Effects of a penthiopyrad and picoxystrobin fungicide mixture on phoma stem canker (*Leptosphaeria* spp.) on UK winter oilseed rape

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Abstract In the UK, fungicides are often used to control phoma stem canker on winter oilseed rape. Field trials were established near Boxworth, Cambridgeshire for four cropping seasons (2011/2012, 2012/2013, 2013/2014 and 2014/15) to test the efficacy of a new fungicide mixture Refinzar® (penthiopyrad + picoxystrobin) by comparison to an existing fungicide Proline 275® (prothioconazole) against phoma stem canker (*Leptosphaeria* spp.) and effect on winter oilseed rape (cv. Catana) yield. In each season, weather data were collected from a weather station at Boxworth and the release of ascospores was monitored using a nearby Burkard spore sampler. The patterns of ascospore release differed between seasons and related to weather conditions. Fungicides penthiopyrad + picoxystrobin and

prothioconazole were applied in October/November when 10% plants had phoma leaf spotting (T1, early), 4/8 weeks after T1 (T2, late) or at both T1 and T2 (combined). When phoma leaf spot symptoms were assessed in autumn/winter, penthiopyrad + picoxystrobin and prothioconazole both decreased numbers of phoma leaf spots caused by *L. maculans*; there were few leaf spots caused by *L biglobosa*. Penthiopyrad + picoxystrobin and prothioconazole both reduced phoma stem canker severity before harvest compared to the untreated control but did not increase yield in these seasons when epidemics were not severe. In 2013/2014, the presence of *L. maculans* and *L. biglobosa* in upper stem lesions or stem base cankers was determined by species-specific PCR. The proportions of stems with *L. maculans* DNA were much greater than those with *L. biglobosa* DNA for both upper stem lesions and basal stem cankers. These results suggest that both penthiopyrad + picoxystrobin and prothioconazole can decrease phoma stem canker severity of winter oilseed rape in severe disease seasons.

Keywords

Phoma stem canker, winter oilseed rape, fungicides, DMI, QoI, SDHI

Introduction

Phoma stem canker is a disease of oilseed rape, which is caused by closely related fungal species *Leptosphaeria maculans* and *L. biglobosa* (Fitt, et al. 2006a; Stonard et al. 2010). Both pathogens follow a monocyclic disease cycle in the UK with phoma leaf spotting symptoms in autumn/winter and stem base canker in spring/summer. Severe cankers inhibit the flow of water and nutrients to the seed, and thus decrease seed yield and quality. Oilseed rape is the third most valuable arable crop grown in the UK and has a total annual value of > £600 M and an average on-farm yield of 3.5-4.0 t/ha (AHDB Cereals & Oilseeds 2015). Globally, phoma stem canker has been calculated to annually cause approximately £700M

worth of losses, making it a significant threat to worldwide oilseed rape production and food security (Fitt et al. 2006b).

Generally, *L. maculans* forms damaging stem base cankers and *L. biglobosa* forms less damaging upper stem lesions on UK winter oilseed rape (Fitt et al. 2006a; Huang et al. 2011). This difference is considered a result of differences in timing of ascospore release, with *L. maculans* spores released in early/mid-autumn and *L. biglobosa* spores released in early/mid-winter (Fitt et al. 2006b). More recently, however, *L. biglobosa* has been shown to cause severe upper stem lesions and lodging of crops in some growing seasons (Huang et al. 2014). If this occurs regularly, *L. biglobosa* could become a more important threat to winter oilseed rape yield.

Together with conventional plant breeding strategies that adopt effective resistance genes (Delourme et al. 2006), fungicides are commonly used in the UK to control phoma stem canker on winter oilseed rape. In 2014, 98.1 % of the total area of oilseed rape (674,580 ha) received fungicide treatment for control of disease including phoma stem canker because growers generally expect such treatments to give a yield response (Garthwaite et al. 2012). UK winter oilseed rape experiments have often shown a yield response from fungicide application against phoma stem canker, although, an increase in yield was only registered when canker severity in unsprayed plots was ≥ 3 on a 0-5 disease severity scale (West et al. 2002). Typically, azole fungicides have been applied because of their effective action against *L. maculans* as well as their relatively low cost compared to alternatives. Examples include flusilazole, prothioconazole and tebuconazole (Eckert et al. 2010; Huang et al. 2011). Other fungicides are available to growers; these include quinone outside inhibitor (QoI) fungicides and succinate dehydrogenase inhibitor (SDHI) fungicides, both of which disrupt energy production in the fungal cell (Avenot and Michailides 2010; Bartlett et al. 2002); however, their efficacy against phoma stem canker has not been evaluated.

Legislation from the European Union has forced the withdrawal of some fungicides used to control fungal pathogens in arable crops (Marx-Stoelting et al. 2014). An example is the withdrawal of flusilazole, a chemical widely used for phoma stem canker control in the

UK until 2014. Despite concluding that flusilazole fulfils safety requirements set by Member States, on review the European Commission withdrew usage of Flusilazole across the entire European Union (European Commission 2007). Withdrawal of flusilazole reduced options available to growers for control of phoma stem canker, along with other crop diseases. It is thus imperative to obtain a complete understanding of the effects that novel fungicide mixtures have on phoma stem canker in winter oilseed rape crop.

This paper describes work investigating the efficacy of a new fungicide mixture Refinzar® (a.i. penthiopyrad plus picoxystrobin, an SDHI plus QoI, respectively) to reduce phoma leaf spotting, decrease phoma stem canker severity and improve oilseed rape yield.

Materials and Methods

Weather conditions at the field site

Weather data for the 2011/12, 2012/13, 2013/14 and 2014/15 winter oilseed rape growing seasons were collected at Boxworth, Cambridgeshire, UK (52.259814, -0.025437); near the winter oilseed rape field experiments and the Burkard spore sampler in 2014/15 cropping season and approximately 15 km from the site of the Burkard spore sampler in 2011/12, 2012/13 and 2013/14. Temperature and rainfall data were collected daily using an automated weather station (Campbell Scientific, UK).

