Plant Pathology (2002) **51**, 454–463

Effects of timing of *Leptosphaeria maculans* ascospore release and fungicide regime on phoma leaf spot and phoma stem canker development on winter oilseed rape (*Brassica napus*) in southern England

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At Rothamsted during 1997/98, 1998/99 and 1999/2000 winter oilseed rape growing seasons, numbers of air-borne ascospores of *Leptosphaeria maculans* were often > 4 m^{-3} from autumn (September/October) to spring (April/May), while few or no ascospores were detected during the summer. Mature pseudothecia were generally not observed on debris of the previous crop until September. One-year-old debris (harvested in July 1998) had 95% discharged and 5% mature pseudothecia in August 1999, but by 15 September new pseudothecia (of which 30% were mature) were observed and the first increase in air-borne ascospores (> 4 m^{-3}) occurred. Phoma leaf spotting appeared in untreated field plots 14–25 days after the first increase in air-borne ascospores in autumn. The fungicide mixture difenoconazole plus carbendazim decreased the incidence of new leaf lesions for 1 month after application in autumn and for 2 months in mid-winter. When *L. maculans* was isolated from infected leaves, the growth rate of isolates from leaves to which fungicide was applied was less than that of those from untreated leaves. Foliar applications of fungicide to field plots in the autumn and winter not only decreased the incidence of crown cankers but also reduced the rate of canker development on stem bases in the spring and early summer (when severity of crown cankers increased linearly with time). In untreated crops, when phoma leaf spots appeared early in the autumn, crown cankers developed early in the spring but only became severe enough before harvest to reduce yield greatly in 1997/98. Yield loss was associated with crown cankers that girdled more than half of the stem by harvest (mean severity > 3 on a 0–5 scale). Infections of new leaves produced after stem extension, from January onwards, led to phoma stem lesion development above the crown. In the three seasons, phoma stem lesions became moderately severe (> 2) by harvest only in untreated plots in 1997/98.

Keywords: ascospore numbers, crown canker, *Phoma lingam*, phoma stem lesions, pseudothecia

Introduction

Leptosphaeria maculans causes phoma stem canker (blackleg), a serious disease of oilseed rape (canola) worldwide. It is a damaging disease of winter oilseed rape in western Europe, although the severity of epidemics differs from season to season, from region to region and from crop to crop (Fitt *et al*., 1997; Penaud *et al*., 1999a; West *et al*., 2001). Epidemics are initiated by air-borne ascospores of *L. maculans*, produced in pseudothecia on crop debris, in the autumn (Gladders & Musa, 1980). The ascospores infect leaves to cause phoma leaf spots. Biddulph *et al*. (1999) showed that initial symptoms of infection on inoculated leaves can start to appear after only 5 days at 20°C, 6 days at 16°C, or 14 days at 8°C, but the distinctive pale lesions bearing pycnidia are not visible until some days later. From phoma leaf spots, the pathogen can grow to infect the stem (Hammond *et al*., 1985). The most damaging cankers are formed at the stem base (crown cankers) in spring/summer from leaf infections occurring before stem extension starts in late winter, whereas later leaf infections produce phoma stem lesions higher up the stem (Hammond & Lewis, 1986; Sun *et al*., 2000, 2001). Collectively, crown cankers and phoma stem lesions were termed phoma stem canker by West *et al*. (2001).

Two forms of the fungus occur in Europe; the A-group (Tox$^+$) is generally associated with damaging crown cankers on the stem base, while the B-group (Tox^0) is

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Accepted 22 January 2002

considered to be less damaging (Johnson & Lewis, 1994) and has recently been reclassified as *L. biglobosa* (Shoemaker & Brun, 2001). Most yield losses, due to early senescence and lodging, were associated with the most severe phoma stem cankers (Zhou *et al*., 1999). To develop effective strategies for control of severe epidemics, there is a need to understand the relationships between ascospore maturation and the timing of ascospore dispersal, the development of phoma leaf spots and subsequent development of crown cankers and phoma stem lesions.

In England, control of phoma stem canker depends on the use of fungicides, as resistance in current cultivars is not sufficient for complete control, and decisions about fungicide timing need to be made in the autumn (Gladders *et al*., 1998; West *et al*., 1999). As it is thought that fungicides do not control *L. maculans* once it has reached the stem, it is important to treat crops when the leaves show symptoms (West *et al*., 2001). The period of greatest risk is in the early autumn when temperatures are high and leaves small, as *L. maculans* spreads from leaves to stems more quickly when temperature is higher and leaf petioles shorter (Hammond *et al*., 1985). Up to two applications of fungicides may be advised against phoma stem canker and, even then, the timing of fungicide applications is crucial for effective control of the disease (Gladders *et al*., 1998). The development of accurate forecasts of severe epidemics will assist fungicide spray decisions in the autumn.

A system to improve fungicide spray timing based on host, pathogen and environmental factors has been produced in France, where weather is different from that in southern England. In France, a risk of infection has been associated with occurrence of 7 rain-days (> 1 mm) after sowing of the new crop, completed maturation of pseudothecia on stem debris or the first detection of > 20 *L. maculans* ascospores per day (Penaud *et al*., 1999b), or with the occurrence of 16–19 rain-days since the harvest of the previous crop (Pérès & Poisson, 1997). When one of these criteria is satisfied, a decision on whether or not to spray is made, based on cultivar susceptibility, growth stage, soil type and plant vigour. In England, in the absence of a forecasting scheme, applications of fungicide are usually made in response to the first observation of leaf spots (commonly once a threshold incidence of 20% plants affected is reached) (Gladders *et al*., 1998; West *et al*., 1999). Weekly observations may be required to detect these first leaf spots before the infections become too well established for the disease to be effectively controlled. To produce an effective forecasting scheme, the relationships between host, pathogen and environmental factors in the autumn need to be clearly understood. This paper investigates the relationships between the timings of ascospore release, phoma leaf spotting and phoma stem canker development to guide optimal use of fungicides in southern England.

