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Effects of cultivar resistance and fungicide application on stem canker of oilseed rape (*Brassica napus*) and potential interseasonal transmission of *Leptosphaeria* spp. inoculum

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Abstract

Phoma stem canker is a damaging disease of oilseed rape (*Brassica napus*) that causes annual yield losses to UK oilseed rape growers worth approximately £100 million, despite the use of fungicides. In the UK, oilseed rape is sown in August/September and harvested in the following July. The disease epidemics are initiated by ascospores released from *Leptosphaeria* spp. pseudothecia (ascocarps) on stem stubble in the autumn/winter. Control of this disease is reliant on the use of cultivars with "field resistance" and azole fungicides. This study investigated the effects of cultivar resistance and application of the fungicide prothioconazole on the severity of stem canker before harvest and the subsequent production of pseudothecia on the infected stubble under natural conditions in the 2017/2018, 2018/2019, and 2019/2020 cropping seasons. The application of prothioconazole and cultivar resistance decreased the severity of phoma stem canker before harvest, and the subsequent production of *Leptosphaeria* spp. pseudothecia on stubble in terms of pseudothecial density. Results showed that stems with less severe stem cankers produced fewer mature pseudothecia of *Leptosphaeria* spp. on the infected stubble. This investigation suggests that the most sustainable and effective integrated control strategy for phoma stem canker in seasons with low quantities of inoculum is to use cultivars with medium or good field resistance and apply only one spray of prothioconazole when required.

KEYWORDS
blackleg, *Brassica napus*, disease control, integrated pest management, *Leptosphaeria maculans*, oilseed rape

1 | INTRODUCTION

Phoma stem canker (also known as blackleg) is caused by two closely related coexisting ascomycete fungal pathogens, *Leptosphaeria maculans* and *L. biglobosa* (Fitt et al., 2006a; Shoemaker & Brun, 2001). This disease causes damage to oilseed rape worldwide (Fitt et al., 2006b) and accounts for annual yield losses to UK oilseed rape growers worth approximately £100 million, despite the use of fungicides and resistant cultivars (www.cropmonitor.co.uk) (Zhang et al., 2014). In the UK, phoma stem canker is a monocyclic disease that is initiated by ascospores as primary inoculum in autumn or winter. These ascospores are released from pseudothecia (sexual fruiting bodies) that mature after harvest on infected stem debris, such as stubble left in fields from the previous cropping season. Once mature, ascospores are ejected and transported by wind-dispersal from the pseudothecia to infect suitable host plants (Gladders & Musa, 1980; Huang et al., 2005; West et al., 2001). Many ascospores travel less than 1 km, so growers are advised to isolate new crops of oilseed rape 200–500 m from previous crops (West & Fitt, 2005; www.ahdb.org.uk/frag). A successful infection is characterized by the appearance of phoma leaf spots that are usually observed in autumn. *L. maculans* and *L. biglobosa* leaf spots have different phenotypes, with *L. maculans* forming larger grey leaf spots with many black pycnidia (asexual fruiting bodies), while *L. biglobosa* forms smaller black lesions with few or no pycnidia (Fitt et al., 2006a). A recent investigation has shown that incidences of *L. maculans* and *L. biglobosa* were greater when plants were assessed using quantitative PCR than with a visual assessment (Jacques et al., 2021). The pathogens then grow from leaves along the leaf petiole towards the stem, causing stem canker by invading and killing stem cells where they continue to develop until harvest (Hammond et al., 1985; Huang et al., 2006).

It has long been accepted that an integrated approach, combining chemical control, cultivar resistance, and cultural control, is the most effective strategy for managing phoma stem canker epidemics (Gladders et al., 2006a; Huang et al., 2018; Juroszek & von Tiedemann, 2011; West et al., 2002). Growers are advised to apply fungicides only when they are needed. Accurate fungicide application timing is essential for effective control (Gladders et al., 2006a; Huang et al., 2011; West et al., 2002). In the UK, the risk of infection by *Leptosphaeria* spp. is greatest in autumn. Thus, the application of the first fungicide spray is recommended when 10%–20% of plants have *L. maculans* phoma leaf spots in autumn, followed by another fungicide application when reinfection is observed, to prevent the development of cankers in the following June/July (https://ahdb.org.uk/knowledge-library/how-to-manage-phoma-in-oilseed-rape). A range of fungicide active ingredients and modes of action are available commercially and used for protecting oilseed rape against diseases. However, there is a reliance on prothioconazole and tebuconazole, which are both azole fungicides. The use of these two active ingredients, either alone or in co-formulations, is increasing; they accounted for 34% in 2012 and 52% in 2018 of all fungicides applied on oilseed rape in the UK (Garthwaite et al., 2012, 2018). Additionally, growers are encouraged to grow cultivars with good "field resistance" as an economical and environmentally friendly method for phoma stem canker control. These methods are supplemented by cultural practices such as oilseed rape rotations and sowing UK crops by late August (Huang et al., 2018; Marcroft et al., 2004).

