

Citation for published version:

Thomas R. Sewell, Steven Moloney, Mike Ashworth, Faye Ritchie, Alla Mashanova, Yong Ju Huang, Henrik U. Stotz, and Bruce D. L. Fitt, 'Effects of a penthiopyrad and picoxystrobin fungicide mixture on phoma stem canker (*Leptosphaeria* spp.) on UK winter oilseed rape', *European Journal of Plant Pathology*, Vol. 145 (3): 675-685, July 2016.

DOI:

<https://doi.org/10.1007/s10658-016-0916-8>

Document Version:

This is the Accepted Manuscript version.
The version in the University of Hertfordshire Research Archive may differ from the final published version.

Copyright and Reuse:

© 2016 Koninklijke Nederlandse Planteziektenkundige Vereniging

This manuscript version is made available under the terms of the Creative Commons Attribution licence CC BY 4.0 (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

Enquiries

If you believe this document infringes copyright, please contact Research & Scholarly Communications at rsc@herts.ac.uk

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

3

4 **Thomas R Sewell • Steven Moloney • Mike Ashworth • Faye Ritchie • Alla Mashanova**
5 **• Yong Ju Huang • Henrik U Stotz • Bruce DL Fitt**

6

7 T.R. Sewell (correspondent) • S. Moloney • A. Mashanova • Y.J. Huang • H.U. Stotz •
8 B.D.L. Fitt

9 Centre for Agriculture, Food and Environmental Management, University of Hertfordshire,
10 College Lane, Hatfield, Hertfordshire AL10 9AB, UK

11 email t.sewell@herts.ac.uk

12

13 M. Ashworth

14 DuPont (UK) Ltd, Kings Court, London Road, Stevenage, Hertfordshire, SG1 2NG, UK

15

16 F. Ritchie

17 ADAS Ltd, Battlegate Road, Boxworth, Cambridge, CB23 4NN, UK

18

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

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

41

42 Keywords

43

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

45

46 Introduction

47

48 Phoma stem canker is a disease of oilseed rape, which is caused by closely related fungal
49 species *Leptosphaeria maculans* and *L. biglobosa* (Fitt, et al. 2006a; Stonard et al. 2010).
50 Both pathogens follow a monocyclic disease cycle in the UK with phoma leaf spotting
51 symptoms in autumn/winter and stem base canker in spring/summer. Severe cankers inhibit
52 the flow of water and nutrients to the seed, and thus decrease seed yield and quality. Oilseed
53 rape is the third most valuable arable crop grown in the UK and has a total annual value of >
54 £600 M and an average on-farm yield of 3.5-4.0 t/ha (AHDB Cereals & Oilseeds 2015).
55 Globally, phoma stem canker has been calculated to annually cause approximately £700M

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

58 Generally, *L. maculans* forms damaging stem base cankers and *L. biglobosa* forms
59 less damaging upper stem lesions on UK winter oilseed rape (Fitt et al. 2006a; Huang et al.
60 2011). This difference is considered a result of differences in timing of ascospore release,
61 with *L. maculans* spores released in early/mid-autumn and *L. biglobosa* spores released in
62 early/mid-winter (Fitt et al. 2006b). More recently, however, *L. biglobosa* has been shown to
63 cause severe upper stem lesions and lodging of crops in some growing seasons (Huang et al.
64 2014). If this occurs regularly, *L. biglobosa* could become a more important threat to winter
65 oilseed rape yield.

66 Together with conventional plant breeding strategies that adopt effective resistance
67 genes (Delourme et al. 2006), fungicides are commonly used in the UK to control phoma
68 stem canker on winter oilseed rape. In 2014, 98.1 % of the total area of oilseed rape (674,580
69 ha) received fungicide treatment for control of disease including phoma stem canker because
70 growers generally expect such treatments to give a yield response (Garthwaite et al. 2012).
71 UK winter oilseed rape experiments have often shown a yield response from fungicide
72 application against phoma stem canker, although, an increase in yield was only registered
73 when canker severity in unsprayed plots was ≥ 3 on a 0-5 disease severity scale (West et al.
74 2002). Typically, azole fungicides have been applied because of their effective action against
75 *L. maculans* as well as their relatively low cost compared to alternatives. Examples include
76 flusilazole, prothioconazole and tebuconazole (Eckert et al. 2010; Huang et al. 2011). Other
77 fungicides are available to growers; these include quinone outside inhibitor (QoI) fungicides
78 and succinate dehydrogenase inhibitor (SDHI) fungicides, both of which disrupt energy
79 production in the fungal cell (Avenot and Michailides 2010; Bartlett et al. 2002); however,
80 their efficacy against phoma stem canker has not been evaluated.