Materials and methods

Air-borne spores, phoma leaf spot, crown canker and phoma stem lesion development in field plots

Field experiments were established at Rothamsted in southern England in the 1997/98, 1998/99 and 1999/2000 seasons (Table 1). Winter oilseed rape cultivars Capitol and Lipton were sown in late August in four randomized blocks of five main plots, with each main plot split between the two cultivars. The UK rating for resistance to *L. maculans*, based on phoma stem canker symptoms, for Capitol was 6 and that for Lipton was 5 (Anonymous, 1997). These cultivars were chosen because they were thought to be susceptible to different pathogenicity groups of *L. maculans* (M-H Balesdent, unpublished data) and to be resistant to light leaf spot (*Pyrenopeziza brassicae*) and downy mildew (*Peronospora parasitica*). Each subplot was 15×3 m, including a 9 m^2 sampling area and a central area of 12 m^2 for harvest yield. Guard rows, 3 m wide, between plots and areas of the field surrounding the experiment were sown with cv. Capitol. To supplement naturally occurring ascospore inoculum each

Table 1 Dates of cultural procedures, including fungicide applications, in field experiments to study factors affecting phoma stem canker (*Leptosphaeria maculans*) on winter oilseed rape (cvs Capitol and Lipton) during three seasons at Rothamsted

	Dates of cultural procedures		
Fungicide regimes	1997/98	1998/99	1999/2000
Control	none	none	none
A1	23 Oct, 13 Nov, 24 Feb	15 Oct	11 Oct
A2	26 Nov, 16 Jan, 24 Feb	19 Oct, 23 Nov	27 Oct, 25 Nov
A3	23 Oct, 26 Nov, 16 Jan, 24 Feb	19 Oct, 23 Nov, 13 Jan	25 Nov, 4 Mar
SF	24 Sep, 23 Oct, 26 Nov, 16 Jan, 24 Feb	28 Sep, 19 Oct, 23 Nov, 13 Jan, 4 Mar	30 Sep, 27 Oct, 25 Nov, 10 Jan, 4 Mar
Sowing (seeds m^{-2})	26 Aug (120)	27 Aug[1] (80)	27 Aug[1] (80)
Desiccation	10 July	9 July	12 July
Harvest	19 July	17 July	20 July

[1]Sowing density was decreased after the first season to decrease height of plants and thus the risk of lodging.

season, a line around the experiment, *c.* 3 m from the plots, was inoculated in early September with infected winter oilseed rape stem base debris (*c.* 200 stems in total each season). The stem debris had been collected at the end of the previous season and kept outdoors. The plots received different fungicide regimes, designed to produce different phoma stem canker epidemics, ranging from untreated to five fungicide applications from September to February (SF plots). The other three fungicide regimes were adjusted from season to season in response to the natural epidemics of phoma leaf spotting to allow leaf infection at certain times but to prevent it at other times (Table 1). The fungicide used was a mixture of difenoconazole (as 'Plover', at 62·5 g a.i. ha^{-1}) plus carbendazim (as 'Campbell's carbendazim 50% flowable' in 1997/98, 'Stefes C-Flo 2' in 1998/99, and 'Bavistin DF' in 1999/2000, all at 125 g a.i. ha^{-1}) applied in water at 220 L ha^{-1}. Plots received other pesticides and fertilizer according to local commercial practice and were desiccated with 'Reglone' (diquat at 1·5 g L^{-1} a.i.) prior to harvest, combine harvested in mid-July and yields determined.

Each month, the growth stage (GS) of the crop was evaluated using the scale derived by Sylvester-Bradley & Makepeace (1985) and 25 plants, sampled at random from the sampling area of each plot, were assessed for incidence (percentage of plants affected) and severity of all diseases present. The severity of phoma leaf spotting was recorded for each plant as number of leaves affected. Phoma stem canker (crown cankers and phoma stem lesions, as defined by West *et al.*, 2001) was scored on a 0–5 scale (where 0 = uninfected, 1 = < 25% stem circumference girdled, 2 = 25–50% girdled, 3 = > 50% girdled, stem firm, 4 = > 50% girdled, stem weak, 5 = plant dead or lodged) and the mean severity score for plants with a score ≥ 1 was calculated. Crown cankers were associated with the scars of rosette leaves produced before GS 2,1 and were typically < 5 cm above the crown, while phoma stem lesions were associated with scars of leaves produced after GS 2,1 and typically > 5 cm above the crown (Zhou *et al.*, 1999). The numbering of leaves to record GS and the association of crown cankers with leaves below 5 cm in height (rosette leaves) was verified by counting and measuring the vertical distance from the cotyledon leaf scars to each successive leaf scar on four stems (cv. Capitol) at the end of the season. Disease assessment data were analysed by analysis of variance using the Genstat statistical package (Payne *et al.*, 1993).