Previous research has shown that the greater the quantity of primary inoculum at the start of the cropping season, the more severe the stem cankers are before harvest (Bousset et al., 2019; Lô-Pelzer et al., 2009b; Marcroft et al., 2004). Therefore, there is a need to reduce the quantity of primary inoculum produced on stem debris after harvest for effective control of phoma stem canker. However, there is limited knowledge on the effect of integrated management approaches on the interseasonal transmission of pathogen inoculum. It is not known how cultivar resistance and application of fungicides affect the production of primary inoculum in the UK. The aim of this investigation was to study the effects of fungicide application and cultivar resistance on the severity of phoma stem canker before harvest and on the subsequent development of pseudothecia on the crop stubble after harvest.

2 | MATERIALS AND METHODS

2.1 | Field experiments with different cultivars and fungicide treatments

Six cultivars with different field resistance ratings for phoma stem canker were selected for field experiments at Terrington St Clement, Norfolk. They were selected based on the AHDB Recommended List (RL) resistance ratings (0–9 scale, with 9 being good resistance) (https://ahdb.org.uk/knowledge-library/recommended-lists-for-cereals-and-oilseeds-rl-harvest-results-archive). To study the effects of cultivar resistance, the six cultivars were classified into three groups: good resistance, medium resistance, or susceptible. Cultivar Quartz (resistance rating 8) had good resistance, while cvs Barbados (resistance rating 7), Django (resistance rating 6), and Hunivers (resistance rating 7) had medium resistance, and cvs Charger and Flamingo (both resistance rating 4) were susceptible to *Leptosphaeria* spp. The experiments were sown in late August or early September in each of the 2017/2018, 2018/2019, and 2019/2020 cropping seasons. Each plot was 10 × 3 m. Plots were arranged in a randomized block design. Each of the six cultivars had untreated and prothioconazole-treated plots, each with three replicates: in total 36 plots. All other standard farm inputs and treatments were applied uniformly across the experiments. Proline 275 (Bayer Crop Science UK; prothioconazole-desthio 275 g/L) was used for this work because it is a triazole fungicide and representative of the main chemical group used to control oilseed rape diseases in the UK (Garthwaite et al., 2018). A half-label dose rate (0.315 L/ha) was used in two applications (early spray and late spray, respectively). The timing of the early spray was dependent on the timing of 10%–20% incidence of phoma leaf spot (% plants affected) on the susceptible cultivar Flamingo and the second spray was applied 4–6 weeks after the first spray when the soil was accessible, the wind speed was low, and there was no rain. The first sprays were applied on 25 October 2017, 16 November 2018, and 5 November 2019 and the second sprays were applied on 28 November 2017, 11 December 2018, and 16 December 2019 in the 2017/2018, 2018/2019, and 2019/2020 cropping seasons, respectively.

2.2 | Phoma stem canker assessments

In June/July each season before harvest, phoma stem canker was assessed by pulling up 20 plants randomly from each plot. The stems were cut at the crown collar and the severity of stem base canker on each plant was scored using a 0–7 scale (modified from the 1–6 scale of Lô-Pelzer et al., 2009a), whereby 0 = 0%, 1 = 1– 5%, 2 = 6%–25%, 3 = 26%–50%, 4 = 51%–75%, 5 = 76%–99%, 6 = 100% stem cross-section affected and plant still alive; 7 = 100% cross-section affected, dead stem with a hollow or severely necrotic pith. Stem cankers assessed in this work refer to stem basal cankers which were classified as cankers at the root crown or <10 cm above it (Mitrousia et al., 2018). Mean disease severity score per plot was calculated from the 20 plants per plot assessed.

2.3 | Stem incubation for pseudothecial production

Oilseed rape stems with stem cankers were sampled from the field experiments every year after harvest for pseudothecial production, as described by Huang et al. (2005). Stems from each treatment were collected together and placed in a free draining plastic tray around a Burkard spore sampler in a field plot at Bayfordbury, Hertfordshire so that pseudothecia could mature under natural conditions. The stems were collected and dried when major ascospore release events were observed using the Burkard spore sampler because the major release of ascospores indicated that most of the pseudothecia were mature (October/November). Once dried, five stems were selected randomly from each treatment. For each stem, the stem base (<10 cm above the root crown) was cut into 'stubble sticks' (5 × 0.5 cm). Four sticks from each stem were placed in a Petri dish for counting pseudothecia.

2.4 | Pseudothecial density assessment

Using a bifocal dissecting microscope at 10× magnification, the total number of mature pseudothecia on each 5 × 0.5 cm stubble stick was counted. The pseudothecial density (number of pseudothecia per cm^2) on each stubble stick was calculated. A characteristically shaped and sized *Leptosphaeria* spp. pseudothecium was considered mature when the neck was formed or the ostiole was open (i.e., ascospores were released) (Toscano-Underwood et al., 2003). The average pseudothecial density on each stem was calculated using a mean of the four stubble sticks. The average pseudothecial density per treatment was calculated using the means of the five stems per treatment (e.g., 20 stubble sticks per treatment were assessed).

