase their durability.

Host resistance against *P. brassicae* can be either isolate-specific (qualitative) or nonspecific (quantitative). There have been several studies that focused on analysing the genetic basis of resistance in oilseed rape against *P. brassicae*. Some of these studies have used pathogen populations, in the form of natural ascospore (sexual spore) inoculum or conidia (asexual spores) of isolate mixtures taken from diseased leaf samples (Boys et al.,

2012; Bradburne et al., 1999; Pilet et al., 1998). A very few studies have been performed with single-spore isolates of *P. brassicae*, including the assessment of seedling resistance of *B. napus* (Bradburne et al., 1999; Karolewski, 1999) and cross-infectivity of *P. brassicae* between different host *Brassica* species (Boys, 2009; Maddock et al., 1981). Simons and Skidmore (1988) have provided experimental evidence for the presence of differential interactions (specific resistance and corresponding virulence) between *Brassica oleracea* and *P. brassicae*. However, there have been no further studies on isolate-specific resistance against the LLS pathogen since then. The limited number of experiments with individual isolates limits the knowledge of the genetic basis of resistance against *P. brassicae*, which is important for successful deployment of cultivar resistance.

One of the challenges associated with investigating isolate-specific interactions in the *Brassica napus–P. brassicae* pathosystem is the detection and quantification of phenotypic changes related to *P. brassicae* infection. In general, LLS severity assessments are based on the amounts of *P. brassicae* acervuli (asexual sporulating structures) on the plants, measured as the percentage area covered with sporulation or using disease severity assessment scales (Boys et al., 2012; Bradburne et al., 1999; Karolewski, 1999; Pilet et al., 1998). Although some other changes linked with *P. brassicae* infection such as necrotic responses (Boys et al., 2012; Bradburne et al., 1999), leaf deformations (e.g., leaf curling, leaf distortion, petiole elongation), and stunting of the plants (Ashby, 1997, 2000) have been reported, these features have not been directly accounted for in current LLS assessments. It is also not clear if and how these changes are linked with each other and whether they reflect different defence mechanisms against *P. brassicae*. Therefore, it is essential to investigate these resistance/susceptibility phenotypes to gain a basic understanding of *B. napus–P. brassicae* interactions and to improve cultivar resistance through selective breeding for effective management of LLS.

The experimental work described in this paper, which used single-spore isolates of *P. brassicae*, aimed to investigate specific host–isolate interactions and different phenotypic changes associated with LLS to aid the identification and selection of different sources of host resistance. A better understanding of these aspects can lead to more effective management of LLS.

2 | MATERIALS AND METHODS

To investigate *B. napus* cultivar/line interactions with single-spore *P. brassicae* isolates, two glasshouse experiments were performed. The experiments had four replicates and were arranged in split-plot designs. In the first experiment, there were 10 *B. napus* cultivars/lines tested against eight *P. brassicae* isolates. In the second experiment, the same eight isolates were tested against another eight cultivars/lines together with four control cultivars. In both experiments, there were the same resistant and susceptible control

cultivars/lines, so that the results of both experiments could be analysed together.

2.1 | Selecting *P. brassicae* isolates

The single-spore (single-conidial) isolates of *P. brassicae* were obtained from oilseed rape crops. Diseased *B. napus* leaves were collected and incubated at 4°C for 5 days in the dark to induce *P. brassicae* asexual sporulation. After incubation, single acervuli were harvested from leaves using a sterile needle and placed in a 0.5 ml microfuge tube containing 30 μl of sterilized distilled water. Tubes were vortexed briefly to liberate the conidia and the spore suspension was placed onto a potato dextrose agar plate and spread across the plate using a sterile L-shaped plastic spreader (Sterilin). Inoculated plates were incubated at 15°C in the dark for 2 days and observed for spore germination. Plates were further incubated for 1 week and single-spore isolates were selected for subculturing. A mycelial plug was taken from each selected *P. brassicae* isolate and placed in a 2 ml Eppendorf tube containing 200 μl of sterilized distilled water. The mycelial plug was ground using a sterilized plastic pestle and macerated mycelia were inoculated onto malt extract agar plates (100 μl inoculum per plate). Inoculated plates were incubated at 15°C in the dark for 3 weeks for spore (conidia) production.

Spore suspensions were prepared by adding 10 ml of sterilized distilled water onto each of the *P. brassicae* culture plates. All the mycelia were scraped off and macerated in water using a sterile L-shaped plastic spreader and then left for 5 min to liberate spores. Spore suspensions were filtered through sterile Miracloth (Calbiochem) into 50 ml Falcon tubes and a haemocytometer (Bright-Line; Hausser Scientific) was used to determine the spore concentration. Spore suspensions from single-spore isolates were diluted to obtain the required concentration of 10^5 spores/ml and stored at −20°C until needed. A total of 18 single-spore isolates of *P. brassicae* were obtained from diseased leaf samples collected in 2014/2015 or 2015/2016 cropping seasons and eight isolates were selected based on their cultivar of origin and geographic location in the UK (Table 1).