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

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

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

93

94 Materials and Methods

95

96 Weather conditions at the field site

97

98 Weather data for the 2011/12, 2012/13, 2013/14 and 2014/15 winter oilseed rape growing
99 seasons were collected at Boxworth, Cambridgeshire, UK (52.259814, -0.025437); near the
100 winter oilseed rape field experiments and the Burkard spore sampler in 2014/15 cropping
101 season and approximately 15 km from the site of the Burkard spore sampler in 2011/12,
102 2012/13 and 2013/14. Temperature and rainfall data were collected daily using an automated
103 weather station (Campbell Scientific, UK).

104

105 Numbers of ascospores in the air

106

107 The numbers of *Leptosphaeria* ascospores in the air were estimated using a 7-day volumetric
108 spore sampler (Burkard Manufacturing Co. Ltd, UK). For the 2011/12, 2012/13 and 2013/14
109 cropping seasons, the spore sampler was located at Whittlesford, Cambridgeshire, UK
110 (52.109299, 0.156023). For the 2014/15 cropping season, the spore sampler was located at
111 Boxworth, Cambridgeshire (52.270127, -0.027112). The spore sampler accommodated a

112 rotating drum (2 mm per hour) that held a strip of Melinex tape. The tape was lined with a
113 thin layer of petroleum jelly and hexane paste mixture (10 g petroleum jelly, 20 ml hexane).
114 After 7 days of sampling, the rotating drum was removed and the Melinex tape was divided
115 into seven 24-hour segments. Each segment was then cut horizontally, with one half stored at
116 -20 °C for molecular analysis and one half mounted for microscopy to count spore numbers.
117 The slide-mounted tape was stained with trypan blue solution (0.4% w/v in water, Sigma-
118 Aldrich, UK) so that the ascospores were visible under a light microscope (100x total
119 magnification). Counting was done in three longitudinal traverses across the slide and the
120 number of ascospores recorded for each traverse. The concentration of ascospores in the air
121 was calculated according to equation described by Lacey and West (2006).

122

123 Winter oilseed rape field experiments

124

125 Field experiments were established near Boxworth, Cambridgeshire, UK for the 2011/12,
126 2012/13, 2013/14 and 2014/15 cropping seasons. The winter oilseed rape cultivar Catana
127 (Dekalb, UK) was used because of its susceptibility to *L. maculans* (resistance rating of 4 in
128 the UK North region on a 1-9 scale; where 9 is very resistant) but good resistance against
129 *Pyrenopeziza brassicae* the cause of light leaf spot (AHDB Cereals & Oilseeds 2015).

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

140 alternative to the azole fungicides that are used widely in the UK on winter oilseed rape.
141 Sanction® (DuPont UK Ltd; a.i. flusilazole 250g/l) was used for the first two cropping
142 seasons before its active ingredient flusilazole was withdrawn. It was replaced by another
143 azole fungicide, Proline 275® (Bayer Crop Science UK Ltd; a.i. prothioconazole 275 g/l), for
144 the 2013/14 and 2014/15 cropping seasons. To represent the components of Refinzar®,
145 Galileo® (DuPont UK Ltd; a.i. picoxystrobin 250 g/l) and LEM17® (DuPont UK Ltd; a.i.
146 penthiopyrad 200 g/l) were also applied but data are not presented. The fungicide spray
147 timings differed from season to season, with the first application (T1) taking place in autumn
148 when 10% of plants were affected with phoma leaf spots. The second application (T2) was
149 made 8 weeks after T1 in 2011/2012 season and 4 weeks after T1 in 2012/13, 2013/14 and
150 2014/15 seasons. All plots except the untreated control received a spring-flowering spray (T3)
151 against the pathogen *Sclerotinia sclerotiorum*, the causal agent of sclerotinia stem rot.

152 (Table 1 here)

153

154 Phoma leaf spotting, stem canker and yield assessment

155

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

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

168 and scoring the cross-sectional area of necrotic tissue according to a 0-6 scale (Huang et al.
169 2011), modified from Lô-Pelzer et al. (2009). Upper stem lesions were cut at the centre point
170 of the lesions and assessed on the same scale. Desiccated plots were harvested using a small
171 plot harvester and yield (t/ha) recorded. Presence of light leaf on stems was also noted.