2.5 | Statistical analysis

The statistical analyses of the data were done using GenStat (General Statistics) (www.vsni.co.uk). The 3-year data sets were analysed by fitting a mixed-model procedure. The fitted model consisted of the constant term and the full factorial combination of cultivar, fungicide, and year to determine the main effects of application of prothioconazole, choice of cultivar, year, and their two-way and three-way interactions on severity scores of stem canker replicated three times in each year (balanced design) and the density data of pseudothecia on crop stubble replicated between four and five times in each year (unbalanced design). The pseudothecial density data were $\log_e$-transformed to make the data more normally distributed and the variance homogenous. To analyse the effects of the three resistance groups, the fitted model consisted of the constant term and the full factorial combination of resistance, fungicide, and year. The post hoc test was done using the least significant difference (LSD) calculated at $p = 0.05$ by residual maximum likelihood (REML). The relationship between stem canker severity score and the density of pseudothecia on the sampled stems was analysed using linear regression and linear regression with groups.

3 | RESULTS

3.1 | Phoma stem canker severity

The three-way interaction of cultivar × fungicide × year was not significant, but the main effects of cultivar, fungicide, and cropping year and the effects of all two-way interactions were all significant ($p < 0.01$; Table 1). The significance of interaction between cultivar and fungicide means that the difference between fungicide treatments in stem canker severity depended on cultivars (Table 2). For example, cvs Quartz, Charger, Flamingo, and Django had significantly less severe canker in treated plants than in untreated plants, while cvs Barbados and Hunivers had no difference between the treated and untreated plants. The significance of interaction between cultivar and cropping season means that the difference among cultivars in stem canker severity depended on cropping seasons (Table 2). For example, cvs Barbados, Django, Flamingo, and Hunivers had no significant difference in canker severity scores in all three cropping seasons. However, cvs Charger and Quartz had a significantly higher canker score in 2017/2018 than in 2018/2019 and 2019/2020 cropping seasons.

When the means of cultivar × fungicide were compared, untreated cvs Charger and Flamingo had significantly greater stem canker severity scores than all other treatments tested. However, the treatment that had the most severe stem canker was untreated cv. Charger (2.46); this was significantly greater than the stem canker severity score of untreated cv. Flamingo (1.85) (Table 2). The stem canker severity score of treated cv. Charger (1.27) was significantly greater than that of untreated cv. Django (0.88), but the stem canker severity scores of untreated cvs Hunivers (1.17) and Quartz (0.97) and treated cv. Flamingo (1.14) were not significantly different from either treatment. There was no significant difference in the stem canker severity scores between untreated and treated cv. Hunivers (0.87), but the stem canker severity score of untreated cv. Barbados (0.52) was significantly less severe than that of untreated cv. Hunivers but not different from treated cv. Hunivers. There was no significant difference in stem canker severity score between treated cvs Quartz (0.48), Django (0.37), and Barbados (0.26). These treatments had significantly less severe canker scores than all other treatments, except for untreated cv. Barbados (Table 2).

When the six cultivars were divided into three resistance groups, neither the three-way interaction of resistance × fungicide × year nor the two-way interaction of resistance × year were significant

TABLE 1 Testing outputs of significant probability levels by fitting a mixed model for the main effects of cultivar, fungicide, and cropping season, the two-way interactions, and the three-way interaction

Factor	Wald statistic	df_{num}	F statistic	df_{den}	F probability
Stem canker severity score					
Cultivar	193.76	5	38.75	70.0	<0.01
Fungicide	61.62	1	61.62	70.0	<0.01
Year	27.01	2	13.50	70.0	<0.01
Cultivar × fungicide	17.62	5	3.52	70.0	<0.01
Cultivar × year	28.76	10	2.88	70.0	<0.01
Fungicide × year	14.08	2	7.04	70.0	<0.01
Cultivar × fungicide × year	13.53	10	1.35	70.0	0.22
Log_e (number of pseudothecia/cm^2)					
Cultivar	220.85	5	44.17	124.4	<0.01
Fungicide	33.69	1	33.69	124.0	<0.01
Year	50.92	2	25.46	124.2	<0.01
Cultivar × fungicide	16.21	5	3.24	124.6	<0.01
Cultivar × year	49.61	10	4.96	124.8	<0.01
Fungicide × year	4.82	2	2.41	124.9	0.10
Cultivar × fungicide × year	18.58	10	1.86	124.5	0.06

Abbreviations: df_{num}, numerator degrees of freedom; df_{den}, denominator degrees of freedom.