Numbers of ascospores in the air

The numbers of *Leptosphaeria* ascospores in the air were estimated using a 7-day volumetric spore sampler (Burkard Manufacturing Co. Ltd, UK). For the 2011/12, 2012/13 and 2013/14 cropping seasons, the spore sampler was located at Whittlesford, Cambridgeshire, UK (52.109299, 0.156023). For the 2014/15 cropping season, the spore sampler was located at Boxworth, Cambridgeshire (52.270127, -0.027112). The spore sampler accommodated a

rotating drum (2 mm per hour) that held a strip of Melinex tape. The tape was lined with a thin layer of petroleum jelly and hexane paste mixture (10 g petroleum jelly, 20 ml hexane). After 7 days of sampling, the rotating drum was removed and the Melinex tape was divided into seven 24-hour segments. Each segment was then cut horizontally, with one half stored at -20 ^{o}C for molecular analysis and one half mounted for microscopy to count spore numbers. The slide-mounted tape was stained with trypan blue solution (0.4% w/v in water, Sigma-Aldrich, UK) so that the ascospores were visible under a light microscope (100x total magnification). Counting was done in three longitudinal traverses across the slide and the number of ascospores recorded for each traverse. The concentration of ascospores in the air was calculated according to equation described by Lacey and West (2006).

Winter oilseed rape field experiments

Field experiments were established near Boxworth, Cambridgeshire, UK for the 2011/12, 2012/13, 2013/14 and 2014/15 cropping seasons. The winter oilseed rape cultivar Catana (Dekalb, UK) was used because of its susceptibility to *L. maculans* (resistance rating of 4 in the UK North region on a 1-9 scale; where 9 is very resistant) but good resistance against *Pyrenopeziza brassicae* the cause of light leaf spot (AHDB Cereals & Oilseeds 2015).

In each growing season, seeds of cv. Catana were sown in mid/late August at a seed rate of 5 kg/ha and a drilling depth of 1 cm. To test the efficacy of a new fungicide mixture (penthiopyrad + picoxystrobin), by comparison to existing fungicides (flusilazole or prothioconazole), for control of phoma stem canker (*Leptosphaeria* spp.) and impact on winter oilseed rape yield, experiments were arranged in a randomised block design with three replicates. Each plot received one of 14 treatments (four different fungicides applied under three different timing regimes (T1, T2 or T1 and T2 combined), one untreated throughout the cropping season, one treated with a spring spray only, T3), thus totalling 42 plots (Table 1). The fungicide Refinzar® (DuPont UK Ltd; a.i. penthiopyrad 160 g/l plus picoxystrobin 80 g/l) was used in all four cropping seasons. The product has been marketed as a potential

alternative to the azole fungicides that are used widely in the UK on winter oilseed rape. Sanction® (DuPont UK Ltd; a.i. flusilazole 250g/l) was used for the first two cropping seasons before its active ingredient flusilazole was withdrawn. It was replaced by another azole fungicide, Proline 275® (Bayer Crop Science UK Ltd; a.i. prothioconazole 275 g/l), for the 2013/14 and 2014/15 cropping seasons. To represent the components of Refinzar®, Galileo® (DuPont UK Ltd; a.i. picoxystrobin 250 g/l) and LEM17® (DuPont UK Ltd; a.i. penthiopyrad 200 g/l) were also applied but data are not presented. The fungicide spray timings differed from season to season, with the first application (T1) taking place in autumn when 10% of plants were affected with phoma leaf spots. The second application (T2) was made 8 weeks after T1 in 2011/2012 season and 4 weeks after T1 in 2012/13, 2013/14 and 2014/15 seasons. All plots except the untreated control received a spring-flowering spray (T3) against the pathogen *Sclerotinia sclerotiorum*, the causal agent of sclerotinia stem rot.

(Table 1 here)

Phoma leaf spotting, stem canker and yield assessment

Phoma leaf spotting was assessed by randomly sampling ten plants per plot in the 2011/12 and 2012/13 cropping seasons and 15 plants per plot in the 2013/14 and 2014/15 cropping seasons; as described in Steed et al. (2007). The sampling was done regularly between November and February each cropping season. The total numbers of *L. maculans* (large grey lesions with pycnidia) and *L. biglobosa* (small dark lesions with few or no pycnidia) leaf spots on each leaf were recorded, together with growth stage of the plant.

Phoma stem canker severity assessment was done once in the 2011/12 and 2012/13 cropping seasons (25 July 2012 and 9 July 2013), twice in the 2013/14 cropping season (27 May and 1 July 2014) and twice in the 2014/15 cropping season (1 June and 29 June 2015). A random sample of either 10 (2011/12 and 2012/13), 25 (2013/14) or 15 (2014/15) plants was collected from each of the 42 plots using the method described in Steed et al. (2007). The severity of basal cankers was assessed by cutting the stem at the base of each sampled plant

and scoring the cross-sectional area of necrotic tissue according to a 0-6 scale (Huang et al. 2011), modified from Lô-Pelzer et al. (2009). Upper stem lesions were cut at the centre point of the lesions and assessed on the same scale. Desiccated plots were harvested using a small plot harvester and yield (t/ha) recorded. Presence of light leaf on stems was also noted.