172

173 Stem canker subsampling, DNA extraction and species-specific PCR

174

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

189

190 Statistical analysis

191

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

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

197

198 **Results**

199

200 **Rainfall**

201

202 Rainfall patterns differed between the four seasons during autumn/winter (phoma leaf spot
203 development stage) and summer (phoma stem canker development stage). In the 2011/12
204 cropping season, the autumn and winter months were dry compared with the 2013/14
205 cropping season. In August and September, 73 mm of rainfall was recorded. Periods of
206 prolonged rainfall did not commence until December 2011 and there were never periods of
207 heavy rainfall. In the summer, it was predominantly wet, with heavy rainfall in April (101
208 mm), June (103 mm) and July (115 mm) (Figure 1b). In the 2012/13 cropping season,
209 prolonged rainfall occurred much earlier, with periods of substantial rainfall commencing in
210 mid-September and continuing to mid-February with the occasional short dry period. In
211 August and September, 70 mm of rainfall was recorded. The spring and summer were dry
212 with occasional periods of short-term rainfall (Figure 1d). In the 2013/14 cropping season,
213 rainfall pattern was similar to that of the 2012/13 growing season in the autumn/winter.
214 Rainfall started in early autumn, with increases in August and September over a few days and
215 then continued for a period between October and mid-November. In August and September,
216 91 mm of rainfall was recorded. A period of prolonged rainfall occurred between December
217 and February (202 mm over 88 days) (Figure 1f). In the 2014/15 cropping season high rainfall
218 commenced early (8 August) with a period of very heavy rainfall (112.6 mm) causing flash
219 floods in the region. In August and September, 192 mm of rainfall was recorded although
220 58 % of this was on 8 August. Rainfall in the winter months was more sporadic than in the
221 previous seasons, with no periods of particularly prolonged rainfall between December and
222 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 °C 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 °C) occurred on 10 and 11 of February. Average temperature between 1 October and 31
230 May was 7.8 °C (Figure 1b). In 2012/13, a similar pattern was observed, but low temperature
231 (-4.4 °C) 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 °C were recorded during this period. Average temperature between 1
234 October and 31 May was 5.7 °C (Figure 1d). In 2013/14, there was no period of particularly
235 cold weather, with average daily temperature never < 0 °C. Average temperature between 1
236 October and 31 May was 8.3 °C (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 °C on two occasions (-0.7 °C and -0.4 °C on 19 January and 22
239 January, respectively). Average temperature between 1 October and 31 May was 7.2 °C
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

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

260 (Figure 1 here)

261 Field experiments

262
263 In all four cropping seasons, the spring flowering spray had no affect on leaf spotting, canker
264 severity or yield when compared to the control; therefore, the untreated control data presented
265 are a mean of untreated plots and spring spray only (T3) plots. Penthiopyrad alone produced
266 similar results to penthiopyrad + picoxystrobin and therefore has been excluded from the
267 analysis. Picoxystrobin alone produced similar results to the untreated control and therefore
268 has been excluded from the analysis and data are not presented.

270 Phoma leaf spotting

271
272 In the 2011/12, 2012/13 and 2014/15 cropping seasons, incidence of phoma leaf spotting in
273 unsprayed plots did not increase in severity on winter oilseed rape leaves until March and
274 phoma leaf spotting was never severe during the autumn/winter; therefore, data are not
275 shown. In 2013/14, the phoma leaf spotting started earlier and incidence (% plants affected)
276 was much greater in unsprayed plots in the autumn/winter months compared to the previous
277 two winter oilseed rape cropping seasons (Figure 2). Experimental plots treated with

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

319

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

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

337 (Table 2 here)

338

339 Discussion

340

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

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

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

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

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

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

390

391 **Acknowledgements** This work was financially supported by the DuPont UK Ltd, Perry
392 Foundation, Felix Thornley Cobbold Agricultural Trust and the Biotechnology and Biological
393 Sciences Research Council (BBSRC). This research was also supported by ADAS Ltd and
394 University of Hertfordshire. The authors thank Aiming Qi for statistical advice, Avice Hall
395 for supervision and Coretta Klöppel and Georgia Mitrouisia for assisting with data collection.

