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Patterns of ascospore release in relation to phoma stem canker epidemiology in England (*Leptosphaeria maculans*) and Poland (*Leptosphaeria biglobosa*)

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Abstract

Experiments over five growing seasons at Rothamsted (1998/99–2002/03), four seasons at Boxworth (1998/99, 1999/2000, 2001/02, 2002/03) in England (*Leptosphaeria maculans*) and three seasons (1998/99–2000/01) at Poznan in Poland (*Leptosphaeria biglobosa*) suggest that differences in the development of phoma stem canker epidemics between England and Poland relate to differences in weather patterns between the two countries. The duration of ascospore release was longer in England, where winter weather is mild and wet, than in Poland, where winters are cold and often with snow cover, but there was little difference between two sites in England (Rothamsted and Boxworth). Wetness provided by rainfall was essential for release of ascospores of both *L. maculans* in England and *L. biglobosa* in Poland. Temperature did not affect release of ascospores over the range 5–20 °C. Diurnal periodicity in release of ascospores of *L. maculans* in England and *L. biglobosa* in Poland was similar. The timing (date) of first release of ascospores of *L. maculans* or *L. biglobosa* in autumn was related to rainfall in August and September; with increasing rainfall the date was earlier. The incubation periods from first release of ascospores to first appearance of phoma leaf spots for both *L. maculans* in England and *L. biglobosa* in Poland, and from first leaf spots to first stem base canker in England, were described using a thermal time (degree-day) approximation.

Introduction

Phoma stem canker (blackleg), caused by *Leptosphaeria maculans* (anamorph *Phoma lingam*), is an important disease of oilseed rape (*Brassica napus* ssp. *oleifera*) world-wide (West et al., 2001). It is a damaging disease of winter oilseed rape in Europe, although the severity of epidemics differs from season to season, from region to region and from crop to crop (Fitt et al., 1997). The pathogen population comprises at least two genetically distinct species (Williams and Fitt, 1999; Mendes-Pereira et al., 2003), formerly known as A-group and B-group *L. maculans* and recently named as *Leptosphaeria maculans* (*senso stricto*) and *Leptosphaeria biglobosa* (Shoemaker and Brun, 2001). *L. biglobosa* is generally less damaging, causing upper stem lesions (Jedryczka et al., 1999; West et al., 2002a); *Leptosphaeria maculans* is considered more damaging, causing stem base canker (Johnson and Lewis, 1994; West et al., 2002a).

Two areas of Europe with severe phoma stem canker epidemics, but different pathogen populations and climate, are England and Poland. Both *L. maculans* and *L. biglobosa* are present in England, but *L. maculans* is the predominant cause of the severe stem base cankers observed in summer before harvest (Gladders and Musa, 1980; West et al., 2002a). In contrast, in Poland *L. biglobosa* is the predominant cause of the mid and upper stem

lesions observed before harvest (Jedryczka et al., 1999; West et al., 2001; Karolewski et al., 2002). Winter weather is usually colder in the winter oilseed rape growing regions of Poland than in such regions in England, but springs and summers are warmer in Poland than England (Figure 1). It is not clear whether differences in phoma stem canker epidemics between England and Poland relate to differences in weather conditions or differences in pathogen populations.

In western Europe, including England, epidemics of phoma stem canker on winter oilseed rape are initiated in autumn by air-borne ascospores released from pseudothecia formed on infected oilseed rape debris from previous crops (Gladders and Musa, 1980; West et al., 1999; Brun et al., 2000). Under favourable conditions, ascospores infect leaves to produce lesions (phoma leaf spots) from which the pathogen grows down petioles into stems to initiate stem base cankers or upper stem lesions (Hammond et al., 1985). Early leaf infection can often lead to the development of severe stem base canker before harvest, resulting in yield loss (Zhou et al., 1999; Sun et al., 2001). West et al. (2002b) suggested use of *L. maculans* ascospore release information to optimise fungicide timing in England. In contrast, there is little information about release of *L. biglobosa* ascospores in Poland. In controlled conditions, *L. biglobosa* ascospores germinated faster than *L. maculans* ascospores; the ascospore germination pattern and rate of penetration of leaf stomata differed from that of *L. maculans* (Huang et al., 2001, 2003). However, little work has been done to compare relationships between patterns of ascospore release, onset of phoma leaf spotting and development of phoma stem canker epidemics in winter oilseed rape crops in Poland (predominantly *L. biglobosa*) and England (predominantly *L. maculans*).