A Burkard spore trap (surrounded by eight trays, each ~0·5 m^2 and containing 100 infected stems similar to those used to inoculate the field experiment), situated 1 km from the field experiment, was used each season to monitor the daily concentration of air-borne *L. maculans* ascospores. Development of *L. maculans* pseudothecia on the stem debris was investigated by microscopic examination of 25 pseudothecia, taken from five different stems (five per stem) each week from late August onwards. Pseudothecia were classed as mature when their asci contained differentiated ascospores with more than four cells per spore. In autumn 1999, numbers of mature pseudothecia on stems from the crop harvested in 1999 were compared with numbers on stems from crops harvested in 1998 and kept outdoors subsequently. Meteorological data were collected by the Rothamsted synoptic meteorological station situated *c.* 1 km from the experiments in each season.

Fungicide activity

The activity of the fungicide mixture used in the field experiment was investigated, both in the crop and in controlled environment experiments. In October 1999, eight plants (cv. Lipton) bearing leaves upon which the first lesions of the season were beginning to appear were collected 1 week and 3 weeks after the first fungicide application was made (30 September 1999) from treated (SF) plots and untreated (control) plots. On each occasion, leaves with lesions present were surface sterilized in NaOCl (*c.* 1% available chlorine) for 2 min and a section (0·5 × 0·5 cm) from each lesion was placed in a Petri dish containing 1·5% distilled water agar (DWA). The growth rates of mycelium produced from the lesion sections at 20°C were assessed and the colonies identified.

In controlled conditions (15°C, photoperiod 12 h, light intensity 200 μmol/m^2 s^{-1}), leaves six and seven of 36 plants (cv. Lipton) were each inoculated with a drop of *L. maculans* ascospore suspension (2×10^3 spores mL^{-1}, i.e. *c.* 25 spores per drop) at one point on each leaf. The inoculation points had been rubbed gently with a pencil eraser to ensure that the drops of spore suspension would remain in place. The inoculation points were central and adjacent to the mid-rib of each leaf. The plants were grown in a peat-based compost (75% peat, 12% loam, 10% grit, 3% vermiculite, plus 2 kg 'Osmocote Plus' per m^3 and 1·5 kg 'PG mix' per m^3; Petersfield Products, Cosby, Leicester, UK) in 9 cm diameter plastic pots. Plants were kept inside polyethylene chambers to maintain 100% relative humidity for the first 3 days following inoculation. Each week, subsets of six inoculated plants were temporarily removed and sprayed with the fungicide mixture at the concentration used in the field experiment (0·28 g L^{-1} difenoconazole plus 0·57 g L^{-1} carbendazim). Isolations on DWA were made from the site of inoculation and from sections of leaf taken at distances of 1, 2, 4, 6, 8 and 10 cm from the inoculation site along the mid-rib, in the direction of the stem. These isolations were made from three untreated plants and from three fungicide-treated plants (two leaves per plant), 1 day after the fungicide application was made and from another three treated plants 1 week after the fungicide application.

Results

Air-borne spores and phoma leaf spotting in field plots

After harvest in July, mature pseudothecia were first observed on infected stem debris on 26 September 1997, 23 July 1998 (at sites of severe crown cankers only) and 22 September 1999. In August 1999, stem debris that was > 1 year old was found to have predominantly (95%)

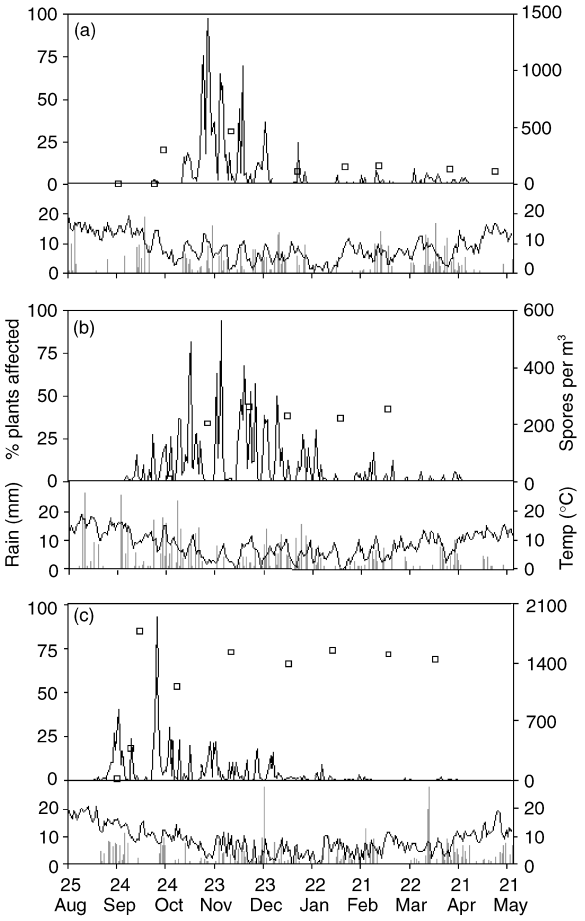

Figure 1 Changes with time in the numbers of ascospores of *Leptosphaeria maculans* (line on upper axis) in the air and incidence (% plants affected) of phoma leaf spots (□) in untreated winter oilseed rape (mean of cvs Capitol and Lipton) in relation to daily rainfall (vertical bars) and temperature (line on lower axis) at Rothamsted in (a) 1997/98, (b) 1998/99 and (c) 1999/2000.

empty (discharged) pseudothecia with occasional mature pseudothecia present. By 15 September, new immature pseudothecia were observed on the 1 year old stem debris and the incidence of mature pseudothecia had increased to 30% of those sampled. This was 1 week before mature pseudothecia were first detected on the stem debris from crops harvested in July 1999.