2.2 | Selecting *B. napus* cultivars/lines

A set of 18 oilseed rape cultivars/lines was selected. Selection of commercial oilseed rape cultivars was based on the resistance rating against *P. brassicae* in AHDB RL trials. Cultivars Bristol and Marathon were added as the susceptible controls and cultivars Imola and Cuillin were resistant controls, included in both experiments. In addition to cultivar Imola, which harboured a major gene locus for resistance, three lines were selected from a doubled haploid (DH) population and parents of two other DH populations with diverse genetic backgrounds were also included. Detailed descriptions of each cultivar/line, along with specific reasons for selecting it, are given in Table S1.

2.3 | Plant growth and inoculation

Seeds of the selected *B. napus* cultivars/lines were pregerminated at 20°C in the dark for 48 h in a Petri dish containing a damp filter paper. Pregerminated seeds were then sown in 40-cell seed trays filled with compost prepared by mixing Miracle-Gro all-purpose compost (The Scotts Company) and John Innes No. 3 (LBS Horticulture) in a 1:1 ratio. After sowing, the compost surface was covered with a thin layer of vermiculite to maintain its moisture. Seed trays were placed in a plastic outer tray containing a capillary mat and watered regularly from underneath. They were maintained in a controlled-environment cabinet (FITOCLIMA D1200; ARALAB) with a 12 h day-length at 250 μmol·m^{-2}·s^{-1}, 60% relative humidity and 20°C day/18°C night temperatures until seedlings reached growth stage 1,0–1,1 (i.e., cotyledons unfolded and first true leaf emerged; Sylvester-Bradley & Makepeace, 1984).

Seedlings were then transplanted into 9 cm diameter pots containing the same compost mixture and transferred to a temperature-regulated glasshouse at 16°C day/14°C night temperatures with natural daylight supplemented by a 12 h photoperiod. Plants were arranged in a split-plot design generated using Experiment Design Generator and Randomiser (EDGAR) (Brown, 2004). *P. brassicae* isolates were randomly assigned to main plots (blocks) and cultivar/

TABLE 1 List of *Pyrenopeziza brassicae* isolates, their origin and mating type information, that were used to investigate the interactions with *Brassica napus*

| Isolate name | Origin | | Year | Mating type |
	Oilseed rape cultivar	Location		
15WOSR64-SS1	Bristol	Hereford, Herefordshire	2015	*MAT1-1*
15WOSR81-SS1	Temple	Hereford, Herefordshire	2015	*MAT1-2*
17WOSR-I1[a]	Imola	Morley, Norfolk	2016	*MAT1-2*
15WOSR76-SS2	Cracker	Hereford, Herefordshire	2015	*MAT1-2*
15WOSR78-SS1	Anastasia	Hereford, Herefordshire	2015	*MAT1-2*
17WOSR-I4[a]	Imola	Morley, Norfolk	2016	*MAT1-2*
15WOSR5.2–SS2	Catana	Boxworth, Cambridgeshire	2015	*MAT1-2*
17WOSR-CUI[a]	Cuillin	Morley, Norfolk	2016	*MAT1-2*

[a]These isolates were obtained by reinoculating a field *P. brassicae* population collected from Morley, Norfolk, UK onto various oilseed rape cultivars/lines in a glasshouse experiment.

line treatments were randomly assigned to the subplots (pots) within each of the main plots. Four replicate plants from each cultivar/line were included for each of the isolates. At the 1,4–1,5 growth stage, plants were spray-inoculated with conidial suspensions (10^5 spores/ml) incorporated with 0.005% Tween 80 just before the inoculation. Average spray volume of 1.2 ml per plant was applied until leaves were fully covered with fine droplets of conidial suspensions. Main plots (blocks) were kept individually covered with clear polyethylene sheets during inoculation to prevent possible cross-contamination and inoculated plants were covered for 48 h after inoculation to maintain high humidity to facilitate spore germination and infection. Because there was a large number of cultivar-by-isolate combinations included in this experiment (8 isolates × 18 cultivars/lines), tests were done on two occasions with four appropriate resistant or susceptible controls included on each occasion (Table S1) to monitor the uniformity of experimental conditions being provided.

2.4 | LLS assessment

Plants were monitored regularly and the timing and appearance of *P. brassicae* infection-related changes were recorded. At 24 days postinoculation (dpi), plants were destructively harvested by cutting at the stem base above the compost surface, individually placed in polyethylene bags with a dampened paper towel and incubated at 4°C for 5 days to induce sporulation. The final disease assessment was made at 29 dpi. Disease severity was measured as percentage leaf area covered with *P. brassicae* sporulation (acervuli) and plants were also given a LLS score using a 1–6 scale, where 6 was most susceptible; Table S2). In addition, the number of leaves with deformations (leaf curling, leaf distortion, petiole elongation) and the presence of necrotic responses were recorded for each plant.