TABLE 2 Severity of phoma stem canker from field experiments in the 2017/2018, 2018/2019, and 2019/2020 cropping seasons

			Stem canker severity (0–7 scale)			
Cultivar	Resistance rating[a]	Treatment	2017/2018	2018/2019	2019/2020	Mean of cultivar × fungicide
Barbados	7	Untreated	0.68	0.42	0.47	0.52 ef
		Treated	0.25	0.12	0.40	0.26 f
		Mean of cultivar × year	0.47 ghi	0.27 i	0.43 ghi	–
Charger	4	Untreated	3.78	2.02	1.58	2.46 a
		Treated	1.53	1.08	1.20	1.27 c
		Mean of cultivar × year	2.66 a	1.55 b	1.39 bc	–
Django	6	Untreated	1.18	0.57	0.90	0.88 d
		Treated	0.35	0.22	0.55	0.37 f
		Mean of cultivar × year	0.77 eh	0.39 hi	0.73 eh	–
Flamingo	4	Untreated	1.88	1.65	2.02	1.85 b
		Treated	1.03	1.22	1.17	1.14 cd
		Mean of cultivar × year	1.46 bc	1.43 bc	1.59 b	–
Hunivers	7	Untreated	1.70	0.80	1.02	1.17 cd
		Treated	0.83	0.90	0.87	0.87 de
		Mean of cultivar × year	1.27 bcd	0.85 dg	0.94 def	–
Quartz	8	Untreated	1.37	0.72	0.83	0.97 cd
		Treated	0.78	0.23	0.43	0.48 f
		Mean of cultivar × year	1.08 cde	0.48 ghi	0.63 fi	–

Note: The six winter oilseed rape cultivars were either untreated or treated with the fungicide prothioconazole. 'Mean of cultivar × fungicide' represents the mean phoma stem canker severity scores over three seasons of the two-way interaction between cultivar and fungicide. 'Mean of cultivar × year' represents the mean phoma stem canker severity scores across two treatments of the two-way interaction between cultivar and year. Least significant differences (LSD) were calculated at $p = 0.05$ and used to separate the mean stem canker severity score between the various treatment combinations in the two-way interactions. Values that do not share a common letter are significantly different at $p = 0.05$.

[a]Cultivar AHDB recommended list phoma resistance rating on 0–9 scale; resistance rating 8–9 was classed as good resistance, 6–7 as medium resistance, and <5 as susceptible.

($p = 0.78$ and 0.52, respectively), but the main effects of resistance, fungicide, and cropping season and the effects of the other two two-way interactions of resistance × fungicide and fungicide × year were significant ($p < 0.05$). All the three resistance groups significantly benefitted from receiving prothioconazole treatment (Figure 1). However, there was no significant difference between the 'Good' and 'Medium' resistant groups within the same fungicide treatment.

3.2 | Pseudothecial density on stem stubble

The three-way interaction of cultivar × fungicide × year and the two-way interaction of fungicide × year were not significant ($p = 0.06$ and 0.10, respectively), but the main effects of cultivar, fungicide, and cropping season and the effects of the other two two-way interactions of cultivar × fungicide and cultivar × year were significant ($p < 0.01$; Table 1). The significance of the interaction between cultivar and fungicide means that the difference between fungicide treatments in pseudothecial density depended on cultivars (Table 3). For example, cvs Barbados, Charger, Hunivers, and Quartz had significantly fewer pseudothecia per cm^2 in treated than in untreated plants, while cvs Django and Flamingo had no difference between the treated and untreated plants.

The significance of interaction between cultivar and cropping season means that the difference among cultivars in pseudothecial density was dependent on cropping season (Table 3). For example,

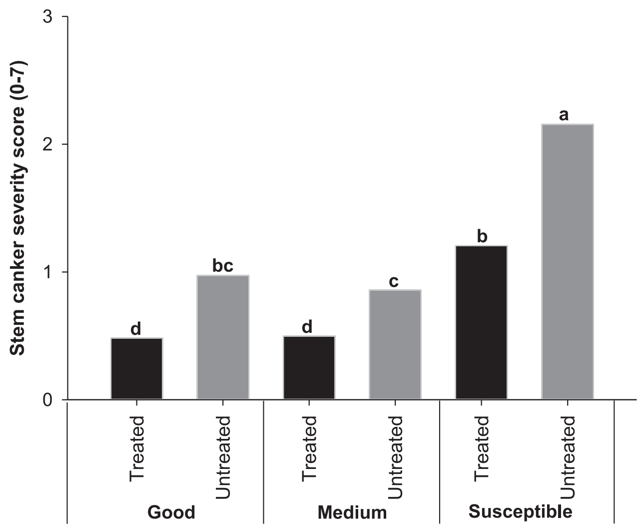

FIGURE 1 Mean severity of phoma stem canker of different fungicide treatments within resistance groups from winter oilseed rape field experiments in the 2017/2018, 2018/2019, and 2019/2020 cropping seasons. The resistance group 'Good' contained cv. Quartz, 'Medium' contained cvs Barbados, Django, and Hunivers, and 'Susceptible' contained cvs Charger and Flamingo. They were either untreated or treated with the fungicide prothioconazole. Least significant differences (LSD) were calculated at $p = 0.05$ and used to separate the mean stem canker score between different treatment combinations in the two-way interaction of resistance × fungicide. Bars that do not share a common letter are significantly different at $p = 0.05$

cvs Barbados and Charger had significantly greater pseudothecial density in 2017/2018 and 2018/2019 than in 2019/2020 while cvs Django, Hunivers, and Quartz had no significantly greater pseudothecial density in 2017/2018 than in 2018/2019 and 2019/2020. There was no significant difference in pseudothecial density for cv. Flamingo between all three cropping seasons.