Stem canker subsampling, DNA extraction and species-specific PCR

To investigate whether the phoma stem cankers were caused by *L. maculans* and/or *L. biglobosa*, stems with basal stem canker or upper stem lesion symptoms were subsampled for DNA extraction and *Leptosphaeria* species-specific PCR. Approximately three stems per plot were selected from basal stem canker and upper stem lesion samples from all 42 plots of the 2013/14 field experiment. Using a scalpel, thin shavings of the basal canker or upper stem lesion tissue were cut away from each stem and placed in 2 ml Eppendorf tubes (Sigma-Aldrich Co LLC, UK). The subsamples were stored at -20 ^{o}C after freeze-drying for 24 hours. The subsamples were then ground into a powder using a mortar and pestle. A sub-sample of the powdered stem material was transferred into 2 ml Eppendorf tubes and DNA was extracted using a DNA extraction kit (DNAMITE Plant kit; Microzone Ltd, UK) and quantified using a Nanodrop ND-1000 spectrophotometer (Labtech International, UK). Identification of species was done using end-point PCR with species-specific PCR primers LmacF/LmacR for *L. maculans* and LbigF/LmacR for *L. biglobosa* (Liu et al. 2006). Gel electrophoresis was done to identify the presence of *L. maculans* and/or *L. biglobosa* DNA.

Statistical analysis

The R software was used to for statistical analyses of data (R Development Core Team 2011). Linear mixed effects models were done on leaf spotting, canker severity and yield data. Two-way mixed effect ANOVA was done on spray timing and fungicide treatment. One-way

mixed effect ANOVA was done independently on spray timing and then fungicide treatment. Residuals were tested for normality using the Shapiro-Wilk test of normality.

Results

Rainfall

Rainfall patterns differed between the four seasons during autumn/winter (phoma leaf spot development stage) and summer (phoma stem canker development stage). In the 2011/12 cropping season, the autumn and winter months were dry compared with the 2013/14 cropping season. In August and September, 73 mm of rainfall was recorded. Periods of prolonged rainfall did not commence until December 2011 and there were never periods of heavy rainfall. In the summer, it was predominantly wet, with heavy rainfall in April (101 mm), June (103 mm) and July (115 mm) (Figure 1b). In the 2012/13 cropping season, prolonged rainfall occurred much earlier, with periods of substantial rainfall commencing in mid-September and continuing to mid-February with the occasional short dry period. In August and September, 70 mm of rainfall was recorded. The spring and summer were dry with occasional periods of short-term rainfall (Figure 1d). In the 2013/14 cropping season, rainfall pattern was similar to that of the 2012/13 growing season in the autumn/winter. Rainfall started in early autumn, with increases in August and September over a few days and then continued for a period between October and mid-November. In August and September, 91 mm of rainfall was recorded. A period of prolonged rainfall occurred between December and February (202 mm over 88 days) (Figure 1f). In the 2014/15 cropping season high rainfall commenced early (8 August) with a period of very heavy rainfall (112.6 mm) causing flash floods in the region. In August and September, 192 mm of rainfall was recorded although 58 % of this was on 8 August. Rainfall in the winter months was more sporadic than in the previous seasons, with no periods of particularly prolonged rainfall between December and February (Figure 1h).

223

224 Average temperature

225

226 Across the four seasons, average temperature followed a typical pattern, with temperature
227 decreasing to ≤ 0 ºC in December, January and February. Periods of particularly low
228 temperatures differed among seasons. In the 2011/12 cropping season, a low temperature (-
229 7.1 ºC) occurred on 10 and 11 of February. Average temperature between 1 October and 31
230 May was 7.8 ºC (Figure 1b). In 2012/13, a similar pattern was observed, but low temperature
231 (-4.4 ºC) occurred a month earlier on 14 January. One notable difference in this cropping
232 season was an uncharacteristic period of cold weather in mid-late March. Snowfall and
233 temperatures < 0 ºC were recorded during this period. Average temperature between 1
234 October and 31 May was 5.7 ºC (Figure 1d). In 2013/14, there was no period of particularly
235 cold weather, with average daily temperature never < 0 ºC. Average temperature between 1
236 October and 31 May was 8.3 ºC (Figure 1f). The 2014/2015 cropping season was similar to
237 the previous season in that there was no period of particularly cold weather, with average
238 daily temperature only < 0 ºC on two occasions (-0.7 ºC and -0.4 ºC on 19 January and 22
239 January, respectively). Average temperature between 1 October and 31 May was 7.2 ºC
240 (Figure 1h).

241

242 Ascospore numbers

243

244 The numbers of ascospores in the air and the period in which most ascospores were released
245 differed among growing seasons. In 2011/12 and 2012/13, there was a major discharge of
246 spores in November and a large discharge of spores in January; the discharge in November
247 was longer in 2012/13 (Figure 1a, c). In 2013/14, the spore release pattern was similar to
248 2012/13 but differed in timing; ascospore dispersal occurred over a longer period in the
249 autumn, with a large release in the winter of both seasons; however, in 2013/14, the autumn

release of spores was a month before the equivalent release in 2012/13 (November in 2012/13 and October in 2013/14). Similarly, a large release of spores in the winter occurred a month earlier in 2013/14 than 2012/13 (January in 2012/13 and December in 2013/14) (Figure 1c, e). Due to accessibility issues in 2014/2015 cropping season, spore release data commenced at the start of November. Nonetheless, two large releases were recorded at the end of November and mid/late January (Figure 1g). A common pattern among all four seasons was the relationship between rainfall and spore release. In most seasons, spore release commenced in large numbers after a period of prolonged or heavy rainfall. For example, heavy rainfall at the start of November 2011 was associated with ascospore release later that month. However, some spores were also released after periods of light rainfall, such as in December 2013.

(Figure 1 here)

Field experiments

In all four cropping seasons, the spring flowering spray had no affect on leaf spotting, canker severity or yield when compared to the control; therefore, the untreated control data presented are a mean of untreated plots and spring spray only (T3) plots. Penthiopyrad alone produced similar results to penthiopyrad + picoxystrobin and therefore has been excluded from the analysis. Picoxystrobin alone produced similar results to the untreated control and therefore has been excluded from the analysis and data are not presented.