Although models to predict release of ascospores of *L. maculans*, based on rainfall and air temperature, have been developed (Pérès and Poisson, 1997; Salam et al., 2003), little experimental work has tested the effects of temperature and wetness on the relative timing or pattern of release of ascospores of *L. maculans* and *L. biglobosa*. In France, a model based on rain days predicts the first *L. maculans* ascospore release will occur when 16–19 rain days have elapsed since harvest (Pérès and Poisson, 1997). In Australia, a model has been developed based on temperature and rainfall, indicating that there is a risk of *L. maculans* ascospore release after a total of 43

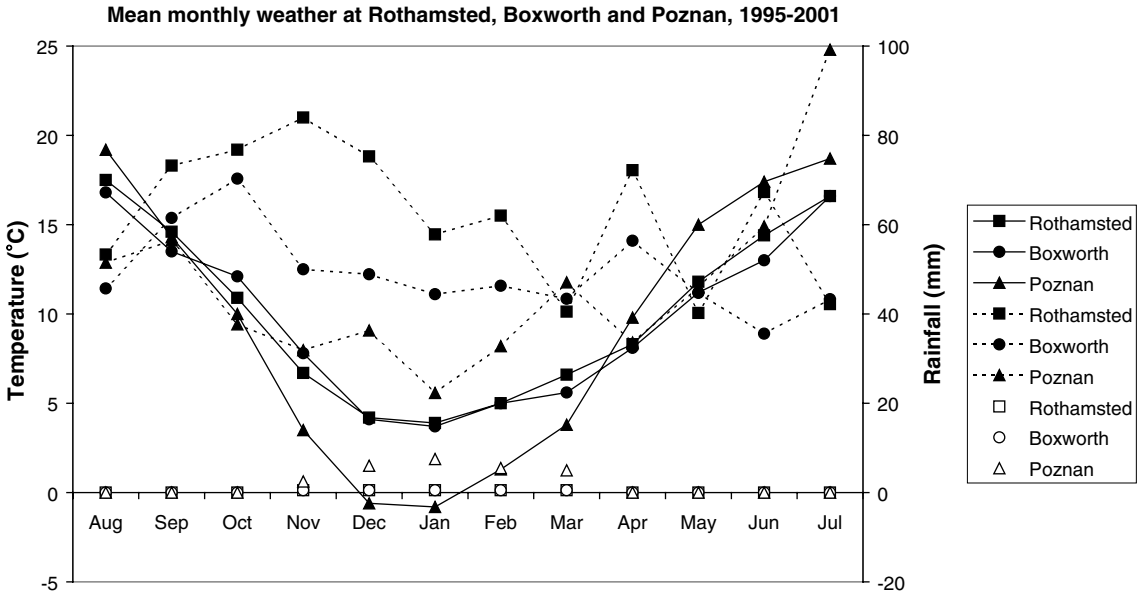

Figure 1. Air temperature (—), rainfall (. . .) and snow cover (□, ○, △) in England [Rothamsted (■, □) and Boxworth (●,○)] and Poland (Poznan, ▲, △); air temperature and rainfall are mean monthly values, snow cover (days) is the mean of monthly maximum and minimum days with snow cover, over the period 1995–2001.

days with temperature < 22 °C and rainfall ≥ 4 mm have elapsed since harvest (Salam et al., 2003). In England, empirical models using disease survey and weather data have suggested that the rainfall in the previous August and September is related to the subsequent incidence of stem base canker at harvest (Gladders and Symonds, 1995). Controlled environment and field work on *L. maculans* in England suggested that incubation periods were relatively constant over seasons when expressed in thermal time (degree-days), with 130–160 degree-days from inoculation with ascospores to appearance of phoma leaf spotting (Biddulph et al., 1999) and 1120–1240 degree-days from leaf spotting to appearance of stem base canker (Sun et al., 2001). However, it is not clear whether similar relationships apply to *L. biglobosa* in Poland. This paper compares the patterns of ascospore release, onset of phoma leaf spotting and development of phoma stem canker epidemics in England (*L. maculans*) and Poland (*L. biglobosa*), and investigates effects of temperature on release of ascospores from mature pseudothecia on oilseed rape debris.

Materials and methods

Preparation of infected oilseed rape stem debris

Diseased winter oilseed rape stems (30–50 cm long) were collected within 2 weeks after harvest from untreated plots in field experiments at Rothamsted, Hertfordshire or Boxworth, Cambridgeshire (70 km from Rothamsted) in England (with stem base cankers) and Poznan in Poland (with upper stem lesions), respectively. Diseased stems were identified by observation of the visible tissue discolouration and the presence of pycnidia of the anamorph, *Phoma lingam*. The stems were placed in freely draining plastic trays (45 × 75 cm). The trays with diseased stems were incubated outdoors to allow pseudothecia and ascospores to mature in natural conditions. In each season, the daily temperature and rainfall were recorded by weather stations at each site. Experiments have shown that the stem bases of oilseed rape debris from Rothamsted and Boxworth predominantly produce *L. maculans* (> 95% of ascospore isolates); the upper stems of oilseed rape debris from Poznan produce almost entirely *L. biglobosa* (> 99% of ascospore isolates) (Jedryczka et al., 1999; Toscano-Underwood et al., 2001; Huang et al., 2003). For debris used in controlled environment experiments, isolations were made from stem tissues before pseudothecia were produced and from ascospores ejected from mature pseudothecia to confirm that UK debris was producing only *L. maculans* ascospores and Polish debris producing only *L. biglobosa* ascospores.