2.5 | Data analysis

Analysis of variance (ANOVA) was based on a standard split-plot design in which the isolate treatments were the main plots and the cultivar/line treatment was randomly arranged in each main plot. The residual error from the main plot (i.e., error A) was used to test the effect of isolate and to calculate the least significant difference (LSD) to compare the differences between isolates. The residual error from the subplot (i.e., error B) was used to test the effect of cultivar/line and the interaction of isolate × cultivar/line, and to calculate the LSD to compare the differences between cultivars/lines and between combinations of the two-way interactions. These data analyses on different trait measurements were completed using GENSTAT 18th edition statistical software for Windows (Payne et al., 2011). LLS severity (percentage leaf area covered with *P. brassicae* sporulation) and leaf deformation (percentage leaves deformed, calculated using the number of leaves with deformations and the total number of leaves per plant) data were transformed by taking the arcsine of the

square root of the proportion value (Sokal & Rohlf, 1995), so that the variance was more homogeneous across treatments and measurements were normally distributed, before the ANOVA was done. If the *F* test showed a significant effect of any factor, the standard error of the difference and the LSD were calculated and presented at a probability level of 5% ($p \leq 0.05$). For analysis of the relationships between different measures (LLS score, percentage leaf area covered with *P. brassicae* sporulation, and percentage leaves deformed), simple linear regression analyses were done using calculated means for different cultivars/lines. Differences between different cultivars/lines or different isolates were tested using comparative analysis of position and parallelism of linear regression.

Two parameters were used to assess LLS severity: percentage leaf area with *P. brassicae* sporulation (acervuli) and disease score on 1–6 scale. There was a positive correlation between the two parameters (Figure S1). However, percentage leaf area with acervuli appeared to be a better quantitative measure than disease score when assessing the differences in disease severity, especially at the high disease severity levels, because acervuli can appear without any lesions associated with them. Therefore, the percentage leaf area with sporulation was taken as the preferred parameter for analysing cultivar/line-by-isolate interactions. Resistant interactions between oilseed rape cultivars/lines and *P. brassicae* isolates were identified by calculating LSD values of the means of cultivar/line-by-isolate interactions at probability levels of 1% and 5% ($p \leq 0.01$ and $p \leq 0.05$, respectively; Cherif et al., 2007; Ghaneie et al., 2012). Based on the transformed LLS severity (percentage leaf area with *P. brassicae* sporulation) values, the smallest mean value (0%) was used as the resistant control and, with reference to that, the cultivar/line-by-isolate interactions with mean values that were not greater than LSD values at 1% (LSD = 16.57) and 5% (LSD = 12.59) probability levels were identified as moderately resistant and resistant, respectively.

Interactions between *B. napus* genotypes and *P. brassicae* isolates were further analysed using the linear regression analysis described by Ghazvini and Tekauz (2008) to identify differential responses. Resistant/susceptible responses of the *B. napus* genotypes (different cultivars/lines) measured quantitatively as the percentage leaf area with *P. brassicae* sporulation were analysed using the regression model expressed as:

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

where, in this study, $\underline{Y}_{ij}$ is the mean percentage leaf area with sporulation of the *i*th cultivar on the *j*th isolate, μ_i is the mean percentage leaf area with sporulation 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 percentage leaf area with sporulation of all cultivars/lines with the *j*th isolate minus the grand mean, and δ_{ij} is the sum of deviations from regression of the *i*th cultivar/line with the *j*th isolate. Regression slope parameter and deviation mean squares for each cultivar/line were taken as measures of disease severity response
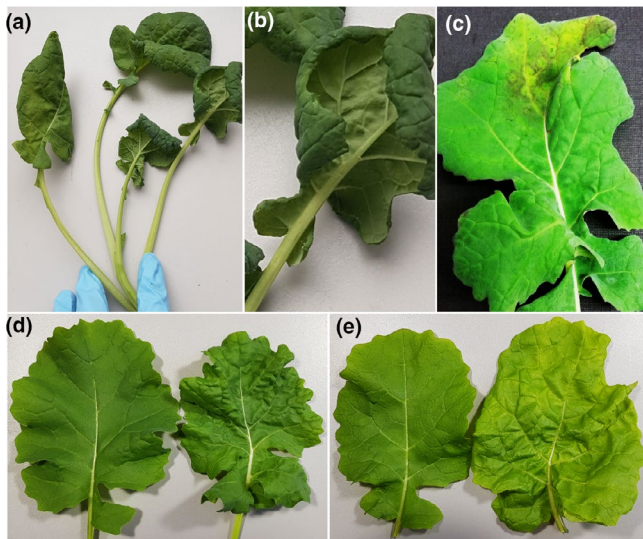
towards increased isolate virulence and the specificity of the resistance/susceptibility of the cultivars/lines, respectively.
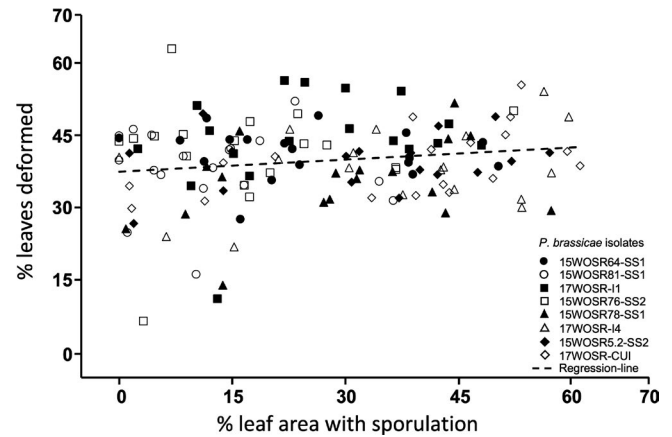
3 | RESULTS