396

397 **References**

398

399 AHDB Cereals & Oilseeds. (2015). Oilseed rape guide. *Agriculture and Horticulture*
400 *Development Board*. Warwickshire, UK. 31

401 Avenot, H. F., & Michailides, T. J. (2010). Progress in understanding molecular mechanisms
402 and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in
403 phytopathogenic fungi. *Crop Protection*, 29, 643–651.

404 Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M., & Parr-Dobrzanski, B.
405 (2002). The strobilurin fungicides. *Pest Management Science*, 58, 649–662.

406 Delourme, R., Chevre, A. M., Brun, H., Rouxel, T., Balesdent, M. H., Dias, J. S., et al.
407 (2006). Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape
408 (*Brassica napus*). *European Journal of Plant Pathology*, 114, 41–52.

409 Eckert, M. R., Rossall, S., Selley, A., & Fitt, B. D. L. (2010). Effects of fungicides on *in vitro*
410 spore germination and mycelial growth of the phytopathogens *Leptosphaeria maculans*
411 and *L. biglobosa* (phoma stem canker of oilseed rape). *Pest Management Science*, 66,
412 396–405.

413 European Commission. (2007). Review report for the active substance flusilazole. *EC Review*
414 *Reports 6850/VI/97 final*. Brussels, Belgium. 24.

415 Evans, N., Baierl, A., Semenov, M. A., Gladders, P., & Fitt, B. D. L. (2008). Range and

416 severity of a plant disease increased by global warming. *Journal of the Royal Society*
417 *Interface*, 5, 525–531.

418 Fitt, B. D. L., Huang, Y.-J., van den Bosch, F., & West, J. S. (2006a). Coexistence of related
419 pathogen species on arable crops in space and time. *Annual Review of Phytopathology*,
420 44, 163–82.

421 Fitt, B. D. L., Brun, H., Barbetti, M. J., & Rimmer, S. R. (2006b). World-wide importance of
422 phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape
423 (*Brassica napus*). *European Journal of Plant Pathology*, 3–15.

424 Garthwaite, D. G., Hudson, S., Barker, I., Parrish, G., Smith, L., & Pietravalle, S. (2012).
425 Pesticide Usage Survey Report. *Arable Crops in the United Kingdom 2012*, York, UK.
426 87.

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

431 Huang, Y.-J., Karandeni-Dewage CS, & Fitt, B. D. L. (2014). Importance of *Leptosphaeria*
432 *biglobosa* as a cause of phoma stem canker on winter oilseed rape in the UK. *Aspects of*
433 *Applied Biology*, 127, 117–122.

434 Huang, Y.-J., Qi, A., King, G. J., & Fitt, B. D. L. (2014). Assessing quantitative resistance
435 against *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape)
436 in young plants. *PloS One*, 9, e84924.

437 Lacey, M. E., & West, J. S. (2007). *The air spora: a manual for catching and identifying*
438 *airborne biological particles*. Springer US. United States of America. 156.

439 Liu, S. Y., Liu, Z., Fitt, B. D. L., Evans, N., Foster, S. J., Huang, Y.-J., et al. (2006).
440 Resistance to *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed

441 rape) induced by *L. biglobosa* and chemical defence activators in field and controlled
442 environments. *Plant Pathology*, 55, 401–412.

443 Lô-Pelzer, E., Aubertot, J. N., Bousset, L., Pinochet, X., & Jeuffroy, M. H. (2009). Phoma
444 stem canker (*Leptosphaeria maculans*/*L. biglobosa*) of oilseed rape (*Brassica napus*): is
445 the G2 Disease Index a good indicator of the distribution of observed canker severities?
446 *European Journal of Plant Pathology*, 125, 515–522.

447 Marx-Stoelting, P., Niemann, L., Ritz, V., Ulbrich, B., Gall, A., Hirsch-Ernst, K. I., et al.
448 (2014). Assessment of three approaches for regulatory decision making on pesticides
449 with endocrine disrupting properties. *Regulatory Toxicology and Pharmacology*, 70,
450 590–604.

451 R Development Core Team, R. (2011). R: A Language and Environment for Statistical
452 Computing. (R. D. C. Team, Ed.)*R Foundation for Statistical Computing*. R Foundation
453 for Statistical Computing. doi:10.1007/978-3-540-74686-7

454 Steed, J. M., Baierl, A., & Fitt, B. D. L. (2007). Relating plant and pathogen development to
455 optimise fungicide control of phoma stem canker (*Leptosphaeria maculans*) on winter
456 oilseed rape (*Brassica napus*). *European Journal of Plant Pathology*, 118, 359–373.