Phoma leaf spotting

In the 2011/12, 2012/13 and 2014/15 cropping seasons, incidence of phoma leaf spotting in unsprayed plots did not increase in severity on winter oilseed rape leaves until March and phoma leaf spotting was never severe during the autumn/winter; therefore, data are not shown. In 2013/14, the phoma leaf spotting started earlier and incidence (% plants affected) was much greater in unsprayed plots in the autumn/winter months compared to the previous two winter oilseed rape cropping seasons (Figure 2). Experimental plots treated with

278 penthiopyrad + picoxystrobin or prothioconazole had significantly less *L. maculans* type leaf
279 lesions per plant when compared with the untreated control, except when fungicides had only
280 just been applied (T2 only plots at December 2013 assessment) or when their activity had
281 decreased over time (T1 application at February 2014 assessment). The penthiopyrad alone
282 treatment was statistically similar to the picoxystrobin + penthiopyrad treatment.

283 The two fungicides significantly decreased number of *L. biglobosa* type lesions,
284 compared with the untreated control, in December 2013 on T1 only and on T1 plus T2 plots,
285 and in February 2014 on T2 only treated plots. When comparing the efficacy of the two
286 fungicides, there was no significant difference in the numbers of *L. maculans* type lesions
287 present between penthiopyrad + picoxystrobin and prothioconazole treated plots (Figure 2a, c,
288 e). Furthermore, there was no significant difference in the numbers of *L. biglobosa* type leaf
289 lesions present between penthiopyrad + picoxystrobin and prothioconazole treated plots
290 (Figure 2b, d, f).

291 (Figure 2 here)

292

293 Stem canker severity

294

295 In the 2011/12, 2012/13 and 2014/15 cropping seasons, stem canker was not severe (Figure
296 3). Severity was never more than 1.5 on a 0-6 scale for either upper stem lesions or basal stem
297 cankers in these three cropping seasons. Fungicide application did not significantly decrease
298 stem canker severity in 2011/12 and only prothioconazole at the combined T1/T2 application
299 timing significantly reduced severity compared to the control in 2012/13. In the 2013/14
300 cropping season (Figure 3c), canker was more severe than in other seasons. There were
301 significant differences in the severity of basal stem cankers between fungicide treatments and
302 between timings ($P < 0.05$, 12 df), however, there was no significant difference in upper stem
303 lesion severity between fungicide treatments and timings (data not shown). Unlike
304 prothioconazole, penthiopyrad + picoxystrobin did not decrease the severity of basal stem
305 cankers when applied at the T1 spray timing only when compared to untreated ($P < 0.05$, 4

306 df). Nonetheless, at T2 and T1/T2 timings, both penthiopyrad + picoxystrobin and
307 prothioconazole reduced severity equally. Penthiopyrad + picoxystrobin at T1/T2 and
308 prothioconazole at T1/T2 performed similarly, reducing basal stem canker severity more than
309 if they were applied at T1 only or T2 only. Although there were significant differences
310 between fungicide treatments and between timings, the interactions were not significant and
311 were removed from the final model.

312 No other diseases were severe in the field experiments across all four growing
313 seasons; although, in 2014/15 cabbage stem flea beetle affected winter oilseed rape
314 establishment in the Cambridgeshire region and may have had an affect on the field
315 experiments. Light leaf spot was present but not severe.

316 (Figure 3 here)

317

318 Yield

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320 Improvement in yield of fungicide-treated plots was sometimes positive and sometimes
321 negative when compared with the control over the four cropping seasons (Figure 4). Despite
322 effects of treatment on stem canker severity across all cropping seasons, there was no
323 significant effect of fungicide treatment on yield in any season.

324 (Figure 4 here)

325

326 Proportion of stems with *L. maculans* or *L. biglobosa*

327

328 A total of 133 basal stem canker samples and 74 upper stem lesion samples was analysed by
329 PCR. The proportions of upper stem lesions and basal stem cankers with *L. maculans* DNA
330 detected in the sample was much greater than those with *L. biglobosa* DNA detected (Table
331 2). Out of 74 samples of upper stem lesions, 45 had only *L. maculans* DNA detected, two
332 samples had only *L. biglobosa* DNA detected and 11 samples had both species DNA detected.
333 No *L. maculans* or *L. biglobosa* DNA was detected in 16 upper stem samples. Of 133 basal

stem canker samples, 102 had only *L. maculans* DNA detected and four samples had both species detected. No samples had only *L. biglobosa* DNA recorded. No *L. maculans* or *L. biglobosa* DNA was detected in 27 basal stem canker samples.

(Table 2 here)

Discussion

These results suggest that in cropping seasons when there are moderately severe phoma stem canker epidemics, penthiopyrad + picoxystrobin and prothioconazole are both effective at reducing phoma stem canker severity *in situ*. Severe canker results in yield loss because transport of water and nutrients up the stem is decreased by girdling, thus resulting in premature ripening and shrivelled seed pods (West et al. 2002). These results show that penthiopyrad + picoxystrobin or prothioconazole both prevent the formation of severe cankers, potentially allowing good pod development.

Furthermore, they show that foliar application of penthiopyrad + picoxystrobin or prothioconazole in the autumn reduced the number of *L. maculans* type leaf lesions that formed on leaves. Application of either fungicide when incidence of *L. maculans* leaf spotting reached 10% plants affected (T1) significantly reduced the number of lesions; a further application one or two months later (T2) appears to have had a smaller but still significant effect on the number of lesions. Work with GFP-labelled *L. maculans* has shown that if the phoma leaf spot stage is prevented, the pathogen does not grow along the leaf petiole to form stem cankers (Huang, et al. 2014). Thus, this early stage inhibition stops the later development of cankers; exemplified here by the T1 and T2 application of either penthiopyrad + picoxystrobin or prothioconazole, which significantly reduced the number of lesions on leaves in November and December and significantly reduced stem canker severity in the following July.