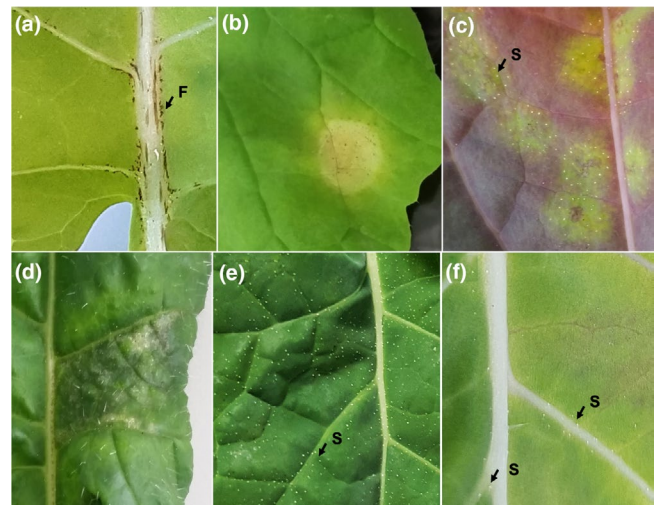### 3.1 | Symptoms of *P. brassicae* infection on host plants

According to the observations made on *P. brassicae* infection-related phenotypic changes in host plants, the first visible sign of infection of plants was leaf deformations that appeared at c.7 dpi. Such deformation included leaf curling, leaf distortions, and petiole elongations (Figure 1a–c) and could be seen on both resistant and susceptible cultivars/lines (Figure 1d,e). Interestingly, leaf deformation incidence (percentage leaves deformed) did not correlate with LLS severity measured as percentage leaf area with *P. brassicae* sporulation (Figure 2) or with the disease score, even though leaf deformations were more prominent on some cultivars/lines than on the others. There was no immediate cell death or hypersensitive response upon *P. brassicae* infection in any of the cultivars/lines. However, some of the cultivars/lines produced a necrotic response (black necrotic flecking) that became visible at c.10–14 dpi (Figure 3a). Black flecking was more prominent along the midribs and lateral veins but was also present on the leaf lamina. *P. brassicae* asexual sporulation (acervuli) was first observed at 16–18 dpi and occasionally there were yellowish, pale-green, or grey coloured patches observed on the leaf lamina (Figure 3b–d). Acervuli were mostly observed with no lesions

FIGURE 2 Relationship between percentage leaves deformed (y) and light leaf spot severity measured as the percentage leaf area covered with *Pyrenopeziza brassicae* asexual sporulation (x). Regression of light leaf spot severity (percentage leaf area with *P. brassicae* sporulation) against percentage leaves deformed for each of the cultivar/line-by-isolate combinations (arcsine back-transformed data) for eight *P. brassicae* isolates used to study the relationships between these two parameters in glasshouse experiments with 18 cultivars/lines. Analysis showed that there was no significant correlation ($p = 0.14$) for all eight isolates tested. ($R^2 = 0.02$, $n = 144$)

FIGURE 1 Leaf deformations associated with *Pyrenopeziza brassicae* infections in oilseed rape plants inoculated with single-spore isolates of *P. brassicae* in glasshouse experiments. (a) Petiole elongation on cv. Darmor, (b) leaf curling on Darmor, (c) leaf distortion on cv. Bristol, (d) leaf deformation on susceptible cv. Excel (right) compared to an uninoculated control (left), (e) leaf deformation on resistant cv. Imola (right) compared to an uninoculated control (left). Pictures (a–c) were taken at 24 dpi. Pictures (d) and (e) were taken at 7 dpi. dpi, days postinoculation

FIGURE 3 Necrosis, leaf lesions, and sporulation associated with *Pyrenopeziza brassicae* infections in oilseed rape plants inoculated with single-spore isolates of *P. brassicae* in glasshouse experiments. (a) Necrosis (black necrotic flecking) (F) on cv. Imola, (b) yellowish lesion on cv. Darmor, (c) green islands on senescing leaves associated with *P. brassicae* sporulation (S) on cv. Marathon, (d) grey lesion on line Q83, (e) *P. brassicae* sporulation on cv. Hearty with no visible lesions, (f) line Q2 showing limited *P. brassicae* sporulation confined mainly to and along the midrib and lateral veins of a leaf. All the pictures were taken at 29 dpi. dpi, days postinoculation

associated with them (Figure 3e) and appeared in concentric ring-like patterns. On resistant cultivars/lines, acervuli observed were mostly confined to and along the midribs and lateral veins (Figure 3f).

3.2 | LLS severity; cultivar/line-by-isolate interactions

Analysis of variance indicated significant differences among cultivars/lines, and significant interactions between oilseed rape cultivars/lines and *P. brassicae* isolates ($p < 0.01$; Table S3). There was a large main effect of cultivar/line, indicating that the differences between different host genotypes accounted for much of the variation. Of the 18 cultivars/lines tested, 14 were either resistant or moderately resistant to at least two isolates and the remaining four cultivars (Excel, Harper, Hearty, and Darmor) were very susceptible (Table 2). Imola and Q69 showed resistant or moderately resistant interactions with all isolates. Other cultivars/lines with resistant or moderately resistant interactions with most isolates were Q2 with seven isolates, Q83 with six isolates, and Cuillin with four isolates. In total, there were 37 resistant and 16 moderately resistant interactions identified from 144 cultivar/line-by-isolate interactions. In addition, several interactions resulted in low mean disease severity (<30% leaf area with sporulation), indicating partial resistance.