Ascospore concentrations

Ascospore numbers in the air were calculated from numbers of ascospores collected each day by a Burkard volumetric spore sampler (Burkard Manufacturing Company Ltd., Rickmansworth, UK). The Burkard sampler was surrounded by 8–10 freely draining plastic trays (45 × 75 cm), each containing about 60 stems (or the equivalent number of stems arranged in a continuous circle on the ground at Boxworth), placed at a distance of 2–3 m from the sampler. The spore sampler sampled air at 10 l min^{-1} through an orifice (14 × 2 mm). A continuous record of spore concentration was obtained, as spores were deposited on Vaseline-coated cellophane tape wrapped around the sampler drum rotating at 2 mm per hour, and changed at 7-day internals. After exposure, the tape was removed from the sampler drum, and cut into pieces 48 mm long (each piece representing a 24 h period). Each piece was mounted on a microscope slide and stained with 0.1% trypan blue in lactophenol. The daily release of ascospores was calculated from the average number of ascospores counted in two longitudinal traverses of each piece of tape as described by McCartney and Lacey (1990).

A Burkard spore sampler was operated from late August to early May in the 1998/99, 1999/2000, 2000/01, 2001/02, and 2002/03 growing seasons at Rothamsted and from late August to late March in 1998/99, 1999/2000, 2001/02, and 2002/03 at Boxworth, England. A Burkard spore sampler was operated from early September to early May in 1998/99, 1999/2000, 2000/01, 2002/03, and 2003/04 at Poznan, Poland.

To study diurnal periodicity of *L. maculans* ascospore release, hourly release of ascospores was counted on 12 dry days (without rain) and 12 rain days (with ≥ 0.5 mm rain) in October/November in both 2000 and 2001 at Rothamsted, using the method described by McCartney and Lacey (1990). Hourly release of *L. biglobosa* ascospores

was counted at Poznan on 10 dry days and 10 rain days in October/November 1998, and 14 dry days and 22 rain days in October/November 2003. Relationships between the date of first ascospore release (>10 ascospores m^{-3}) and weather in August/September were examined by regression of this date on total rainfall, number of days with ≥ 0.5 mm rain and mean temperature, using Genstat statistical software (Payne et al., 1993).

Disease assessments

Disease was assessed in winter oilseed rape plots that did not receive any fungicide applications but were treated with other pesticides and fertilisers according to local commercial practice. At Rothamsted in England in 1998/99 and 1999/2000, development of phoma stem canker epidemics was assessed in four untreated 15×3 m plots sown with each of cvs Capitol or Lipton in late August. The UK rating for resistance to stem canker for Capitol was 6 and that for Lipton was 5 (Anon., 1997). Each month, 25 plants, sampled at random from each plot, were assessed for incidence (% plants affected) of phoma leaf spot from September to March, and incidence and severity (0–4 scale) of stem base canker from March to July. In 2000/01, 2001/02 and 2002/03, phoma stem canker epidemics were assessed in three untreated 15×3 m plots sown with cv. Apex (resistance rating 6) in late August. Ten plants were sampled weekly (September–November) and then monthly (December–March) at random from each plot to assess incidence of phoma leaf spotting. From March to July, ten plants were sampled monthly at random from each plot to assess incidence and severity of stem base canker.

At Boxworth in England, disease assessments were made on winter oilseed rape in four untreated 21×4 m plots, each sown with cv. Apex in late August or early September in 1998/99, 1999/2000, 2001/02, and 2002/03 seasons. Twenty-five plants were sampled at random weekly from September to December and then monthly from December to March from each untreated plot to assess incidence of phoma leaf spots. From March to July, 10 plants per plot were sampled monthly to assess incidence and severity (0–4 scale) of stem base canker.

At Poznan in Poland, disease assessments were made in untreated winter oilseed rape located about 20 km from the Burkard spore sampler. In 1998/99, three untreated 25×3 m plots were sown with each of cvs Capitol or Lipton in late August. In 1999/2000 and 2000/01, four untreated 25×4 m plots were sown with each of cvs with Capitol or Lipton. Each month, 25 plants, sampled at random from each plot, were assessed for incidence of phoma leaf pots from September to early May. The incidence and severity (0–9 scale) of upper stem lesions before harvest were assessed on 50 plants (1999 harvest), or 100 plants (2000 and 2001 harvest).