There were differences between seasons in the numbers and patterns of ascospores released. At the start of each season, few or no ascospores were detected before mid-September. The first days with > 4 ascospores m^{-3} detected were 8 October 1997, 27 September 1998 and 14 September 1999 and ascospores continued to be released until late spring in each season. These first significant releases of ascospores occurred when the daily mean temperatures were < 14°C after at least 3 days without rain and were associated with rainfall (Fig. 1). For example, in 1997/98 (Fig. 1a) the first spore release on 8 October 1997 was after the temperature had fallen to *c.* 12·5°C on the previous 2 days and there had been rain on 7 October. Small numbers of spores were released on wet days for the next week but none were released during the subsequent 20 days without rainfall. The next rainfall on the 4 November was associated with release of many ascospores. The number of rain-days (= 0·2 mm of rain per day) from 20 July (harvest) to the date of first increase in spore release each season was 28, 27 and 17, days, respectively, for 1997/98, 1998/99 and 1999/2000. There were 23, 21 and 16 rain-days from 1 August to the date of first spore release. Subsequent releases of ascospores were associated with occurrence of rain or dew, with maxima of 1465 (19 November 1997), 566 (27 November 1998) and 1941 (22 October 1999) spores m^{-3}.

Phoma leaf spotting was first observed on 22 October 1997 (GS 1,10), 22 October 1998 (GS 1,5) and 28 September 1999 (GS 1,5) (i.e. 15, 25 and 14 days after the first increase in spore release in 1997, 1998 and 1999, respectively). The incidence of phoma leaf spot was often not significantly different between the two cultivars and data are presented as the mean of both cultivars. The development of phoma leaf spotting in untreated plots differed between the seasons; in 1997/98 (Fig. 1a), the incidence of affected plants reached 20% in late October with a maximum incidence of 31% in untreated plots in early December. In 1998/99 (Fig. 1b), although a few leaf lesions were visible in late October, the main leaf spotting epidemic began 3 weeks later than in 1997/98, with incidence reaching 20% in early/mid November and a maximum of 43% in mid-December. In contrast, in 1999/2000 (Fig. 1c) the epidemic was early and severe, with incidence reaching 20% in early October and a maximum of 84% in untreated plots in mid-October. In 1999/2000, a period of dry weather immediately after sowing caused two phases of emergence. Approximately 60% of seedlings emerged 1 week after sowing followed by the remaining 40% about 3 weeks later. As leaf spotting was relatively early and severe, the 84% of plants with leaf spots on 12 October 1999 included plants with lesions on leaves three, four and five (from the first plants to emerge) and those with severe infections on the cotyledons and first two leaves of plants (from the second emergence phase).

The fungicide mixture used decreased the incidence of phoma leaf spot, and produced different phoma leaf spot epidemics under different treatment regimes (Fig. 2). Regular fungicide applications (SF) maintained a low incidence of leaf spotting during the autumn and winter. After individual fungicide applications, new leaf spots did not appear for at least 1 month (e.g. plots of treatment A1 in 1998/99 received one application on 15 October, but incidence of phoma leaf spot did not start to increase until late November, Fig. 2b). The period after fungicide application before new lesions appeared was > 2 months at lower temperatures in winter. For example, the second application to plots of treatment A2 in (1998/99) was made on 23 November but phoma leaf spot incidence did not increase until mid- to late-January (Fig. 2b). Similarly, the second application to plots of treatment A2 in 1999/2000 was made on 25 November, but phoma leaf spot incidence increased only in late January (Fig. 2c). A decrease in

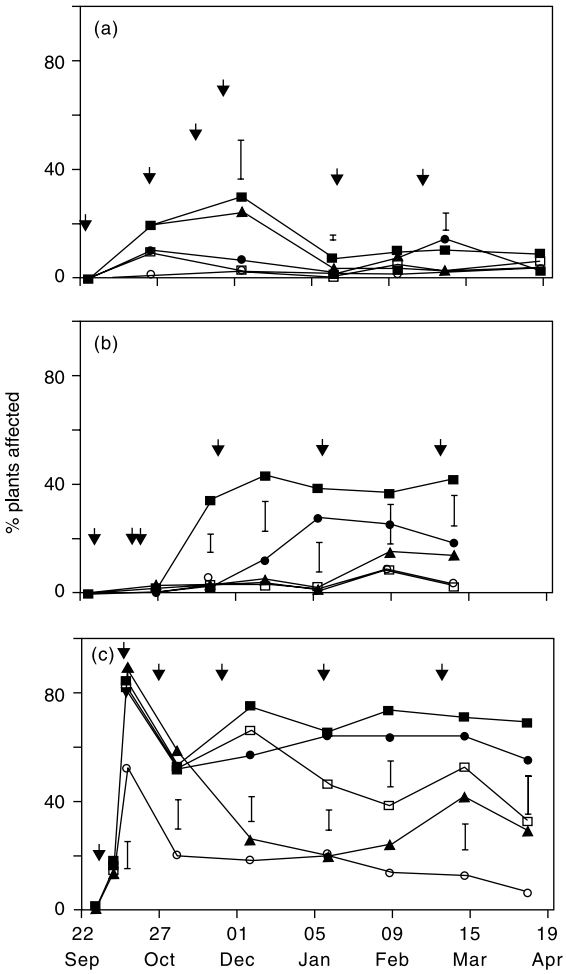

Figure 2 Effects of fungicide regimes (A1, A2, A3, SF) on the incidence (% plants affected) of phoma leaf spot in winter oilseed rape crops (mean of cvs Capitol and Lipton) at Rothamsted in (a) 1997/98, (b) 1998/99 and (c) 1999/2000. Vertical bars represent LSD where differences are significant ($P < 0{\cdot}05$). Untreated control ■, A1 ●, A2 ▲, A3 □, September to February (SF) ○. Dates of fungicide applications are indicated by ▼, and application dates of each fungicide regime are given in Table 1.

the incidence of phoma leaf spotting with time was associated with the shedding of diseased leaves before new leaves had developed symptoms.