The overall mean LLS severity calculated for each cultivar/line across all the isolates reflected the general level of resistance of these cultivars/lines against the *P. brassicae* isolates tested. The most resistant cultivar was Imola, followed by the DH lines Q2, Q69, Q83, and cultivars Yudal and Cuillin. Interestingly, cultivars Excel, Hearty, and Harper, with the *Rlm7* gene that is very effective against *Leptosphaeria maculans* (phoma stem canker) (Mitrousia et al., 2018), showed some of the greatest LLS severity values (44.9%, 42.9%, and 45.5% leaf area with sporulation, respectively). Cultivar Darmor had the greatest percentage leaf area with sporulation (45.7%) when considering the overall response across all the isolates.

On examination of the necrotic responses, cultivar Imola and the DH line Q83 showed a black necrotic flecking phenotype against all the isolates and cultivar Yudal showed black necrotic flecking against seven out of the eight isolates tested. Cultivars Bristol, Trinity, Cabriole', Ningyou7, and the DH lines Q69 and Q2 also produced black necrotic flecking against at least one isolate (Table 2). In total, there were 30 cultivar/line-by-isolate interactions with black necrotic flecking. Of these, 70% ($n = 21$) were associated with resistant or moderately resistant interactions, 23% ($n = 7$) with partial resistance, and 7% ($n = 2$) with susceptible interactions. Considering the mapping population parents that were included, the most remarkable difference was observed between Darmor and Yudal, parents of the DY DH population, in terms of both LLS severity and production of necrotic responses (Figure 4). Yudal had resistant interactions with three isolates and necrotic responses with seven isolates compared to Darmor with no resistant interactions or necrotic responses. The overall mean LLS severity of Darmor calculated across all the isolates was significantly greater than that of Yudal. Parents of the TN DH population, Tapidor and Ningyou7, also differed in their interactions with some of the isolates, even though there was no statistically significant difference in the overall mean LLS severity between the two cultivars (Table 2).

Moreover, we detected significant differences in virulence between *P. brassicae* isolates ($p < 0.01$) on different oilseed rape cultivars/lines; isolate mean ranged from 12.3% to 37.0% leaf area with sporulation (Table 2). Isolate 15WOSR81-SS1 (that originated from cultivar Temple in Herefordshire, UK) was the least virulent, with only two susceptible interactions (>30% leaf area with sporulation). There were 13 resistant or moderately resistant interactions against this isolate and it was recorded to have the greatest number ($n = 6$) of necrotic interactions (black necrotic flecking). Isolates 17WOSR-I4 and 17WOSR-CUI (that originated in Norfolk, UK, from cultivars Imola and Cuillin, respectively) were the most virulent, with 13 susceptible interactions each. There was no significant difference in the overall mean LLS severity between these two isolates.

To identify the differential cultivar/line-by-isolate interactions, different levels of resistant/susceptible responses of cultivars/lines against different *P. brassicae* isolates were analysed using the regression parameters calculated. The mean percentage leaf area with sporulation, regression slope parameters, and deviation mean squares (MS) of cultivars/lines in relation to isolate virulence index are given in Table S4. Regression slope and the deviation MS were plotted against mean percentage leaf area with *P. brassicae* sporulation (mean disease severity) of cultivars/lines (Figure 5). According to the results, Imola, Q69, Yudal, Q63 and Q2 appeared to have smaller regression coefficients compared to the rest of the cultivars/lines (Figure 5a), indicating that these host genotypes may be less sensitive to the increased general virulence of *P. brassicae* isolates. In contrast, cvs Bristol, Temple, Cracker, Excel, Marathon, and Cuillin had greater regression coefficients, suggesting that disease severity on them increased with increasing virulence index of *P. brassicae* isolates. Considering the deviation from regression as a measure of specificity of the resistance/susceptibility, cv. Imola, with smallest deviation MS, appeared to be nonspecifically resistant against all the isolates tested (Figure 5b). The three Q DH lines also showed small deviation MS, indicating nonspecific interactions. In comparison, data for cvs Ningyou7, Bristol, and Tapidor, with high deviation MS, may indicate the presence of differential interactions.