For phoma stem base canker in England, a 0–4 severity scale was used (where 0 = uninfected; 1 = $\leq$ 50% stem circumference girdled; 2 = 50–75% girdled, stem firm; 3 = $>$ 75% girdled, stem weak; 4 = plant dead or lodged) (Zhou et al., 1999). For upper stem lesions in Poland, a 0–9 scale was used (where 0 = uninfected; 1 = 1–2 cm lesion; 2 = 3–4 cm lesion; 3 = 5–6 cm lesion; 4 = 7–10 cm lesion, usually with pycnidia; 5 = 11–15 cm lesion with pycnidia; 6 = 16–20 cm lesion with pycnidia; 7 = 21–25 cm lesion with pycnidia, plant prematurely dead; 8 = main stem and parts of branches prematurely dead and with numerous pycnidia; 9 = whole plant prematurely dead, numerous pycnidia).

In all experiments, the incubation period from first release of ascospores to first phoma leaf spotting was estimated in days and degree-days (Biddulph et al., 1999) and the incubation period from first phoma leaf spotting to first stem base canker was estimated in days and degree-days (Sun et al., 2001).

Effects of rainfall and temperature on release of ascospores from debris

To study the effects of rainfall on release of *L. maculans* ascospores at Rothamsted, seven sets of seven continuous days (3–4 days with rain ≥ 0.5 mm (rain day) followed by 3–4 days without rain (dry day) or 3–4 dry days followed by 3–4 rain days) during the period October–December in 2000 and 2001 were chosen. Numbers of ascospores collected by the Burkard sampler on each day were counted to study patterns of ascospore release on rain days followed by dry days or dry days followed by rain days. To study the effects of

rainfall on release of *L. biglobosa* ascospores at Poznan, three sets of six continuous days (2–3 rain days followed by 2–3 dry days or 2–3 dry days followed by 2–3 rain days) during the period October to December in 2002 were chosen and the number of ascospores collected on each day was counted.

The effects of temperature on release of ascospores of *L. maculans* and *L. biglobosa* were investigated in controlled environment experiments. Rothamsted winter oilseed rape stem base debris (cv. Lipton; confirmed as producing only *L. maculans* ascospores) with mature pseudothecia produced under natural conditions was used to study the release of ascospores of *L. maculans*. Polish upper stem debris (cv. Lipton; confirmed as producing only *L. biglobosa* ascospores) with mature pseudothecia produced under natural conditions was used to study the release of ascospores of *L. biglobosa*. Experiments were done in temperature-regulated incubators (5, 10, 15, or 20 °C) in darkness. Oilseed rape stem debris bearing mature pseudothecia of *L. maculans* or *L. biglobosa* were cut into 1 cm × 3 cm pieces. Pieces of stem debris bearing mature *L. maculans* or *L. biglobosa* pseudothecia were attached to the underside of Petri dish (9 cm diam) lids with Vaseline (six stem pieces per dish). Four sets (each with three dishes) of dishes were prepared.

Sets of the dishes with stem pieces were chosen at random and put in incubators at 5, 10, 15, or 20 °C overnight to pre-condition them. The stem pieces on the lids of Petri dishes were sprayed with distilled water until run-off to induce release of ascospores. The lids were placed over the Petri dish bases and replaced in the incubators in darkness. After 4 h, all the dishes were removed from the incubators and ascospores that had been discharged into the base of the Petri dish were suspended in 1–2 ml water (with 0.05% Tween-80 added) and counted using a haemocytometer slide. To replicate the temperature treatments, the experiment with debris with *L. maculans* pseudothecia was done five times, with new stem debris pieces used each time; the experiment with debris with *L. biglobosa* pseudothecia was done three times, with new stem debris pieces used each time. Incubators were allocated randomly to temperature treatments in each experiment. To assess the effects of temperature on release of ascospores of *L. maculans* and *L. biglobosa*, analyses of variance were done using Genstat (Payne et al., 1993).

Results

Effects of temperature and rainfall on release of ascospores

In controlled conditions, ascospores of both *L. maculans* and *L. biglobosa* were released from mature pseudothecia in darkness at temperatures ranging from 5 to 20 °C (Table 1). For *L. maculans*, slightly greater numbers of ascospores were released (in a 4 h period) at 15 °C, but there were no significant differences between different temperatures ($P = 0.20$; SED 0.42). Similarly, the numbers of *L. biglobosa* ascospores released during a 4 h period in darkness did not differ between different temperatures ($P = 0.72$; SED 0.93). It was estimated that during a 4 h period the number of ascospores of *L. maculans* or *L. biglobosa* released was 600–1100 cm^{-2} of debris.

After pseudothecia had matured, the release of ascospores was associated with rainfall (Figure 2). At Rothamsted, there were few or no *L. maculans* ascospores released in periods of continuously dry days between October and December, even if mature pseudothecia were present, while the number of air-borne ascospores increased greatly after rainfall. However, release of ascospores did

Table 1. Effects of temperature on number of ascospores released over 4 h in darkness from pseudothecia produced on oilseed rape (cv. Lipton) stem debris

Experiment number	Number of ascospores ($\times 10^{-4}$)[a]				
	5 °C	10 °C	15 °C	20 °C	SED
(a) *Leptosphaeria maculans*					
1	2.77	2.33	4.62	2.00	
2	0.72	0.98	1.38	1.07	
3	0.60	0.42	1.07	0.25	
4	0.82	1.07	0.20	1.22	
5	1.02	2.08	2.30	0.45	
Mean	1.18	1.38	1.91	1.00	0.42 (12df)
(b) *Leptosphaeria biglobosa*					
1	2.20	1.37	2.27	1.50	
2	2.57	1.63	2.10	1.87	
3	0.87	1.43	1.10	1.70	
Mean	1.88	1.48	1.82	1.69	0.93 (6df)

[a] 2.77 = 27,700 etc.