Crown canker and phoma stem lesion development in field plots

The incidence and severity of phoma stem canker on the two cultivars were not significantly different and data are presented as means of both cultivars. Differences between treatments in the incidence (Fig. 3) and severity (Fig. 4) of crown cankers (which appeared from early April onwards) reflected earlier differences in the phoma leaf spot epidemics, with reductions in incidence and severity of crown canker produced by most fungicide regimes. In plots with regular fungicide applications (SF), which had a low incidence of phoma leaf spotting in autumn/winter, there was a low incidence of crown canker in the spring/summer. The maximum mean crown canker incidences in untreated plots were 70·5% (16 June 1998), 76·5% (5 July 1999) and 95% (3 July 2000) (Fig. 3a,c and e).

Phoma stem lesions appeared from May onwards (Fig. 3). The beginning of stem extension (GS 2,1) was observed to occur in February in all years. An increase in the separation distance between adjacent leaf scars on plant stems at harvest in 1998 indicated that the first 11–12 leaves were associated with the crown (stem base < 5 cm above ground level), while stem extension raised later leaves higher up the stem (Table 2). Leaf infections of uppermost leaves from February onwards resulted in phoma stem lesions rather than crown cankers. This is illustrated by the A2 treatment in 1998/99, which received its last fungicide application in late November 1998, and had a low incidence of phoma leaf spotting up to January 1999 (Fig. 2b), and a low incidence of crown canker (Fig. 3c). However, the increase in phoma leaf spotting after January was associated with a high incidence of phoma stem lesions (64·5%; Fig. 3d). Fungicides caused a progressively lower incidence of phoma stem lesions when fungicide regimes ended progressively later, especially in 1998/99 (Fig. 3). The maximum mean phoma stem lesion incidences in untreated plots were 77·5% (1 July 1998), 84% (5 July 1999) and 52·5% (3 July 2000) (Fig. 3b,d and f).

The severity of crown cankers at the stem base in untreated plots differed between seasons, although the rate of increase in severity with time was linear and did not differ between seasons (Fig. 4a). Following the relatively late leaf spotting epidemic of 1998/99, crown canker development started later, resulting in a lower final mean severity (Fig. 4a) than in 1997/98 and no yield reduction (Table 3). In addition to reducing incidence of crown canker, fungicide treatments decreased the rate of crown canker development (Fig. 4b, Table 4). Substantial yield loss occurred only when a mean severity of crown canker of at least 3 (on the 0–5 scale) had been reached by early July (Fig. 5). This occurred only once, in untreated plots in 1997/98. In other years there were indications of reduced yields in untreated plots caused by crown canker, but these yields were not significantly different from those of fully treated (SF) plots. Yields of all plots were much lower in 1998 than in 1999 and 2000 (Table 3) due to lodging of the crop. During the three seasons investigated, the mean yields of plots receiving two fungicide applications were never significantly less than those of plots receiving three or more applications. The mean final severity of phoma stem lesions higher up the stems was low (< 2) for all treatments in all seasons, with the exception of the untreated plots in 1997/98, in which they reached a mean severity of 2·6 (data not illustrated). Other diseases which occurred included downy mildew (*P. parasitica*), which was common in early autumn on cotyledons and early leaves. Light leaf spot (*P. brassicae*) was present on leaves, stems and pods of untreated plants. *Botrytis cinerea* and stem rot (*Sclerotinia sclerotiorum*) occurred on stems at very low incidences. Alternaria pod