By contrast, in seasons when there is little early phoma leaf spotting (e.g. 2011/12 and 2012/13), the data suggest that fewer fungicide sprays are needed since canker severity

was very low and it did not affect yield. The timing and severity of basal stem cankers and upper stem lesions has previously been reported to affect the potential yield of winter oilseed rape crops (Zhou et al. 1999). Early, severe basal cankers or upper stem lesions are more likely to cause yield loss than later/slight basal stem cankers or upper stem lesions. The development of later, less severe stem cankers can be associated with a later release of ascospores, as shown by the 2011/12 and 2012/13 cropping seasons, when a large release of ascospores occurred later in the season compared to 2013/14; when there was less rainfall in August and September the release of ascospores was delayed, resulting in a later onset of phoma leaf spotting. Disease severity has previous been linked to yield loss in winter oilseed rape; only when disease severity is high (≥ 3 on a 0 – 5 severity scale) does a yield response occur in fungicide treated plots (West et al. 2002).

The results for timing of ascospore release and leaf spotting suggest that the optimum fungicide application regime differs between seasons. In 2013/14, ascospore release was earlier, due to greater rainfall in August/September, than in the previous two seasons, thus resulting in a more severe canker prior to harvest. These observations are in general agreement with the UK phoma stem canker disease model published by Evans et al. (2008), based on many seasons of data, since the model predicts an earlier date for 10% phoma leaf spotting when rainfall and/or temperature are high during summer. Furthermore, the model predicts the date of onset and severity of canker using thermal time, with greater thermal time between 10% phoma leaf spotting and harvest resulting in more severe cankers. This explains why canker severity was less in 2011/2012 and 2012/13, when winter temperatures were less than in 2013/14.

The low incidence of *L. biglobosa* leaf spots, and small amount of *L. biglobosa* DNA in stem canker samples suggests that the disease was caused predominantly by *L. maculans* in these experiments. It has been suggested that *L. maculans* and *L. biglobosa* have a north-south divide (Stonard et al. 2010), so a smaller amount of *L. biglobosa* in these southern sites was not unexpected. A multiple site study over several years is required to establish more information on the threat that *L. biglobosa* poses to UK oilseed rape production.

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Figure legends

Figure 1. Numbers of ascospores of *Leptosphaeria* spp. (a, c, e, g), average temperature and daily rainfall (b, d, f, h) monitored over four cropping seasons. a-b) 2011/12 cropping season; c-d) 2012/13; e-f) 2013/14; g-h) 2014/15. Weather data were collected at Boxworth, Cambridgeshire, using a day interval automated weather station. The grey line represents average temperature (^{o}C) and black bars represent total daily rainfall (mm). Airborne ascospores (number m^{-3}) were collected using a Burkard spore sampler that was situated at Whittlesford, Cambridgeshire (15 km from site of the field experiment) in 2011/12, 2012/13 and 2013/14 and Boxworth, Cambridgeshire in 2014/15.

Figure 2. Incidence of phoma leaf spotting associated with *Leptosphaeria maculans* (a, c, e) or *L. biglobosa* (b, d, f) type leaf lesions on winter oilseed rape (cv. Catana) plots sprayed with fungicide at T1 (early) (a, b), T2 (late) (c, d) or T1 & T2 (combined) (e, f) in the 2013/14 cropping season near Boxworth, Cambridgeshire. Fifteen winter oilseed rape plants were collected from each plot and assessed for incidence of *L. maculans* and *L. biglobosa* type leaf lesions. Plots were treated with penthiopyrad + picoxystrobin (dotted line), prothioconazole (dashed line) or untreated (solid line). Average number of leaf lesions per leaf was calculated. Standard errors of the means are represented as error bars. Details of spray timings are given in Table 1.

Figure 3. Basal stem canker severity on experimental winter oilseed rape (cv. Catana) plots in a) 2011/12, b) 2012/13, c) 2013/14 and d) 2014/15 cropping seasons near Boxworth, Cambridgeshire. Plots received sprays of penthiopyrad + picoxystrobin or prothioconazole at T1 (early), T2 (late) or T1 & T2 (combined). Basal stem canker severity (scale 0-6; Lô-Pelzer et al., 2009) was scored on 25 plant stems sampled from each plot. Standard errors of the means are represented as error bars (6 df). Details of spray timings are given in Table 1.

Figure 4. Average yield (t/ha) from experimental winter oilseed rape (cv. Catana) plots in a) 2011/12, b) 2012/13, c) 2013/14 or d) 2014/15 cropping seasons near Boxworth, Cambridgeshire. Plots received sprays of penthiopyrad + picoxystrobin or prothioconazole at an early (T1), late (T2) or combined (T1 & T2) timings. Desiccated plots were harvested using a small plot harvester and yield was calculated. Standard errors of the means are represented as error bars (6 df). Details of spray timings are given in Table 1.