457 Stonard, J. F., Latunde-Dada, A. O., Huang, Y.-J., West, J. S., Evans, N., & Fitt, B. D. L.
458 (2010). Geographic variation in severity of phoma stem canker and *Leptosphaeria*
459 *maculans*/*L. biglobosa* populations on UK winter oilseed rape (*Brassica napus*).
460 *European Journal of Plant Pathology*, 126, 97–109.

461 West, J. S., Balesdent, M., Rouxel, T., Narcy, J. P., Huang, Y.-J., Roux, J., et al. (2002).
462 Colonization of winter oilseed rape tissues by A/Tox+ and B/Tox0 *Leptosphaeria*
463 *maculans* (phoma stem canker) in France and England. *Plant Pathology*, 51, 311–321.

464 West, J. S., Fitt, B. D. L., Leech, P. K., Biddulph, J. E., Huang, Y., & Balesdent, M. (2002).
465 Effects of timing of *Leptosphaeria maculans* ascospore release and fungicide regime on
466 phoma leaf spot and phoma stem canker development on winter oilseed rape (*Brassica*

467 *napus*) in southern England. *Plant Pathology*, 51, 454–463.

468 Zhou, Y., Fitt, B. D. L., Welham, S. J., Gladders, P., Sansford, C. E., & West, J. S. (1999).

469 Effects of severity and timing of stem canker (*Leptosphaeria maculans*) symptoms on

470 yield of winter oilseed rape (*Brassica napus*) in the UK. *European Journal of Plant*

471 *Pathology*, 105, 715–728.

472

473

474

475

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 (°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.