4 | DISCUSSION

The evaluation of various host–pathogen responses related to *P. brassicae* infection and their relationships provides important information for identification and differentiation of resistant and susceptible phenotypes, enabling efficient screening of host plant material for breeding purposes and also for the early identification of the disease in oilseed rape crops. Results of this study showed that leaf deformation was a prominent feature linked with *P. brassicae* infection that could be observed as early as 7 dpi. However, although leaf deformation was associated with early stages of pathogen colonization, there was a poor correlation between percentage leaves deformed and percentage leaf area with *P. brassicae* sporulation. This suggested that leaf deformation can be used as an independent indicator in crops to detect LLS early in the growing

TABLE 2 Light leaf spot severity on different oilseed rape cultivars/lines inoculated with single-spore isolates of *Pyrenopeziza brassicae*, measured as percentage leaf area covered with sporulation

| Cultivar/line	Leaf area with sporulation for each isolate (%)[a]								
	15WOSR64-SS1	15WOSR81-SS1	17WOSR-I1	15WOSR76-SS2	15WOSR78-SS1	17WOSR-I4	15WOSR5.2-SS2	17WOSR-CUI	Overall mean
Bristol (S)	21.9	14.8*	24.5	4.7**	**43.1**	42.5	51.8	**59.2**	32.81 g
Cabriolet	23.9	**12.4****	36.3	**8.9****	28.6	34.0	38.3	38.8	28.20 f
Cracker	11.3**	0.0**	21.9	23.6	31.4	44.3	47.4	39.2	27.37 f
Cuillin	11.6**	1.9**	10.3**	16.6*	27.8	37.5	31.7	42.8	22.52 de
Darmor	48.1	23.2	43.6	52.2	46.4	56.1	49.7	53.1	45.71 i
Excel	26.3	18.6	37.3	27.5	57.1	57.1	42.2	60.9	40.88 h
Harper	50.1	36.2	47.9	36.6	44.3	45.9	56.9	46.4	45.54 i
Hearty	38.2	34.4	38.4	36.6	43.5	59.4	42.1	51.7	42.88 hi
Imola	0.0**	0.0**	2.5**	0.0**	0.9**	0.0**	1.4**	1.4**	0.76 a
Marathon (S)	22.9	4.3**	29.9	7.0**	31.8	30.9	30.0	51.1	25.64 ef
Ningyou7	38.4	11.2**	42.1	17.2	11.6**	42.8	30.7	41.2	29.41 fg
Q2	20.2	1.1**	9.5**	1.9**	8.8**	15.2*	14.9*	1.7**	9.39 b
Q69	16.0*	10.2*	13.0*	3.2**	13.7	6.2**	1.9**	11.3**	9.81 b
Q83	8.1**	16.5*	17.3	15.3*	15.9*	22.6	11.1**	13.8*	15.08 c
Tapidor	38.0	8.5**	15.1*	8.5**	36.1	30.4	38.6	49.4	28.38 f
Temple	17.0	4.6**	12.0**	17.3	41.4	53.2	37.0	43.6	28.25 f
Trinity	14.6*	5.5*	30.5	**24.5**	27.0	53.1	39.7	33.4	28.54 fg
Yudal	38.8	**14.6***	**22.5**	**20.0**	**13.6***	**21.1**	**13.8***	**20.6**	20.40 d
Isolate mean	24.67 c	12.26 a	25.36 cd	17.72 b	28.85 d	36.99 f	32.96 e	36.96 f	

Note: Values in bold numbers indicate interactions that produced necrosis (black flecking phenotype). Details of all the cultivars/lines and isolates used in this study are given in Table S1 and Table 1, respectively. S, susceptible control cultivars.

Values marked with a common lower case letter do not differ at $p ≤ 0.05$.

Abbreviation: LSD, least significant difference.

[a] Arcsine back-transformed data for % leaf area covered with *P. brassicae* sporulation.

*Moderately resistant interactions. Values do not differ from the smallest mean value (0%) of cultivar/line-by-isolates interactions at $p ≤ 0.05$ (LSD = 12.59).
**Resistant interactions. Values do not differ from the smallest mean value (0%) of cultivar/line-by-isolates interactions at $p ≤ 0.01$ (LSD = 16.57).
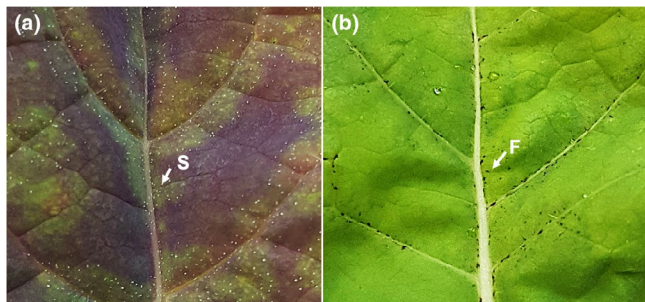
FIGURE 4 Differential phenotypes produced on the parents of the DY (Darmor × Yudal) doubled haploid (DH) population of oilseed rape. In a glasshouse experiment, the parental lines of the DY DH population produced differential phenotypes when spray-inoculated with single-spore isolates of *Pyrenopeziza brassicae*: (a) *P. brassicae* sporulation (S) on cv. Darmor—susceptible phenotype, (b) black necrotic flecking (F) on cv. Yudal—resistant phenotype. Pictures were taken at 29 dpi. dpi, days postinoculation
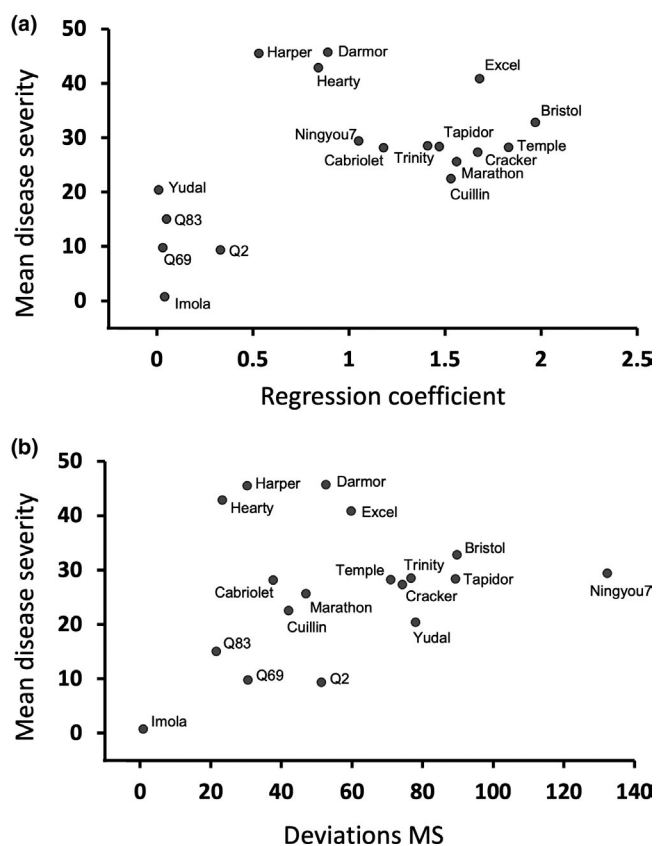
FIGURE 5 Relationships between mean percentage leaf area with *Pyrenopeziza brassicae* sporulation (mean disease severity) and the regression parameters calculated for the interactions between 18 oilseed rape cultivars/lines and eight *P. brassicae* isolates. (a) regression coefficient, (b) deviation mean squares (MS)