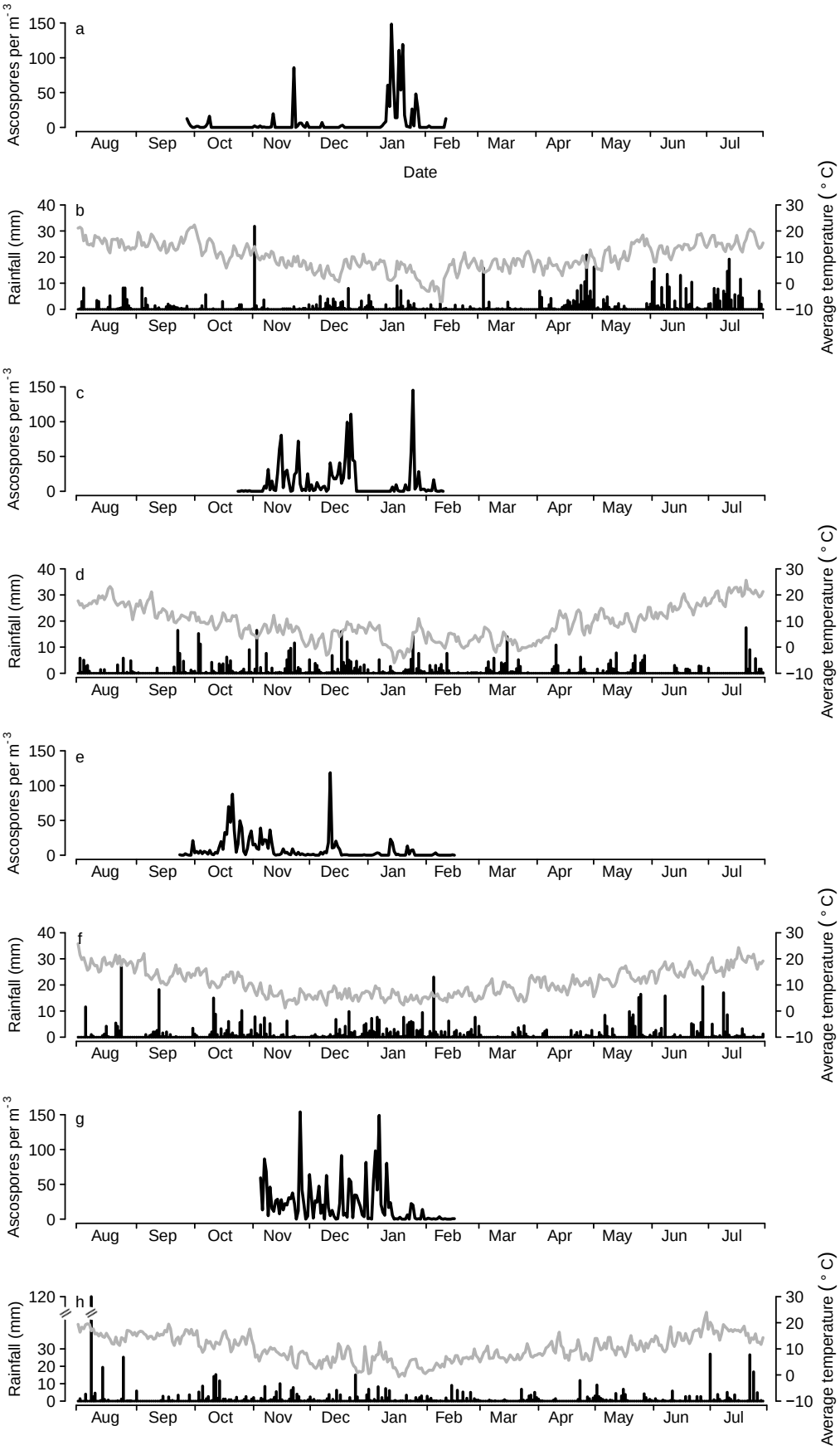
a
b
c
d
e
f
g
h
Ascospores per m-3
Rainfall (mm)
Average temperature (° C)
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Date

Figure 1

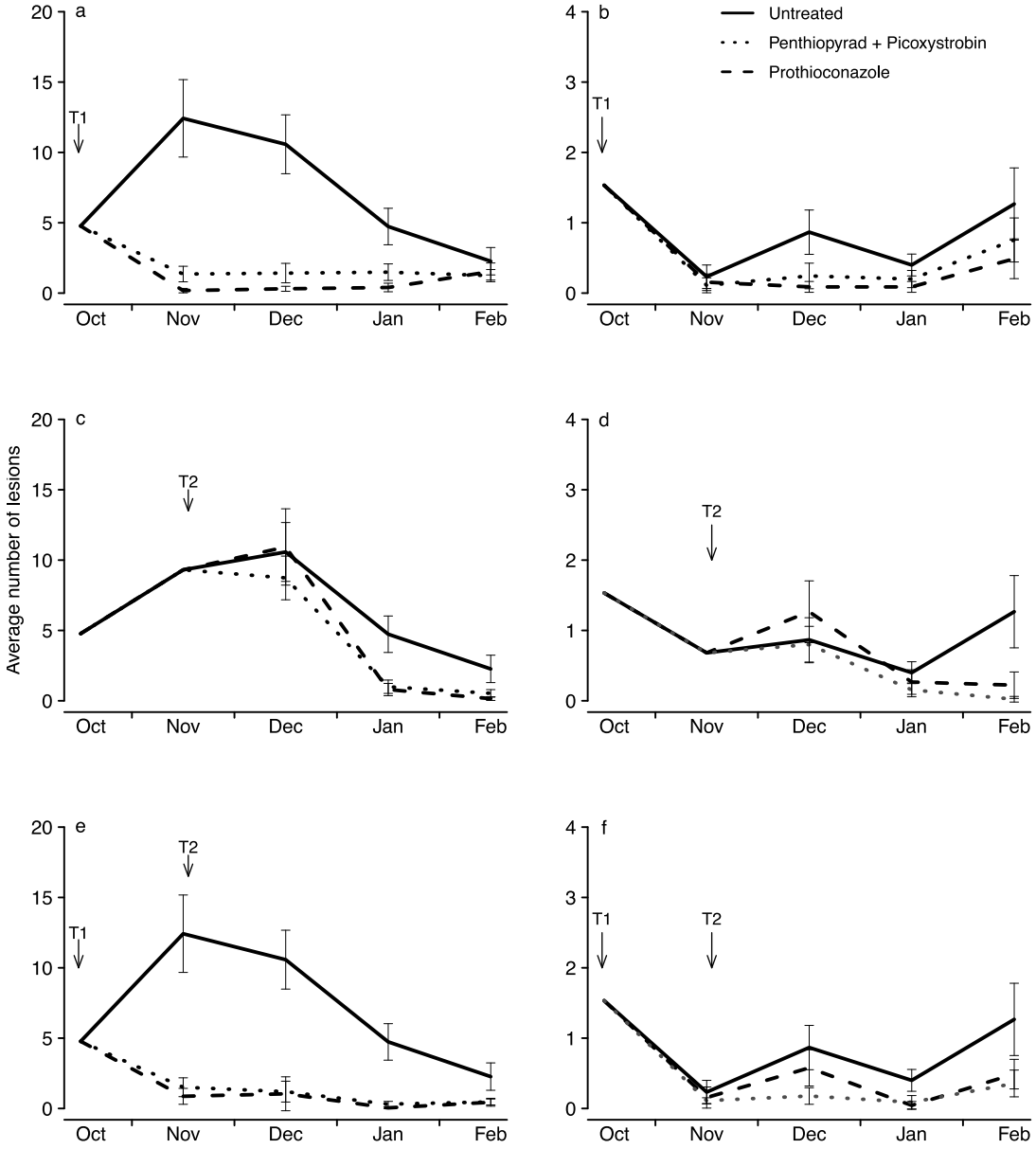
a
b
c
d
e
f
Untreated
Penthiopyrad + Picoxystrobin
Prothioconazole
T1
T2
Average number of lesions
Oct
Nov
Dec
Jan
Feb
0
1
2
3
4
5
10
15
20