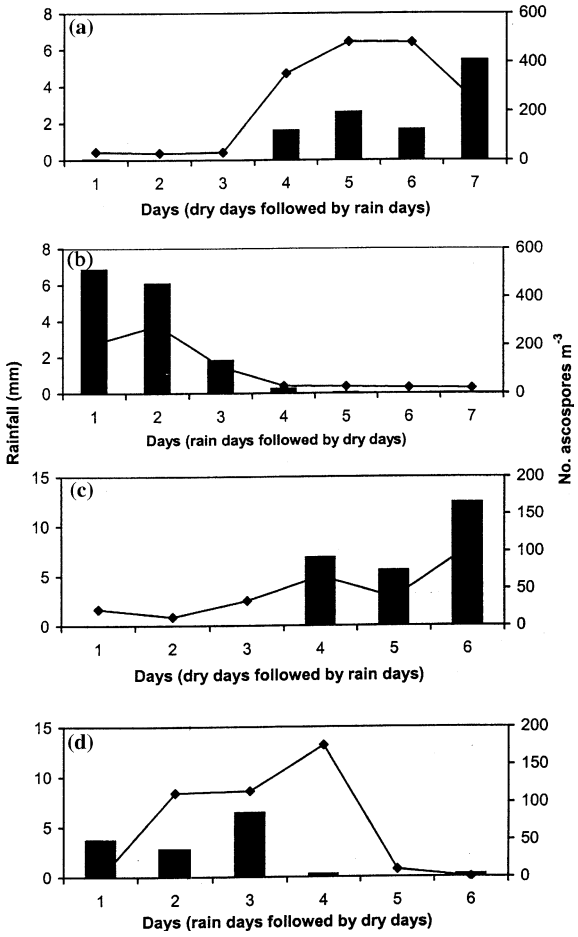

Figure 2. Daily changes in numbers of ascospores (♦) of *Leptosphaeria maculans* (a, b) or *L. biglobosa* (c, d) released from oilseed rape debris in relation to rainfall (mm, vertical bars). *L. maculans* data from Rothamsted, England are daily means of seven sets of 7-day periods during October–December in 2000 and 2001, with either dry days followed by rain days (a) or rain days followed by dry days (b). *L. biglobosa* data from Poznan, Poland are daily means of three sets of 6-day periods during October–December 2002, with either dry days followed by rain days (c) or rain days followed by dry days (d).

not increase during periods of continuous rainfall (Figure 2a). When days with rain were followed by periods of continuously dry days, number of ascospores released decreased rapidly and there were very few or no spores released on the second to fourth days after rain (Figure 2b). Similar results were observed in Poznan with *L. biglobosa*. Few ascospores were released in periods of continuously dry days, while larger number of ascospores were generally released after rainfall (Figure 2c). When days with rain were followed by dry days, the release of ascospores continued on the fourth day when little rain fell and then the number of ascospores released decreased rapidly on the following dry day (Figure 2d). As a consequence of these results, full daily temperature and rainfall data for each site are not presented in this paper.

Seasonal periodicity in L. maculans *ascospore release in England*

There were differences in patterns of ascospore release between seasons, but not between Rothamsted and Boxworth. At the start of each season, few or no ascospores were detected before September at both Rothamsted and Boxworth. The date of the first major release of ascospores (day with >10 spores m^{-3}) differed between seasons, ranging from 14 September to 20 October at Rothamsted, and 24 September to 21 October at Boxworth (Table 2). After the first release of ascospores, ascospores continued to be released until the late spring in each season. However, the majority of ascospores were released in the period from late October to late December (Figures 3 and 4). Ascospores were released from pseudothecia mainly on days with rain (≥0.5 mm) and air temperature >0 °C. At Rothamsted and Boxworth, there were few days with air temperatures <0 °C and ascospores were released throughout the winter.