season; this may be useful for farmers due to the delay in appearance of other symptoms, such as acervuli or leaf lesions, after infection. There have been previous studies that also reported various symptoms such as stunting of plants, stem elongation, leaf curling,

and green island formation with *P. brassicae* infection (Ashby, 1997, 2000) and they have also been commonly observed in field crops infected with *P. brassicae*.

Leaf deformations are considered to be indicative of plant growth regulator imbalance in infected plants (Ashby, 1997, 2000). During the early endophytic phase, the pathogen has to rely on living host tissues to obtain nutrients, maintaining a fine balance in host–pathogen interactions to avoid significant tissue damage that could activate host resistance mechanisms. It has been suggested that in the case of the *P. brassicae–B. napus* interaction, provision of nutrients is facilitated by cytokinins that alter host metabolism and translocate nutrients to the site of infection (Murphy et al., 1997). Experimental evidence suggests that in resistant interactions, the host recognition occurs at a later stage of *P. brassicae* colonization and does not prevent early colonization (Boys et al., 2012); this might provide a possible explanation for the appearance of leaf deformation in resistant as well as susceptible cultivars/lines. However, some host genotypes may be less sensitive to hormonal changes than others, as leaf deformations appeared to be more prominent on some cultivars/lines than others.

Our results suggest that formation of black necrotic flecking and limitation of *P. brassicae* asexual sporulation (acervuli) are generally indicative of host resistance, and these two phenotypes coincided most of the time. This observation was consistent with previous findings that reported a host resistance response against *P. brassicae* associated with black necrotic flecking in oilseed rape cultivar Imola, limited growth of the pathogen during the subcuticular growth phase, and no asexual sporulation (Boys et al., 2012). Furthermore, an earlier report referred to black flecking with limited asexual sporulation (Bradburne et al., 1999). However, for the first time our study enabled comparisons to be made between different oilseed rape cultivars/lines that generate a black necrotic flecking phenotype and their disease response.

Our investigation of this pathosystem revealed significant differences between cultivars/lines, between isolates, and between cultivar/line-by-isolate interactions. The analysis also suggested the presence of isolate-specific resistant interactions. Resistance against *P. brassicae* is often measured quantitatively based on the extent of pathogen sporulation (Boys et al., 2007; Bradburne et al., 1999). Therefore, specific *B. napus–P. brassicae* interactions have to be identified statistically, unlike those in some other pathosystems such as *B. napus–L. maculans*, where cultivar-by-isolate interactions are identified by qualitative assessment of necrotic responses. Even though some interactions resulted in necrotic responses (black necrotic flecking) in this study, limited production of acervuli in some instances appeared to be independent of necrosis, suggesting that there could be different mechanisms of host resistance. For example, there were interactions that produced few acervuli in the absence of a necrotic response (i.e., cultivar Cracker). Additionally, there were two occasions where a necrotic response was observed in susceptible interactions. Hence, percentage leaf area covered by acervuli appeared to be a more reliable measure of host resistance.

Cultivar Imola, which was identified as the most resistant in this study, has been previously described as containing a major gene for resistance against *P. brassicae* (Boys et al., 2012). Even though Imola is not in commercial use, it is likely that there are other oilseed rape cultivars that carry this source of resistance. Additionally, most of the DH lines screened in this study showed a greater level of resistance to *P. brassicae* than commercial cultivars. More importantly, the type of host resistance carried by Imola and those DH lines appeared to be nonspecific and less sensitive towards increased virulence of the isolates tested. Therefore, these could be exploited as effective sources of resistance in oilseed rape breeding programmes. Secondary spread of the disease through conidia substantially contributes to the widespread disease occurrence (Evans et al., 2003; Fitt et al., 1998; Gilles et al., 2001). Conidia dispersed by rain-splashes may also contribute to the spread of the pathogen up crop canopies to infect upper canopy leaves, flowers, and pods (Boys et al., 2007). Therefore, host resistance that prevents or reduces the production of acervuli is a valuable resource for the control of LLS.