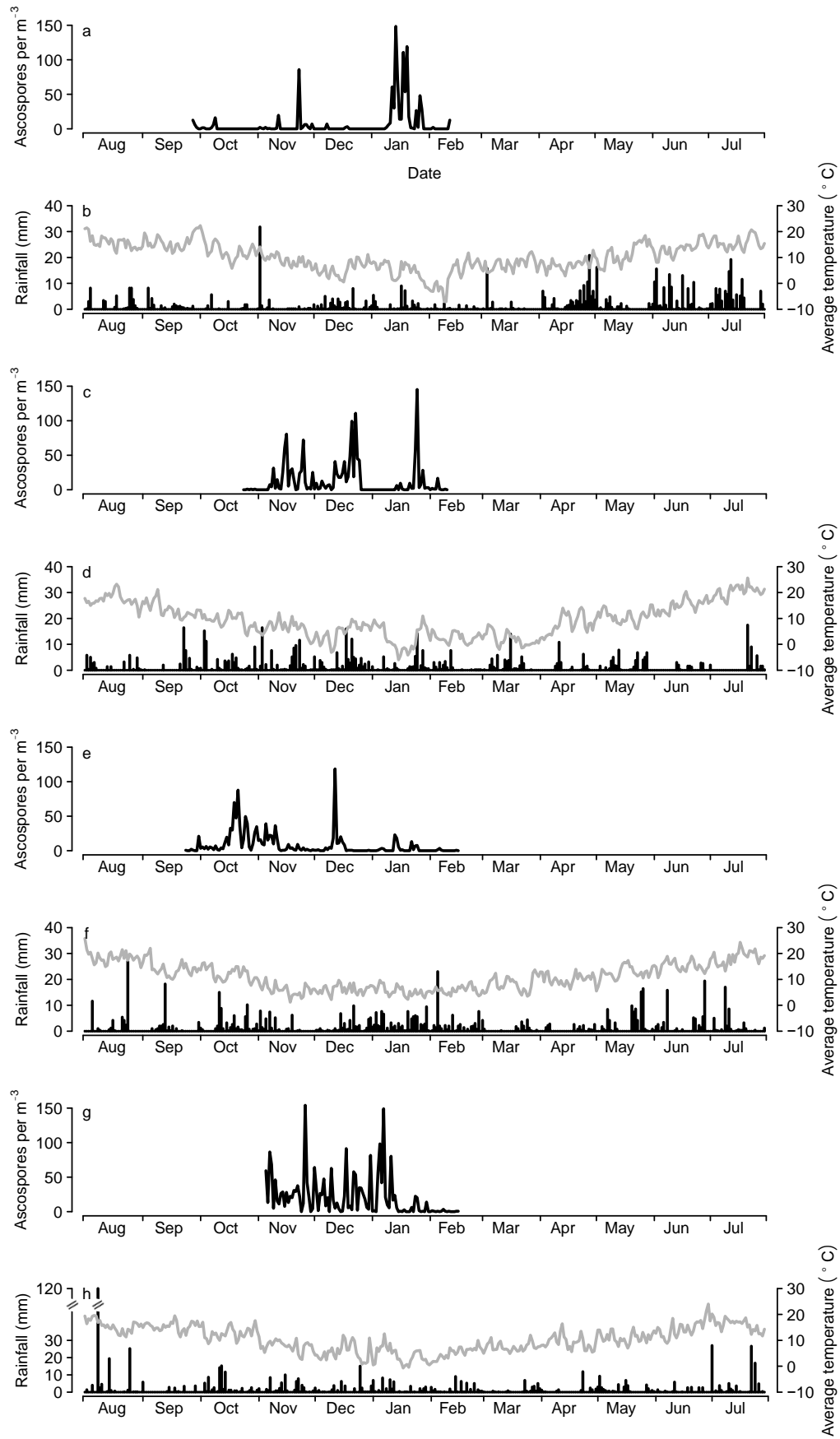


Figure 1

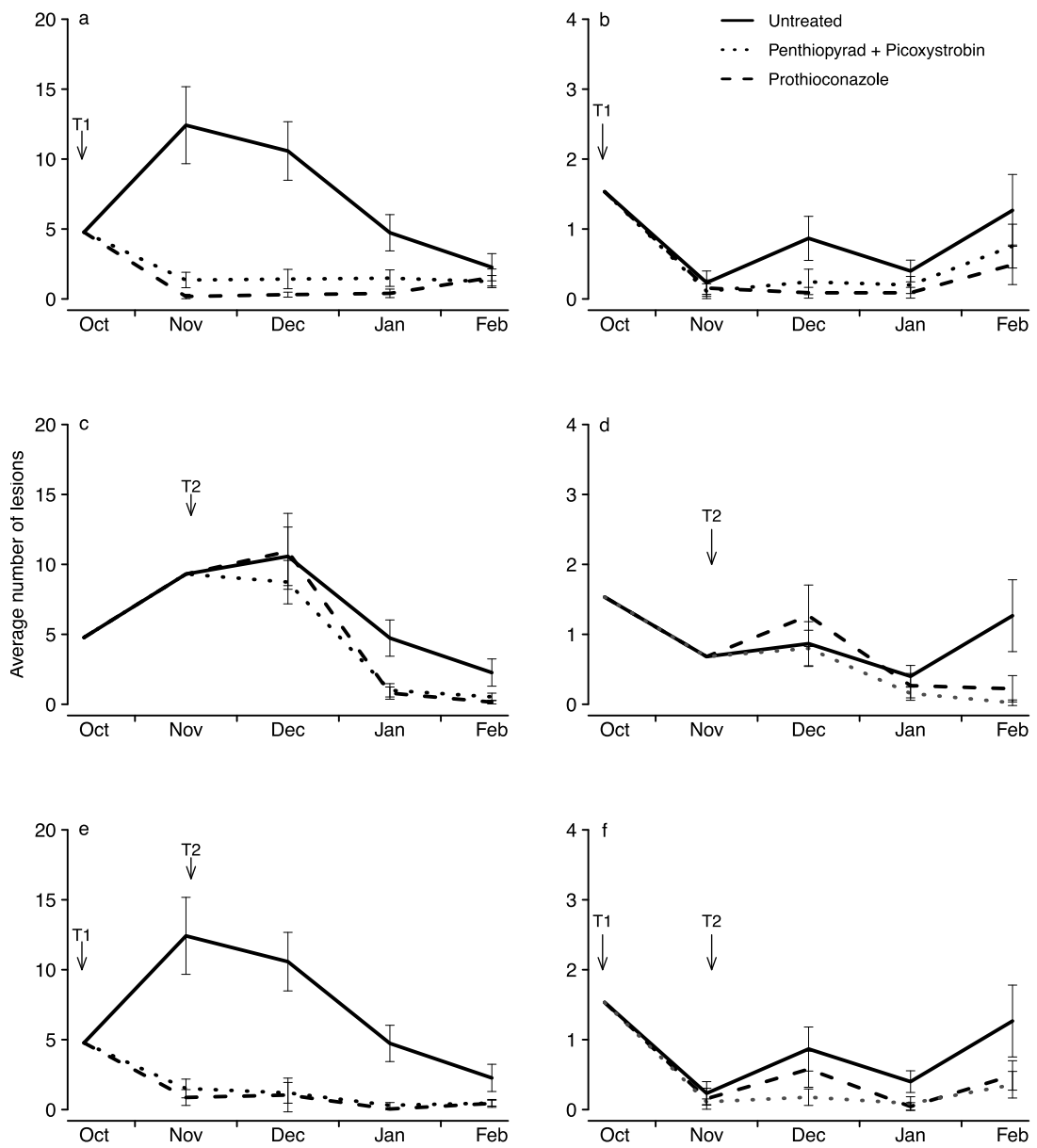


Figure 2

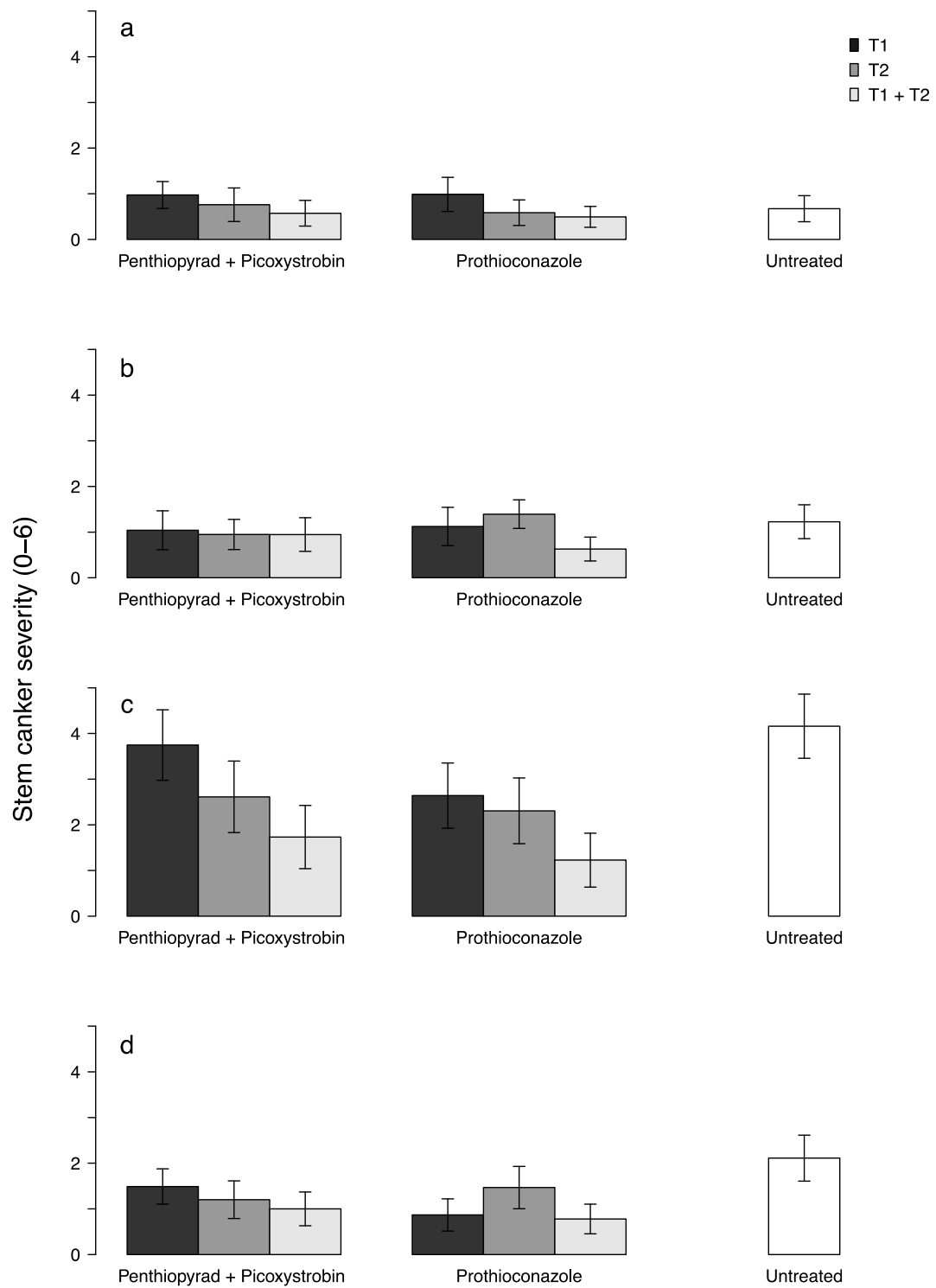