When the means of cultivar × fungicide were compared, untreated cv. Charger (26.02) had the greatest pseudothecial density that was significantly greater than all other treatments (Table 3). There was no significant difference between untreated cvs Hunivers (15.47) and Flamingo (15.04) or treated cv. Flamingo (13.07). The pseudothecial density on treated cv. Flamingo was significantly different from treated cv. Hunivers (5.24) but untreated cv. Quartz (10.17) or treated cv. Charger (10.01) were not significantly different from either treatment. There were significantly more pseudothecia on stubble of untreated than treated cvs Charger and Quartz (Figure 2). There was a significantly smaller pseudothecial density on untreated cv. Barbados (2.89) than treated cv. Hunivers but the pseudothecial density on untreated cv. Django (3.59) and treated cvs Django (5.13) and Quartz (2.83) were not significantly different from that on untreated Barbados. The treatment that had the smallest pseudothecial density was treated Barbados (1.10) (Table 3).

When the six cultivars were divided into three resistance groups, the three-way interaction of resistance × fungicide × year and the two two-way interactions of resistance × fungicide and resistance × year were not significant ($p = 0.36$, 0.39, and 0.25, respectively), but the main effects of resistance, fungicide, and cropping season and the effect of the two-way interaction of resistance × year were significant ($p < 0.05$). Although pseudothecial density did not differ significantly between 'Good' and 'Medium' groups, they had a significantly smaller pseudothecial density than the 'Susceptible' group (Figure 3).

3.3 | Relationship between stem canker severity and pseudothecial density

The relationship was analysed by regressing stem canker severity score against *Leptosphaeria* spp. pseudothecial density (i.e., number of pseudothecia per cm^2; Figure 4). The correlation coefficient (r) between stem canker severity and pseudothecial density was $r = 0.85$, which supported a good simple linear relationship between the two traits ($p < 0.01$). For example, untreated cv. Charger had the greatest stem canker severity score and produced the greatest pseudothecial density (Tables 2 and 3). Stem canker severity scores and pseudothecial densities were greater on untreated stems than on prothioconazole-treated stems of the same cultivar. The stem canker severity scores and pseudothecial densities on susceptible cultivars were greater than those on untreated or treated cultivars with medium or good field resistance. Cultivars with either medium or good resistance ratings after fungicide treatment had the smallest phoma stem canker severity score and subsequently the smallest *Leptosphaeria* spp. pseudothecial density. However, comparison of the positions of lines at the intercept and/or line parallelisms of

TABLE 3 *Leptosphaeria* spp. pseudothecial densities on stubble of six cultivars from the field experiments in the 2017/2018, 2018/2019, and 2019/2020 oilseed rape cropping seasons

			Pseudothecial density (pseudothecia/cm^2)			
Cultivar	Resistance rating[a]	Treatment	2017/2018	2018/2019	2019/2020	Mean of cultivar × fungicide
Barbados	7	Untreated	4.00	3.89	0.27	2.89 f
		Treated	1.62	1.65	0.03	1.10 g
		Mean of cultivar × year	2.81 ef	2.77 ef	0.14	–
Charger	4	Untreated	39.07	20.31	20.10	26.02 a
		Treated	13.06	9.24	7.60	10.01 bc
		Mean of cultivar × year	24.62 a	15.39 abc	13.15 bcd	–
Django	6	Untreated	5.93	1.70	3.25	3.59 ef
		Treated	13.59	3.14	1.74	5.13 ef
		Mean of cultivar × year	9.76 cd	2.43 f	2.49 f	–
Flamingo	4	Untreated	14.26	16.38	14.67	15.04 b
		Treated	8.62	14.57	15.43	13.07 bc
		Mean of cultivar × year	11.75 cd	15.47 ad	15.09 ad	–
Hunivers	7	Untreated	33.78	4.74	5.99	15.47 bc
		Treated	9.20	3.46	3.51	5.24 de
		Mean of cultivar × year	22.85 ab	4.10 ef	4.75 ef	–
Quartz	8	Untreated	19.35	9.44	3.57	10.17 cd
		Treated	3.73	2.22	2.48	2.83 ef
		Mean of cultivar × year	10.67 d	5.83 e	3.09 ef	–

Note: Stems of the six cultivars were collected after harvest from plots that were untreated or treated with prothioconazole and placed in free draining trays to allow pseudothecia to mature under natural conditions. 'Mean of cultivar × fungicide' represents the pseudothecial densities across three seasons of the two-way interaction between cultivar and fungicide. 'Mean of cultivar × year' represents the mean pseudothecial densities across two treatments of the two-way interaction between cultivar and year. Least significant differences (LSD) were calculated at $p = 0.05$ and used to separate the pseudothecial densities between the various treatment combinations in the two-way interactions. Values that do not share a common letter are significantly different at $p = 0.05$.