At Rothamsted, release of ascospores started earlier in 1999 (14 September) and 2001 (17 September) than in 1998 (6 October), 2000 (24 September) and 2002 (20 October) (Figure 3; Table 2). The differences in date of first major release of ascospores were associated with differences in August/September rainfall rather than differences in temperature. Linear regressions of date of first release of ascospores on total rainfall or number of rain days in August and September fitted well to the data and accounted for $>87\%$ (Figure 5a) and $>56\%$ (Figure 5b) of the variance, respectively. However, results showed there was no relationship between date of first release of ascospores and temperature in August and September. In the five seasons investigated, the mean temperatures in August/September ranged from 15.3 to 16.1°C, while the total rainfall and number of days with ≥0.5 mm rain ranged from 77.9 to 193.0 mm and 13 to 31 days, respectively. There was more rain in August/September in 1999,

Table 2. Dates of first release of ascospores in relation to dates of first observed phoma leaf spots and stem base cankers on winter oilseed rape (cvs Capitol and Lipton in 1998–2000, cv. Apex in 2000–2003 at Rothamsted; cv. Apex in 1998–2000, 2001–2003 at Boxworth; cvs. Capitol and Lipton in 1998–2001 at Poznan) in untreated plots in England (*Leptosphaeria maculans*) at Rothamsted and Boxworth and in Poland (*Leptosphaeria biglobosa*) at Poznan

Site/measurement	1998–1999	1999–2000	2000–2001	2001–2002	2002–2003
Rothamsted					
First ascospore release[1] (a)	6 Oct.	14 Sept.	24 Sept.	17 Sept.	20 Oct.
First leaf spotting[2] (b)	26 Oct.	30 Sept.	2 Oct.	26 Sept.	31 Oct.
First stem base canker[3] (c)	12 May	11 April	4 April	19 Mar.	7 May
Days from (a) to (b)	20	16	8	9	11
Degree-days from (a) to (b)	210	234	108	128	105
Days from (b) to (c)	198	191	174	175	188
Degree-days from (b) to (c)	1326	1242	1044	1264	1252
Boxworth					
First ascospore release[1] (a)	6 Oct.	24 Sept.	–	26 Sept.	21 Oct.
First leaf spotting[2] (b)	21 Oct.	5 Oct.	–	15 Oct.	5 Nov.
First stem base canker[3] (c)	15 April	29 Mar.	–	26 Mar.	2 May
Days from (a) to (b)	15	11	–	20	15
Degree-days from (a) to (b)	152	137	–	280	158
Days from (b) to (c)	176	175	–	162	179
Degree-days from (b) to (c)	1095	1122	–	1056	1196
Poznan					
First ascospore release[1] (a)	28 Sept.	29 Oct.	25 Sept.	–	–
First leaf spotting[2] (b)	9 Nov.	28 Feb.	26 Oct.	–	–
Days from (a) to (b)	42	130	31	–	–
Degree-days from (a) to (b)	319	278	387	–	–

[1] First date when ≥ 10 ascospores m^{-3} air were collected by a Burkard spore sampler.

[2] First date when ≥ 5% plants in untreated plots were affected by phoma leaf spotting.

[3] First date when ≥ 5% plants in untreated plots had stem base canker (England); no stem base canker in Poland.

2000, and 2001 than in 1998 and 2002 and release of ascospores was earlier in autumn 1999, 2000 and 2001 than in autumn 1998 and 2002. In 1999 and 2001, the number of ascospores rapidly increased to a maximum in October/November, which was 35–46 days earlier than in 1998 and 2002; the release of ascospores stopped earlier in the following spring. Few ascospores were detected after March in 2000 and 2002, but ascospores were collected in April in 1999 and 2003.

At Boxworth, the patterns of ascospore release were similar to those at Rothamsted in the same seasons (Figure 4). Ascospore release started earlier in 1999 (24 September) and 2001 (26 September) than in 1998 (6 October) and 2002 (21 October). Differences between seasons in the date of the first major release of ascospores were associated with differences in August/September rainfall rather than differences in temperature (Figure 5). In the four seasons investigated, the mean temperature in August/September ranged from 15.3 to 16.8 °C, while the total rainfall and number of days with $\geqslant$ 0.5 mm rain ranged from 27.2 to 149.8 mm and 5 to 27 days, respectively. There was more rainfall in August/September in 1998, 1999, and 2001 than in 2002 and release of ascospores was earlier in autumn 1998, 1999, and 2001 than in autumn 2002. In autumn 1999 and 2001, the number of ascospores rapidly increased to a maximum in October, which was 20–40 days earlier than in autumn 1998 and 2002. In 1999/2000, the release of ascospores stopped earlier in spring than in other three seasons. While few ascospores were detected in early February 2000, ascospores were still detected until early March in 1999, 2002, and 2003.