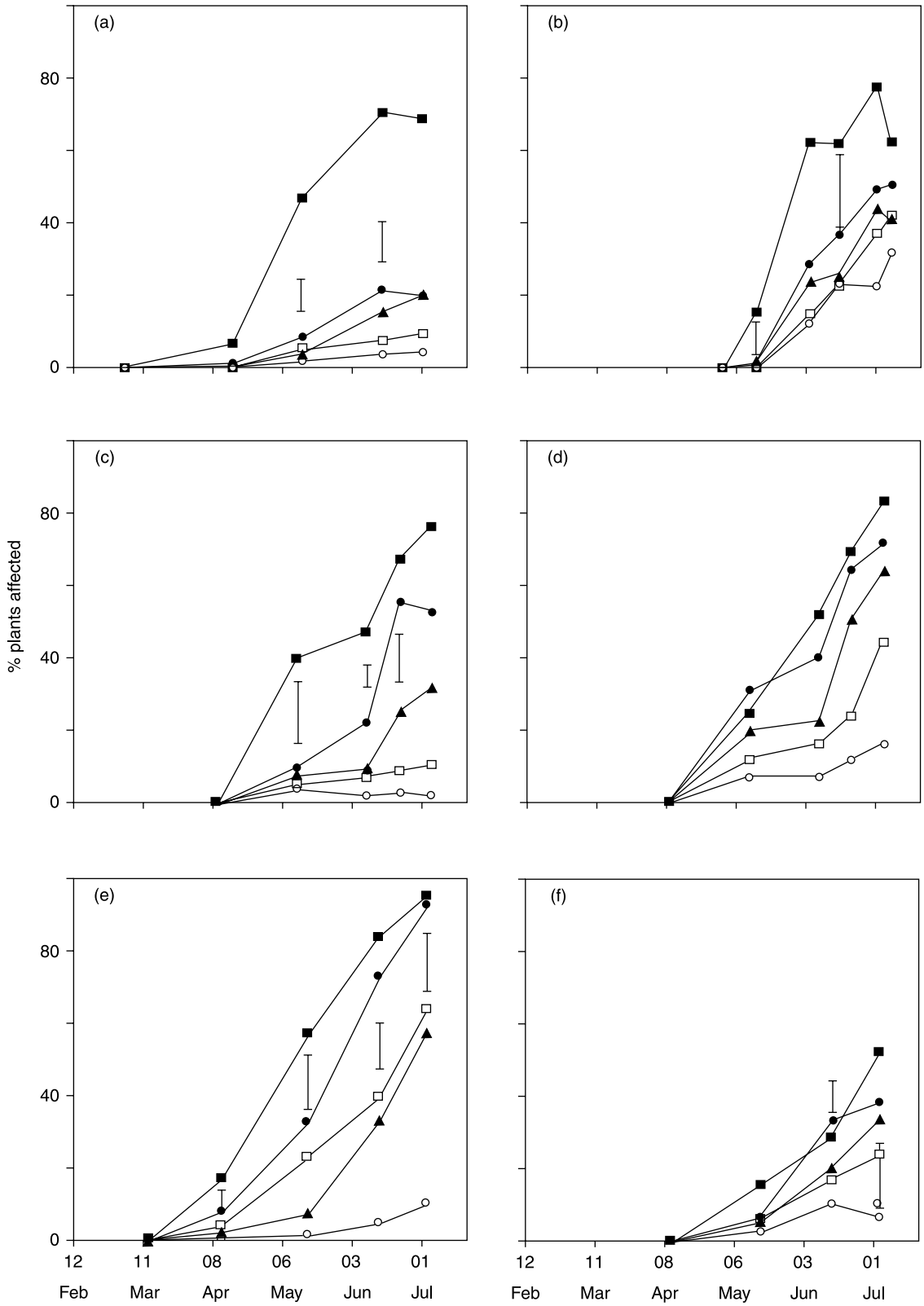

Figure 3 Effects of fungicide regimes (A1, A2, A3, SF) on the incidence (% plants affected) of crown canker (a, c, e) and phoma stem lesions (b, d, f) in winter oilseed rape (mean of cvs Capitol and Lipton) at Rothamsted in 1997/98 (a, b), 1998/99 (c, d) and 1999/2000 (e, f). Vertical bars represent LSD where differences are significant ($P < 0{\cdot}05$). Untreated control ■, A1 ●, A2 ▲, A3 □, September to February (SF) ○. Dates of fungicide applications are given in Table 1.

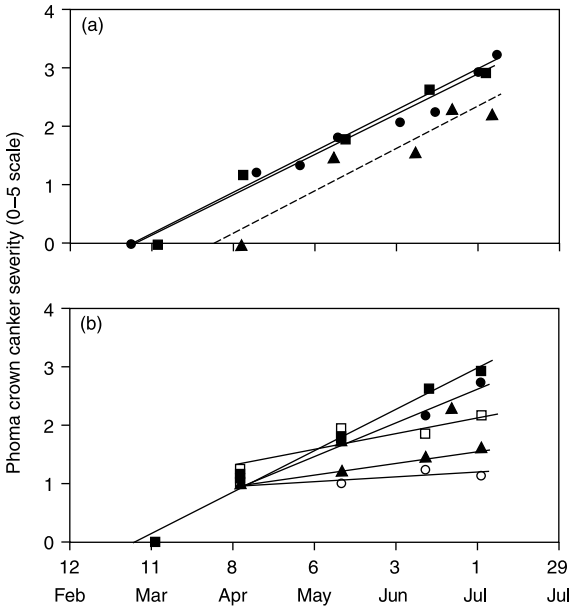

Figure 4 Regressions of crown canker severity (y, 0–5 severity scale, mean severity for plants with score ≥ 1) against time (t, Julian day) in winter oilseed rape (mean of cvs Capitol and Lipton) at Rothamsted; (a) in untreated plots, 1997/98 ●, solid line ($y = 0{\cdot}025t - 1{\cdot}49$; $R^2 = 0{\cdot}97$), 1998/99 ▲, dotted line ($y = 0{\cdot}026t - 2{\cdot}30$; $R^2 = 0{\cdot}89$), and 1999/2000 ■, dashed line ($y = 0{\cdot}024t - 1{\cdot}65$; $R^2 = 0{\cdot}98$); and (b) under five different fungicide regimes in 1999/2000 (untreated control ■, A1 ●, A2 ▲, A3 □, September to February (SF) ○); dates of fungicide applications are given in Table 1.

spot (*Alternaria brassicae*) and powdery mildew (*Erysiphe cruciferarum*) occurred on pods on untreated plants; in 2000, difenoconazole plus carbendazim was applied to all plots on 28 April to control these two diseases.

Fungicide activity

From leaves collected from the field experiment in October 1999 (1 week after spraying with fungicide), 7/8 (seven out of eight) lesions produced mycelium (compared with 8/8 on untreated leaves), but the growth rate of the colonies was lower (mean colony diameter 16·9 mm after 3 days) than that of colonies from untreated leaves (20·7 mm). Three weeks after spraying, 6/6 lesions from leaves in SF plots produced mycelium, but no mycelium was isolated from leaf sections 1 cm or more away from the lesions. However, at the same time, lesions on untreated leaves produced mycelium from leaf sections up to 4 cm away from the lesion. In the controlled environment experiment (Table 5), no colonies were formed from lesions on leaves treated with the fungicide when fungicide was applied 1 week after inoculation. However, colonies were formed from all inoculation sites on leaves when fungicide was applied 3 weeks after inoculation, although these colonies had a lower rate of growth on DWA than those from untreated leaves.