[a]Cultivar AHDB recommended list phoma resistance rating on 0–9 scale; resistance rating 8–9 was classed as good resistance, 6–7 as medium resistance, and <5 as susceptible.

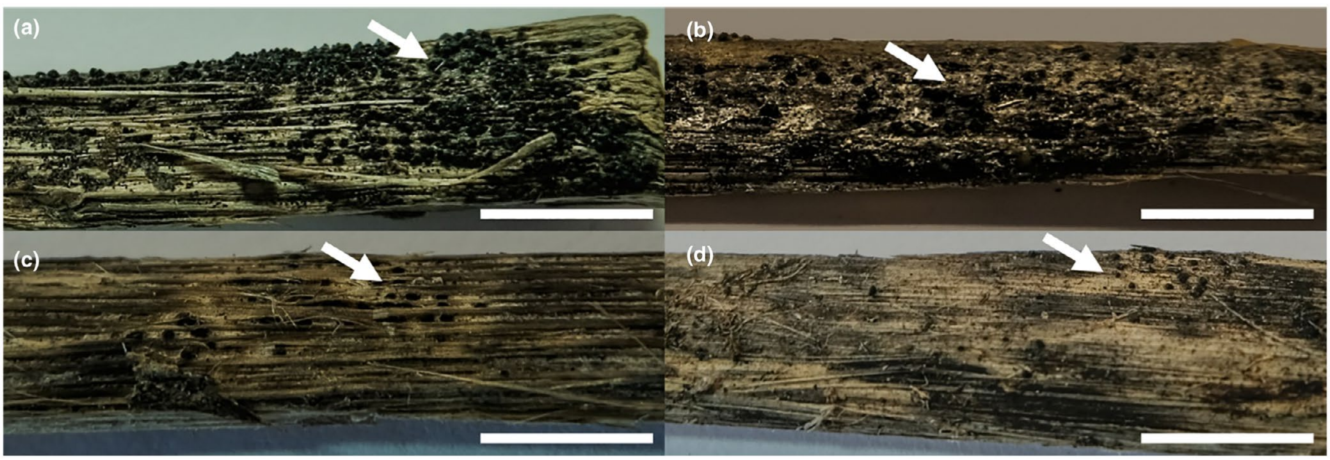

FIGURE 2 *Leptosphaeria* spp. pseudothecia on oilseed rape stem stubble of two cultivars with different levels of 'field resistance'. Stem stubble from susceptible cv. Charger (a, b) and resistant cv. Quartz (c, d) from plots that were untreated (a, c) or treated with fungicide prothioconazole (b, d), from the 2019/2020 cropping season. Stubble was collected after harvest and placed in free draining trays to allow pseudothecia to mature under natural conditions. Scale bars represent 0.5 cm. White arrows indicate the pseudothecia

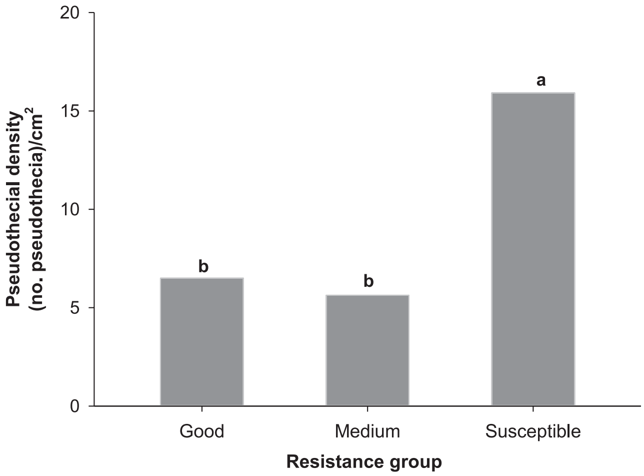

FIGURE 3 Mean *Leptosphaeria* spp. pseudothecial densities on stubble of different resistance groups from winter oilseed rape field experiments in the 2017/2018, 2018/2019, and 2019/2020 cropping seasons. The resistance group 'Good' contained cv. Quartz, 'Medium' contained cvs Barbados, Django, and Hunivers, and 'Susceptible' contained cvs Charger and Flamingo. Stems of these six winter oilseed rape cultivars were collected after harvest from plots that were untreated or treated with prothioconazole and placed in free draining trays to allow pseudothecia to mature under natural conditions. Least significant differences (LSD) were calculated at $p = 0.05$ and used to separate the mean *Leptosphaeria* spp. pseudothecial densities between resistance groups because the two-way interaction of resistance × fungicide was not significant ($p = 0.39$). Bars that do not share a common letter are significantly different at $p = 0.05$