Figure 2

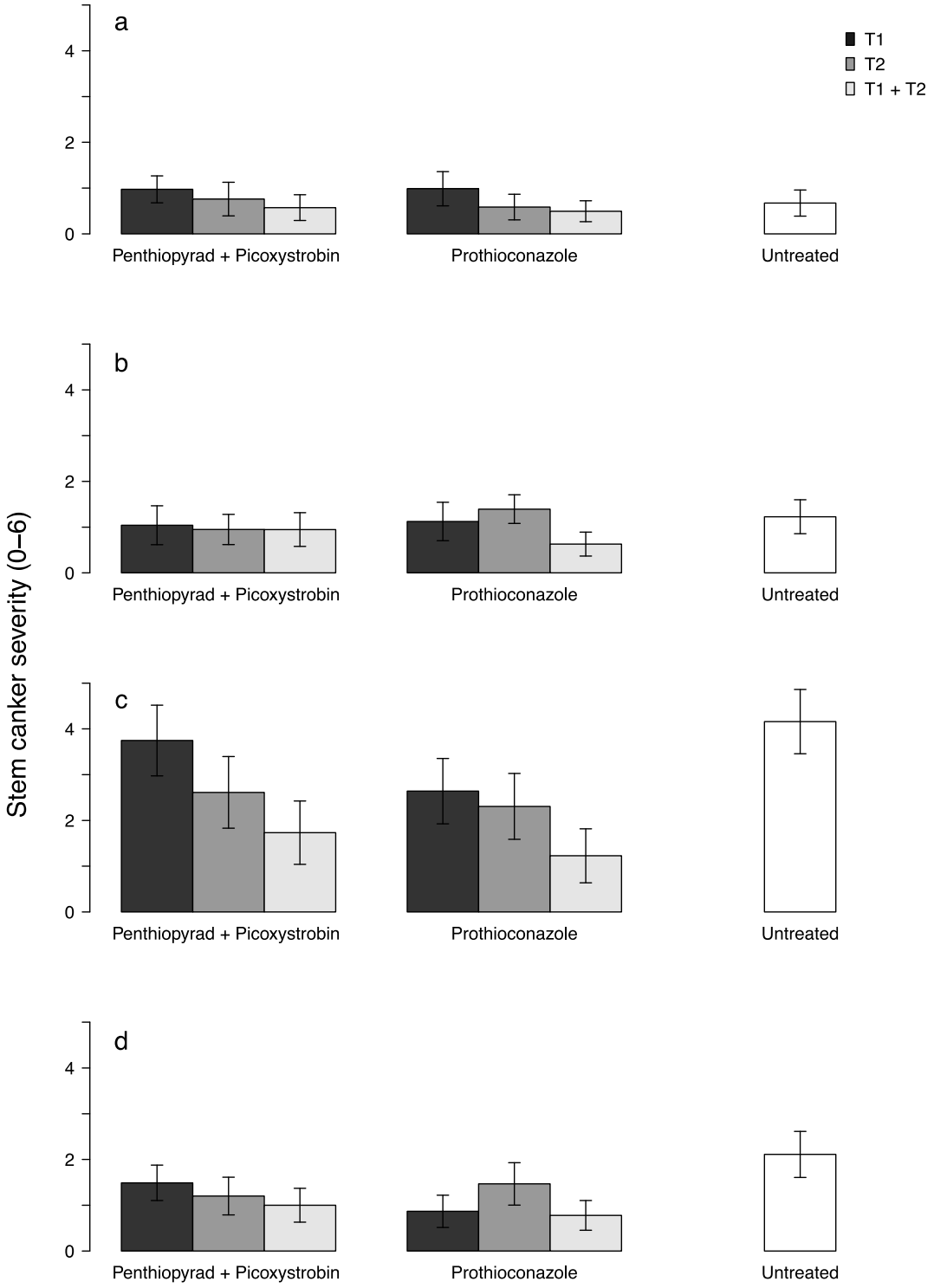
a
b
c
d
T1
T2
T1 + T2
Stem canker severity (0–6)
0
2
4
Penthiopyrad + Picoxystrobin
Prothioconazole
Untreated