Screening of the parents of DH mapping populations enabled the identification of other potential sources of resistance against *P. brassicae*. Our study showed that parents of the DY DH population (Darmor-*bzh* × Yudal) produce differential phenotypes against *P. brassicae*: Yudal was moderately or partially resistant and produced a necrotic response against most of the *P. brassicae* isolates compared to Darmor that showed susceptibility to all the isolates. Previously, Pilet et al. (1998) reported a quantitative trait locus (QTL) for field resistance against *P. brassicae* in the DY DH population under field conditions at Le Rheu, France. According to Pilet et al. (1998), Yudal was considered to be highly susceptible to *P. brassicae* and Darmor-*bzh* was less susceptible, but disease severity varied between their field experiments. Interestingly, their results are in direct opposition to our data. This may have been due to differences in experimental conditions or in pathogen populations. It should be noted that we used cultivar Darmor, which is the progenitor of Darmor-*bzh*, but cultivar Darmor has been reported as more resistant to *P. brassicae* than Darmor-*bzh* (Pilet et al., 1998). Moreover, there has been no previous record of the necrotic response on Yudal. This makes it a very important and interesting case for further investigation of the DY population for resistance against *P. brassicae*. The second DH population that we considered in our study, TN DH (Tapidor × Ningyou7), has not been previously assessed for resistance against *P. brassicae*. Our data indicated resistant/moderately resistant interactions for both the parents with an overall level of partial/intermediate resistance. According to the regression parameters, Ningyou7 showed the greatest deviation MS that may indicate the presence of differential interactions. Therefore, this TN population may also be an important resource for further study of host resistance against the LLS pathogen.

Even though Bristol and Marathon showed some resistant interactions, these cultivars are susceptible to *P. brassicae* under UK field conditions, indicating that *P. brassicae* populations might have evolved to overcome their resistance. This agrees with previous work that suggested that major gene-mediated resistance in cv. Bristol broke down in the early 1990s following its commercial deployment (Boys et al., 2007). The present study also suggested the existence of isolate-specific interactions for cv. Bristol. In the past, breeding for cultivar resistance in oilseed rape has mainly focused on phoma stem canker (*L. maculans*), which was the major disease problem on oilseed rape in the UK for many years, with occurrence of frequent epidemics before LLS become dominant. However, cultivars with good resistance against one pathogen but with poor resistance against other major pathogens are of little value to growers. This presents plant breeders with the challenge of equipping cultivars with resistance against several pathogens. Therefore, it is necessary to evaluate the cultivars with good resistance against *L. maculans* for resistance against *P. brassicae* and vice versa. Our study included three cultivars (Excel, Harper, and Hearty) with good resistance scores for phoma stem canker. These cultivars are known to carry the *Rlm7* gene for resistance against *L. maculans* that is highly effective for controlling phoma stem canker in the UK (Mitrousia et al., 2018). Remarkably, all three cultivars scored some of the greatest overall LLS severities with no resistant interactions. There has been no evidence of a direct effect of the *Rlm7* gene on susceptibility to *P. brassicae*. However, it is important to investigate this further to see whether these three *Rlm7* cultivars have originated from a background susceptible to *P. brassicae*.

The isolates used in this study represent three different oilseed rape-growing areas in the UK: Herefordshire, Cambridgeshire, and Norfolk. There were significant differences between different isolates. In a preliminary experiment with *P. brassicae* populations (conidial suspensions collected from diseased leaves from oilseed rape fields), we observed occasional acervuli on cultivar Imola, in contrast to Boys et al. (2012), who reported no acervuli observed on Imola at any time. It was speculated that acervuli observed on this cultivar in our preliminary experiment might be an indication of the breakdown of resistance and therefore, it was anticipated that single-spore isolates 17WOSR-I1 and 17WOSR-I4 obtained from cv. Imola, would not produce a necrotic response. However, these two isolates induced black flecking on Imola, Yudal, and the DH line Q83. Four of the isolates, including 17WOSR-I1, were able to produce a small number of acervuli along the midribs of Imola leaves. This suggests that the resistance in cv. Imola limits *P. brassicae* asexual sporulation rather than completely eliminating it.

This study provided evidence that different types of resistance against *P. brassicae* are present in different oilseed rape genotypes. We have demonstrated the possibility of using single-spore isolates of *P. brassicae* for pathogenicity assays and for identification of cultivar-by-isolate interactions using statistical methods. This knowledge can be extended to characterize the population dynamics of *P. brassicae*. More importantly, we have identified several sources of resistance that are valuable for oilseed rape breeding programmes. Further investigation of isolate-specific interactions between *B. napus* and *P. brassicae* using a large panel of host plant material and *P. brassicae* isolates, especially those representing different oilseed

rape-growing regions, may provide valuable information for effective deployment of host resistance to manage LLS.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.