Discussion

The results of these experiments indicate that the risk of severe crown canker epidemics in southern England is greatest when there is a high incidence of phoma leaf spotting early in the season. The severity of crown canker epidemics and size of related yield losses were greater in 1997/98 and 1999/2000, when incidence of leaf spotting increased in October, than in 1998/1999, when incidence did not increase until November/December. Other studies in southern England (Hammond & Lewis, 1986; Gladders *et al*., 1998; Zhou *et al*., 1999; Sun *et al*., 2000), France (Poisson & Pérès, 1999) and Australia (Barbetti &

Table 2 Vertical distances (mm) on stems of four winter oilseed rape plants cv. Capitol[1] from the cotyledon scars to the scars associated with successive leaves at harvest in 1998

Leaf position	Plant 1	Plant 2	Plant 3	Plant 4	Mean	Standard error
1	2	2	2	2	2	0
2	4	7	3	4	4·5	0·87
3	7	8	7	7	7·3	0·25
4	8	10	10	9	9·3	0·48
5	15	17	16	17	16·3	0·48
6	22	25	18	20	21·3	1·49
7	27	29	20	25	25·3	1·93
8	30	36	25	31	30·5	2·25
9	33	37	30	33	33·3	1·44
10	34	38	35	35	35·5	0·87
11	41	45	48	42	44·0	1·58
12	46	51	105	48	62·5	14·20
13	86	78	170	81	103·8	22·10
14	146	115	246	149	164·0	28·40
15	195	170	340	189	223·5	39·20
16	267	260	–[2]	258	261·7	2·40
17	350	340	–	345	345·0	2·50

[1]Similar positions of leaf scars were observed on cv. Lipton.
[2]No leaf scar present.

Table 3 Yield of winter oilseed rape (mean of cultivars Capitol and Lipton) (t ha^{-1}) at 90% dry matter, under different fungicide regimes (A1, A2, A3, SF), in harvest years 1998, 1999 and 2000 at Rothamsted

	Yield (t ha^{-1})					
Year	Control	A1[1]	A2	A3	SF	SED (12 df)
1998	2·04	2·56	2·66	2·84	2·72	0·189
1999	5·19	5·14	5·27	4·94	5·35	0·127
2000	4·36	4·56	4·76	4·80	4·88	0·225

[1]Details of dates of fungicide applications are given in Table 1.

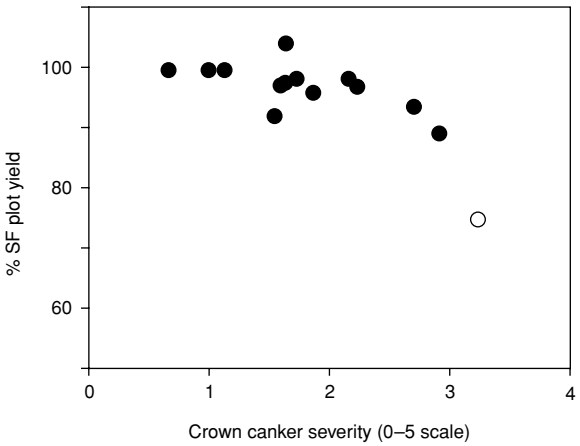

Figure 5 Relationships between yield as a percentage of the SF plot yields and crown canker severity (mean of cvs Capitol and Lipton) in early July, under five different fungicide regimes in 1997/98, 1998/99 and 1999/2000. Yields differed significantly (○, $P < 0·05$) or were not significantly different (●) from the SF yields.

Khangura, 1997) have also shown that the most severe crown cankers develop from spots on cotyledons and young leaves early in the growing season. Infections of early leaves are likely to allow the pathogen to spread rapidly to the stem due to the small size of early leaves and the relatively high temperatures of early autumn in southern England.

Results of these Rothamsted experiments suggest that, if the first increase in leaf lesions is in October, difenoconazole plus carbendazim may prevent the fungus from reaching the stem only if applied immediately the spots appear. Evidence for this is provided by the decrease in the incidence and severity of crown canker associated with October fungicide treatments in field experiments in 1998/99 and 1999/2000. In both the 1999/2000 field experiment and the controlled environment experiment, although the fungicide did not eradicate established infections, it reduced the subsequent growth of the fungus through the leaves. Furthermore, the results suggest that autumn applications may protect uninfected leaves for 1 month; although new lesions appeared immediately after the late September application in 1999, they were presumably initiated by the ascospores released in mid-September before the fungicide was applied.

The field experiment results suggest that, if the first increase in leaf lesions is not until mid-November, the fungicide can give effective control of crown canker even if it is applied some time after the appearance of the lesions. Since leaves are then larger than in early autumn, with longer petioles, and temperatures are lower (the speed of growth of *L. maculans* is slower) it takes longer for the pathogen to reach the stem (Hammond *et al*., 1985; Biddulph *et al*., 1999; Toscano-Underwood *et al*., 2001). Furthermore, the risk that leaf spots which do not appear until mid-November will produce severe crown cankers is less than for those appearing in early October (Hammond & Lewis, 1986; Sun *et al*., 2000; Sun *et al*., 2001). In 1998/99, when the leaf spot epidemic did not start until mid-November, crown cankers did not appear until late April; because cankers subsequently developed at the same rate as in the other two seasons, their severity score was only 2 by early July and they did not cause yield loss. By mid-winter in January/February, the time for which the fungicide protected new leaves may have increased to > 2 months because the incubation period of the pathogen is longer in colder conditions (Biddulph *et al*., 1999; Toscano-Underwood *et al*., 2001). In these Rothamsted experiments, when stem extension started in February, infections of new leaves after mid-winter produced phoma stem lesions rather than crown cankers. Although phoma stem lesions have been considered potentially damaging by Zhou *et al*. (1999), in these experiments their severity score became > 2 in untreated plots only once and fungicide application in mid-winter would not have been justified.