Seasonal periodicity in L. biglobosa *ascospore release in Poland*

Seasonal patterns of ascospore release at Poznan (Figure 6) were different from those in England, although ascospore release also started in the autumn (late September in 1998 and 2000, late

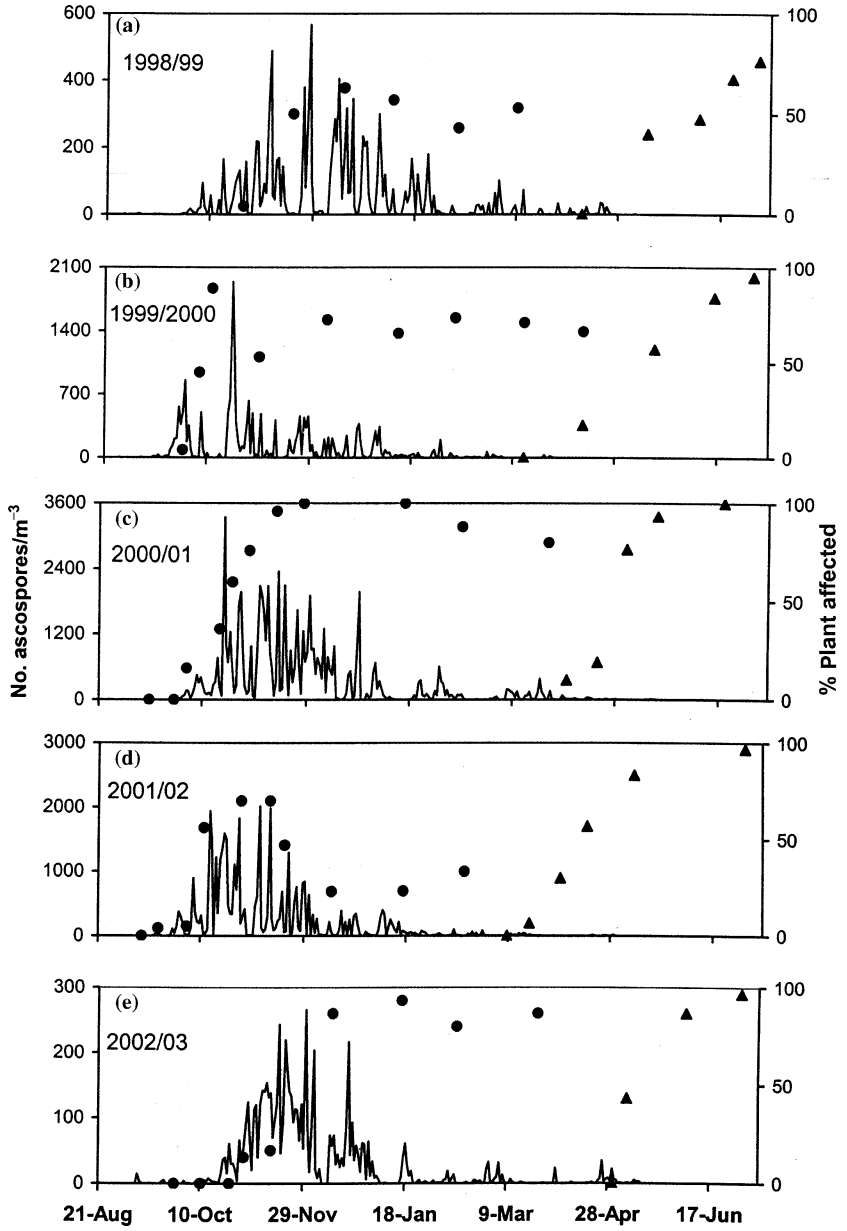

Figure 3. Changes with time in the numbers of air-borne ascospores of *Leptosphaeria maculans* in relation to incidence (% plants affected) of phoma leaf spot (●) and stem base canker (▲) in untreated winter oilseed rape sown at Rothamsted in late August in 1998/99 (a), 1999/2000 (b), 2000/01 (c), 2001/02 (d) and 2002/03 (e).

October in 1999). Differences between seasons in the date of the first release of ascospores were associated with differences in summer rainfall rather than differences in temperature (Figure 5). In the three seasons investigated, the mean temperature in August/September ranged from 15.6 to 17.8 °C, while the total rainfall ranged from 56.7 to 160.3 mm. There was less rainfall in August/September 1999 than in 1998 and 2000 and release of ascospores was later in autumn 1999 than in autumn 1998 and 2000. In Poland, the duration of ascospore release was shorter than in England. Most ascospores were released in October/November and few ascospores were released in the