Figure 3

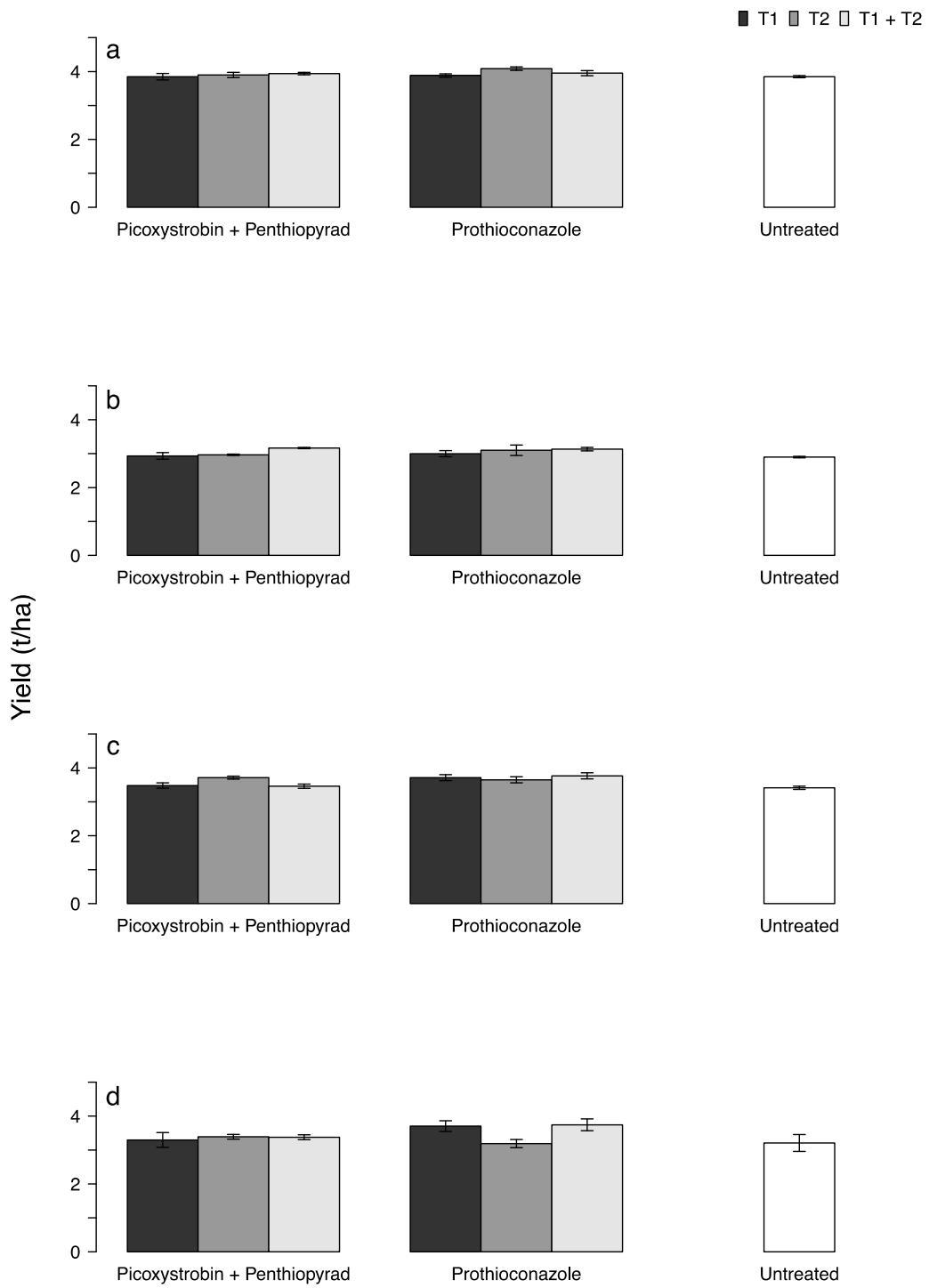


Figure 4

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
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			
	<i>L. maculans</i> only	<i>L. biglobosa</i> 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