Figure 3

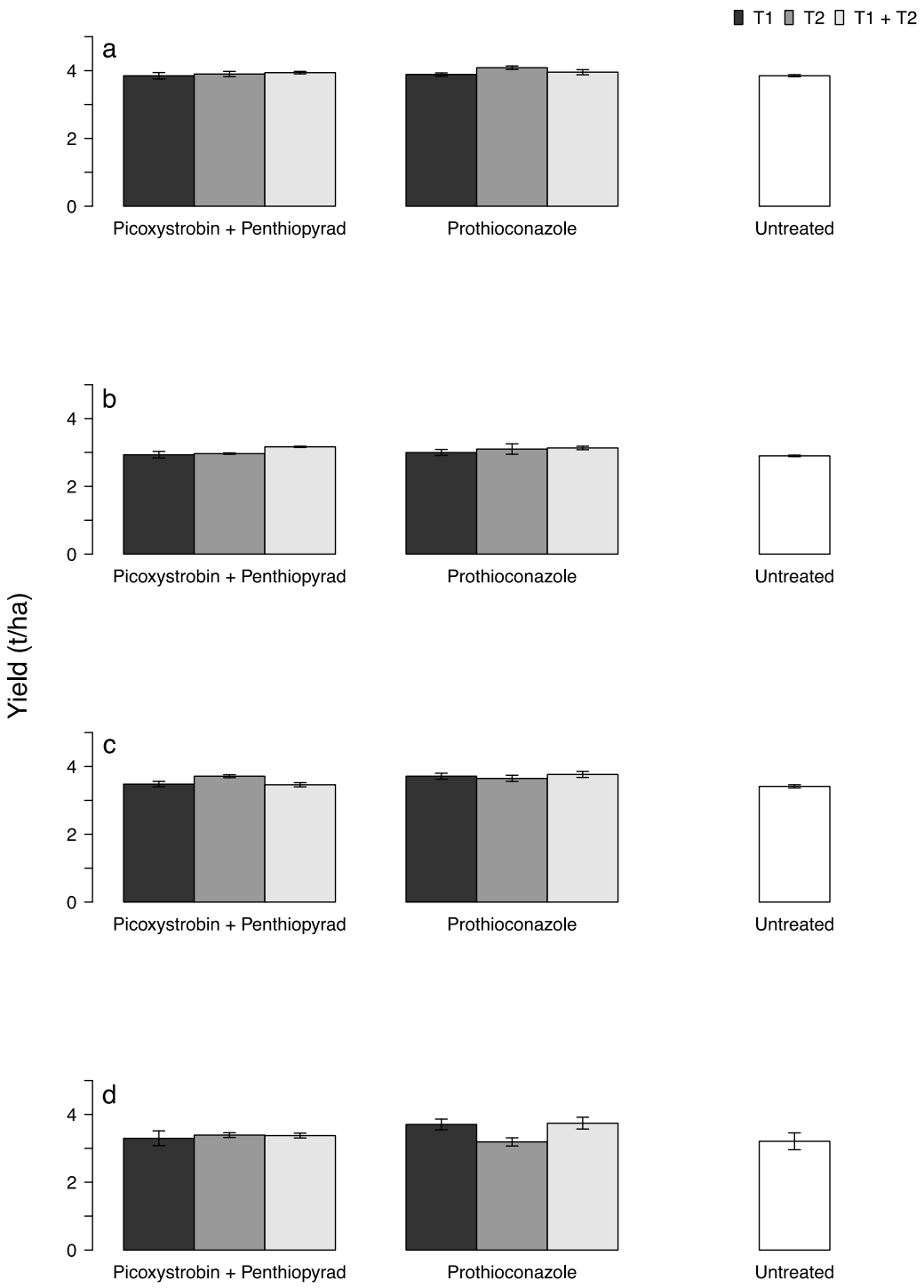
T1
T2
T1 + T2
a
b
c
d
Yield (t/ha)
0
2
4
Picoxystrobin + Penthiopyrad
Prothioconazole
Untreated

Figure 4

Table 1 Treatment list giving fungicides and spray timings used in field experiments at Boxworth, Cambridge over four winter oilseed rape (cv. Catana) cropping seasons. Experiments were arranged in a randomised block design with three replicates. T1 spray was applied in the autumn when 10% of the plants had phoma leaf spotting. T2 spray was applied in the autumn/winter 4 or 8 weeks after T1. A third fungicide spray (T3) targeting sclerotinia stem rot was applied to all treatments except treatment 1, which remained untreated throughout the cropping season. In 2011/12 and 2012/13 cropping seasons, prothioconazole was used as the flowering spray (T3) and in 2013/14 and 2014/15 picoxystrobin was used.

Spray timing	T1 (10% leaf spotting)		T2 (T1 + 4 or 8 weeks)	
Treatment number	Chemical	Rate g a.i/ha	Chemical	Rate g a.i/ha
1	Untreated	-	Untreated	-
2*	Untreated	-	Untreated	-
3^	Flusilazole or Prothioconazole	200 or 176	Untreated	-
4	Penthiopyrad	160	Untreated	-
5	Picoxystrobin	80	Untreated	-
6	Penthiopyrad + Picoxystrobin	160 + 80	Untreated	-
7^	Untreated	-	Flusilazole or Prothioconazole	200 or 176
8	Untreated	-	Penthiopyrad	160
9	Untreated	-	Picoxystrobin	80
10	Untreated	-	Penthiopyrad + Picoxystrobin	160 + 80
11^	Flusilazole or Prothioconazole	200 or 176	Flusilazole or Prothioconazole	200 or 176
12	Penthiopyrad	160	Penthiopyrad^	160
13	Picoxystrobin	80	Picoxystrobin	80
14	Penthiopyrad + Picoxystrobin	160 + 80	Penthiopyrad + Picoxystrobin	160 + 80

* Received T3 flowering spray and therefore differs from treatment 1 which was untreated throughout cropping season.

^ Flusilazole was applied in 2011/12 and 2012/13 until its withdrawal and was replaced by prothioconazole in 2013/14 and 2014/15

Table 2: Numbers (percentage) of winter oilseed rape (cv. Catana) phoma stem canker subsamples with *L. maculans* or *L. biglobosa* DNA present determined by species-specific PCR for *L. maculans* and *L. biglobosa* (subsamples collected stem base cankers* or upper stem lesions sampled from all plots on 1 July 2014 were ground into a powder before DNA was extracted).

	Number (%) of stem canker subsamples with			
	L. maculans only	*L. biglobosa* only	Both	Neither
Upper stem lesion (n = 74)	45 (60.8 %)	2 (2.7 %)	11 (14.9 %)	16 (21.6 %)
Basal stem canker (n = 133)	102 (77 %)	0	4 (2.7 %)	27 (20.3 %)

* three stem base cankers or upper stem lesions per plot