Since leaf spotting early in the autumn poses the greatest risk and accurate timing of fungicide sprays is essential

Table 4 Regression of mean crown canker (*Leptosphaeria maculans*) severity score (0–5 scale, mean score for plants with severity > 1) on time (Julian day) in winter oilseed rape (mean of cvs Capitol and Lipton) plots which were untreated or treated with different fungicide regimes (A1, A2, A3, SF) in harvest years 1998, 1999 and 2000 at Rothamsted. Values of the intercept (*a*), regression slope (*b*) and proportion of variance accounted for (R^2)

	1998			1999			2000		
	a	*b*	R^2	*a*	*b*	R^2	*a*	*b*	R^2
Control	−1·49	0·025	0·97	−2·30	0·026	0·89	−1·65	0·024	0·98
A1	−0·37	0·012	0·64	−2·10	0·022	0·96	−1·46	0·022	0·99
A2	−1·13	0·016	0·85	−1·75	0·019	0·94	−0·65	0·013	0·83
A3	−0·82	0·014	0·70	−1·59	0·018	0·88	−0·85	0·017	0·83
SF	−0·92	0·011	0·60	−1·78	0·018	0·69	−0·29	0·009	0·66

Table 5 Isolation of *Leptosphaeria maculans* from oilseed rape leaves (cv. Lipton, two leaves per plant, positions 6 and 7, on three plants) that were each inoculated on 27 September 1999 with ascospores on a single point, and received fungicide applications after 1, 2, 3 or 4 weeks of incubation at 15°C

Fungicide applied after week	Number of leaf sections from which *L. maculans* was isolated		
	1 day after fungicide application		1 week after application
	Untreated leaves	Treated leaves	Treated leaves
1 (4 Oct)	6/6	0/6	0/6
2 (11 Oct)	6/6	3/6	2/6
3 (18 Oct)[1,2]	6/6	6/6	3/6
4 (26 Oct)[3]	6/6	5/6	(not tested)

[1]Mean diameter of colonies 42·5 mm (SE 2·87) from lesions on untreated leaves and on treated leaves 13·7 mm (SE 2·39) after 8 days.
[2]4/6 sections 1 cm and 3/6 sections 2 cm from the lesions on untreated leaves also produced mycelium but no mycelium grew from pieces 1 cm or more from the inoculation point on treated leaves.
[3]4/6, 3/6 and 1/6 pieces, respectively, 2, 4 and 6 cm from the lesions also produced mycelium on untreated leaves but no mycelium grew from pieces 1 cm or more from the inoculation point on treated leaves.

then to prevent development of severe crown cankers, a method to predict early development of leaf spotting would be invaluable (West *et al*., 2001). These results suggest that the first increase in leaf spotting may be related to the first significant release of ascospores after crop emergence. The observed periods between the initial release of ascospores and the appearance of leaf spotting in the field experiments fitted with results of controlled environment experiments (Biddulph *et al*., 1999; Toscano-Underwood *et al*., 2001). It would be impracticable for growers to assess concentrations of air-borne ascospores on their farms (which requires specialist equipment) or to estimate maturation of ascospores in pseudothecia (which is technically difficult) (West *et al*., 1999). However, in France the risk of early infection has been related to the number of rain-days (16–19) occurring since harvest (Pérès & Poisson, 1997). In these Rothamsted experiments, the number of rain-days (> 0·2 mm) between harvest and the first release of ascospores varied from 17 to 28 in the three seasons. However, the number of rain-days between 1 August and the first ascospore release was more consistent (16–23). In analyses of survey data on phoma stem canker epidemics in eastern England over a 20-year period, rainfall in August and September was found to be an important indicator of epidemic severity the following summer (Gladders & Symonds, 1995). Thus it might be possible to develop a weather-based scheme for predicting the first increase in release of ascospores, and thus predict the initial development of leaf spotting, for use by growers in southern England (West *et al*., 1999). Since the timing of sprays is less critical later in the autumn, observations on the occurrence of leaf spotting on crops should then be sufficient to guide spray decisions.

Results of these and other (e.g. Gladders *et al*., 1998) winter oilseed rape experiments have shown that fungicides can give good control of crown canker epidemics with consequent yield responses. They suggest that the yield responses are likely to be greatest if leaf spotting starts in early October, when accurate timing of fungicide application is essential for it to be effective. If the onset of phoma leaf spotting in the autumn does not occur until November/December (as in the 1998/99 experiment), it seems unlikely that more than one spray against phoma stem canker can be justified. If the incidence of leaf spotting increases rapidly in early October (as in 1999/2000), then a single spray is unlikely to be sufficient to control the epidemic and a two-spray programme is advisable. However, application of more than two fungicide sprays against phoma stem canker in the period from autumn to spring did not increase yield any more in these experiments and is unlikely to be justified in southern England.

Acknowledgements

This paper reports part of the IMASCORE project, funded by the European Union (Fair contract CT96–1669), and work funded by the UK Department of the Environment, Food and Rural Affairs (DEFRA) and the Perry Foundation.

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