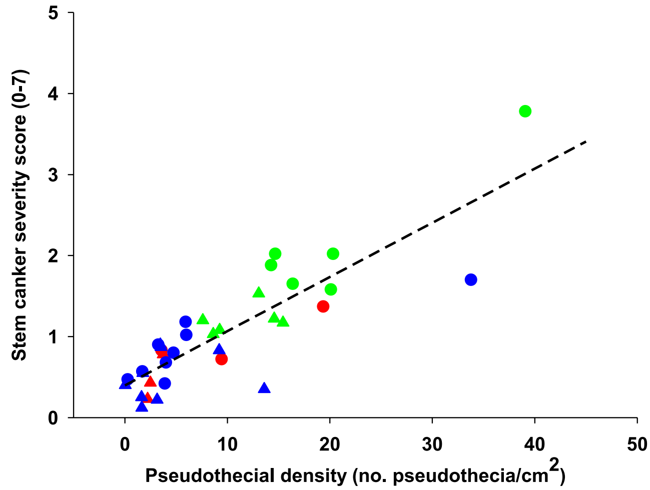

FIGURE 4 Relationship between phoma stem canker severity score before harvest and pseudothecial density of *Leptosphaeria* spp. on stems of six different cultivars from the field experiments in 2017/2018, 2018/2019, and 2019/2020 cropping seasons. The stem samples from untreated (●) or fungicide treated (▲) plots were collected after harvest and placed in free draining trays to allow pseudothecia to mature under natural conditions. The colours for groups represent field resistance rating against *Leptosphaeria* spp.: red = Good (cv. Quartz), blue = Medium (cvs Barbados, Django, and Hunivers), and green = Susceptible (cvs Charger and Flamingo). The fitted relation is $y = 0.40 + 0.067x$ ($R^2 = 71.9\%$, $df = 34$) in which y is the stem canker severity score (0–7 scale) and x the pseudothecial density (no. pseudothecia/cm^2)

the slope showed that separate lines for treated and untreated samples did not significantly improve the common fitted simple line. Fitting separate lines for grouping cultivars into 'Good', 'Medium', and 'Susceptible' field resistance levels did not significantly improve the common fitted simple line. As a result, a common simple linear relation was fitted to the combined data and it accounted for 71.9% of the variance in the observed phoma stem canker severity score by the pseudothecial density (i.e., the coefficient of determination $R^2 = 0.719$, $df = 34$; Figure 4).

4 | DISCUSSION

The application of the fungicide prothioconazole and use of cultivar resistance directly affected the severity of phoma stem canker before harvest, which indirectly affected the subsequent pseudothecial density of *Leptosphaeria* spp. on the stubble after harvest. Cultivars with medium or good resistance had less severe stem canker and subsequently fewer pseudothecia than susceptible cultivars, suggesting that using cultivar resistance will not only control phoma stem canker in the current season but also reduce the inoculum for the following cropping season. Previous studies also showed that greater stem canker severities result in greater pseudothecial densities (Lô-Pelzer et al., 2009b; Marcroft et al., 2004). These previous studies also found that quantitative cultivar resistance reduced the severity of stem canker in crops, but stubble of crops with similar disease severity scores resulted in similar quantities of primary inoculum regardless of the level of quantitative resistance (Lô-Pelzer et al., 2009b; Marcroft et al., 2004). Surprisingly, cv. Quartz with a good resistance rating produced more pseudothecia than cv. Barbados with a medium resistance rating. This may have been due to different types of resistance in these two cultivars, because cv. Quartz has *Rlm7* gene-mediated resistance, whereas cv. Barbados has quantitative resistance. Therefore, the pseudothecia produced on stubble of cv. Quartz may be predominantly *L. biglobosa* because resistance genes like *Rlm7* are effective in controlling phoma stem canker only when caused by *L. maculans* (Huang et al., 2018; Mitrousia et al., 2018). This is supported by previous work showing that more *L. biglobosa* ascospores than *L. maculans* ascospores were discharged from mature pseudothecia on the stems of cvs Roxet and Excel with the resistance gene *Rlm7* in the 2010/2011, 2011/2012, and 2012/2013 cropping seasons (Sidique, 2015). A recent study showed that cultivars with resistance genes against *L. maculans* promote colonization by *L. biglobosa* (Kerdraon et al., 2020). This suggests that use of cultivars with good resistance against *L. maculans* may result in a shift in the ratio of *L. maculans* to *L. biglobosa* in the local *Leptosphaeria* spp. populations.

The application of fungicide prothioconazole effectively reduced the severity of stem cankers and subsequently reduced pseudothecial density at the start of the following cropping season. The application of prothioconazole reduced the severity of stem cankers on all cultivars, suggesting that prothioconazole is still effective for the control of phoma stem canker in the UK. This study showed that when there was little *Leptosphaeria* spp. pathogen inoculum (e.g., 2019/2020 season), using a cultivar with medium field resistance without fungicide application (e.g., cv. Barbados) was more effective in reducing stem canker severity than applying fungicide to a susceptible cultivar (e.g., cv. Charger). This suggests that the use of fungicide may not be necessary or economically viable if there is little *Leptosphaeria* spp. inoculum when using a cultivar with medium or good quantitative resistance. However, the application of fungicide did significantly reduce the subsequent pseudothecial density, suggesting that use of fungicide may not be economically necessary in the current cropping season, but it will help to reduce inoculum for the next season. This study did not distinguish pseudothecia as *L. maculans* or *L. biglobosa*; even if most of the pseudothecia on the stems are *L. biglobosa*, reduction in number of pseudothecia on the crop debris is still important for control of stem canker on the next season's crop, because recent work showed that *L. biglobosa* can cause significant yield losses (Cai et al., 2018). Previous studies in oilseed rape also showed that increases in yield due to fungicide application were not cost-effective when there was little *Leptosphaeria* spp. inoculum (Gladders et al., 2006b). Studies in other crops support this observation. For the control of potato late blight (*Phytophthora infestans*), applying a low fungicide dose to a potato cultivar with good resistance was more effective in reducing disease severity than applying a greater fungicide dose to a susceptible cultivar (Ritchie et al., 2018). Results of this study showed that more cultivars had a significantly greater reduction in stem canker severity after spraying with prothioconazole under high *Leptosphaeria* spp. inoculum concentration than under low pathogen inoculum situations. This suggests that the effectiveness of fungicide application is dependent not only on the choice of cultivar but also on the pathogen inoculum concentration.

Because epidemics of phoma stem canker are initiated by ascospores released from mature pseudothecia in the previous autumn, reducing the density of pseudothecia is crucial for effective control of the disease in the following season. However, development and maturation of pseudothecia are affected by many factors. Differences in the pseudothecial densities between the three cropping seasons suggest that weather conditions affect the development of pseudothecia on crop stubble. Previous studies showed that precipitation and temperature are two major factors affecting the maturation of *L. maculans* and *L. biglobosa* pseudothecia (Huang et al., 2007; Toscano-Underwood et al., 2003). Studies on fungicide sensitivity showed that *L. biglobosa* is less sensitive to triazole fungicides than *L. maculans* (Eckert et al., 2010; Huang et al., 2011), suggesting that application of azole fungicides may favour *L. biglobosa* over *L. maculans* in production of pseudothecia on crop stubble. However, in this study the pseudothecia produced on stubble were not distinguished as *L. biglobosa* or *L. maculans*. Recent work showed that *L. biglobosa* is a key species in microbial communities on oilseed rape stubble, whereas *L. maculans* plays a minor role (Kerdraon et al., 2020). Additionally, *L. biglobosa* is more competitive than *L. maculans* at the necrotrophic growth stage on stem stubble (West et al., 1999). This suggests that the pseudothecial development and speed of maturation on oilseed rape stubble may differ between *L. biglobosa* and *L. maculans* under different environmental conditions and may lead to the changes in predominance of the two species in a local pathogen population.

Results of this study showed that the greater phoma stem canker severity before harvest, the greater the pseudothecial density of *Leptosphaeria* spp. after harvest, suggesting that cultivar resistance of the previous crop had a direct effect on inoculum production for the following cropping season; this agrees with other studies (Bousset et al., 2021; Lô-Pelzer et al., 2009b; Marcroft et al., 2004). However, we also noted that ascospore-bearing pseudothecia were present on stubble even if it was from cultivars with good resistance or treated with fungicide. This suggests that using resistant cultivars and fungicides may not prevent phoma stem canker epidemics but could help to reduce the disease severity and its impact on the yield of the crops. Brun et al. (2010) showed that stem canker severity increased with an increased number of phoma leaf lesions. This suggests that the reduction in initial inoculum concentration (i.e., fewer pseudothecia) could reduce the number of phoma leaf lesions at the beginning of the following cropping season. However, the threshold (i.e., the baseline) values of the initial inoculum that can cause a significant canker severity, or the relationship between the initial inoculum concentration and the final stem canker severity, still needs to be investigated. Increased understanding about initial inoculum concentrations at the beginning of the cropping season could help modellers to model inoculum spread to surrounding oilseed rape fields, because the level of phoma leaf spot in oilseed rape fields was found to be a function of the distance to emitting ascospore sources (Bousset et al., 2021; Zhang et al., 2014).

This investigation suggests that the most sustainable and effective integrated control strategy for phoma stem canker in seasons with little *Leptosphaeria* spp. inoculum is to use cultivars with medium or good resistance, combined with one application of fungicide. These methods could be even more effective if geographical screening work were routinely done to identify *L. maculans* population structures in different regions for effective deployment of resistance (*R*) genes to control phoma stem canker. Deployment of cultivars with different *R* genes will not only reduce phoma stem canker severity before harvest and decrease inoculum concentration for the next cropping season, but can also prolong the lifespan of cultivar resistance (van den Bosch & Gilligan, 2003; Gladders et al., 2006a; Marcroft et al., 2004; Mitrousia et al., 2018). Effective control of severe phoma stem canker epidemics relies on use of fungicides. However, it is important to acknowledge that in the UK fungicides are not applied solely for the control of phoma stem canker; they are also applied for the control of other autumn diseases such as light leaf spot, so it is important for growers to be aware of other diseases to ensure they can be controlled when necessary.

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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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