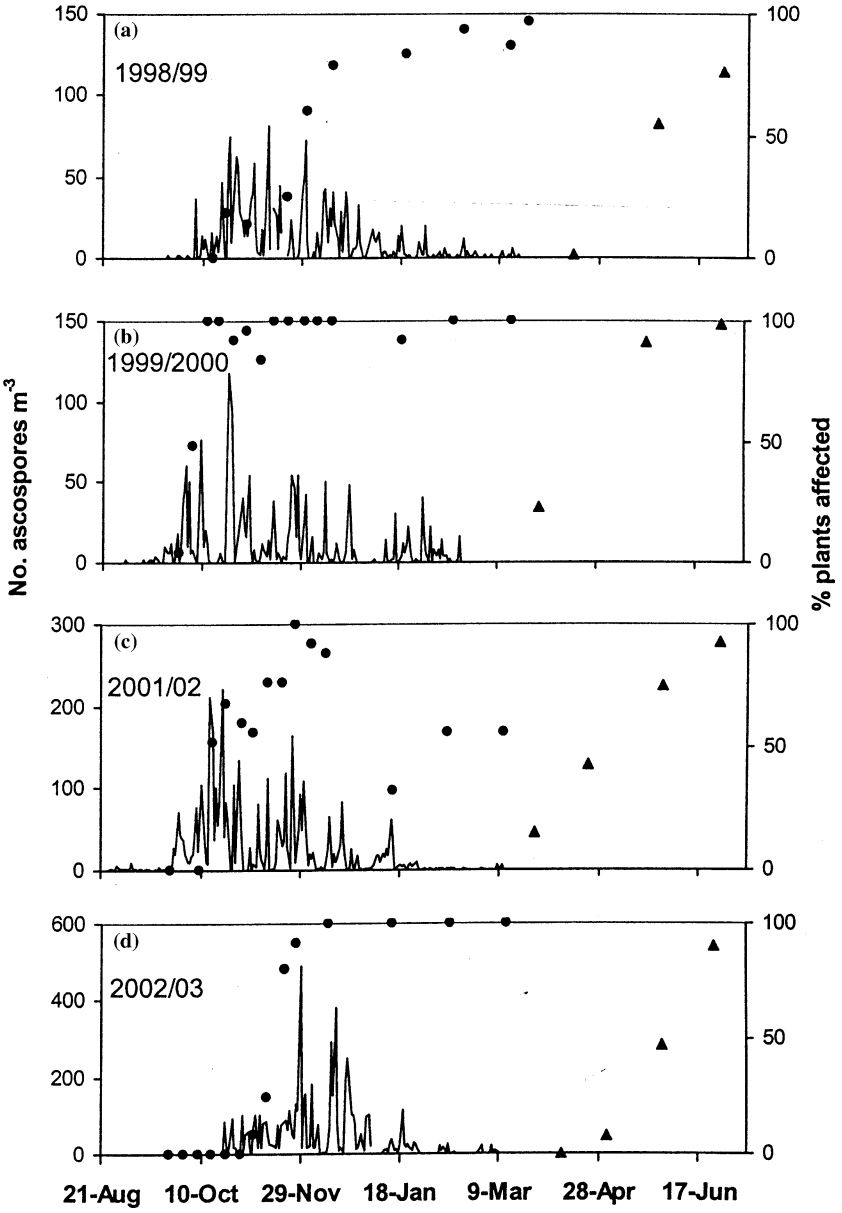

Figure 4. Changes with time in the numbers of air-borne ascospores of *Leptosphaeria maculans* in relation to incidence (% plants affected) of phoma leaf spot (●) and stem base canker (▲) in untreated winter oilseed rape sown at Boxworth from late August/early September onwards in 1998/99 (a), 1999/2000 (b), 2001/02 (c) and 2002/03 (d).

winter and spring (Figure 6). After the first release of ascospores was observed, the number of ascospores increased rapidly to a maximum, particularly in 1998 and 1999. In both 1998/99 and 1999/2000 seasons, no ascospores were detected during periods with sub-zero temperatures and snow from mid-November to early January (Figure 6a, 6b). During winter, ascospores were detected occasionally when air temperature was above 0 °C. When temperature increased in spring, a few ascospores were detected before late March. In the 2000/01 season, the release of ascospores started in late September and continued for 3 months; then the release of ascospores stopped in mid-December when temperature decreased below 0 °C. No ascospores were

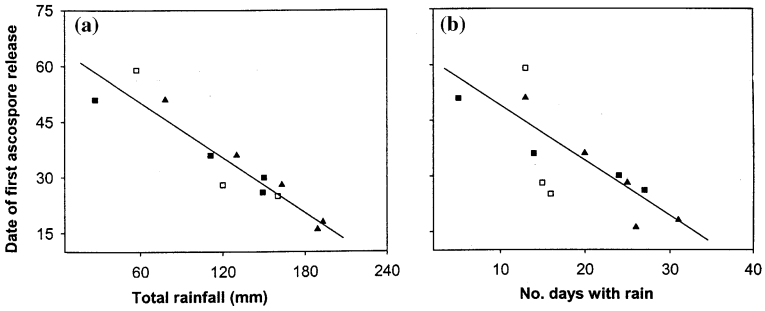

Figure 5. Relationship between the date of the first release of ascospores (> 10 ascospores per m^3 per day) (*f*) and the total rainfall (*r*) (a) or number of days with ≥ 0.5 mm rain (*n*) (b) in August and September 1998–2003 at Rothamsted (▲), Boxworth (■) and Poznan (□). The regression equations were $f = 65.1–0.25\ r$ ($R^2 = 87.3$) for total rainfall and $f = 60.2 - 1.39\ n$ ($R^2 = 56.8$) for days with ≥ 0.5 mm rain. The values for date of first ascospore release on *f* axes are days after 31 August; 15 is 15 September, and 60 is 30 October.

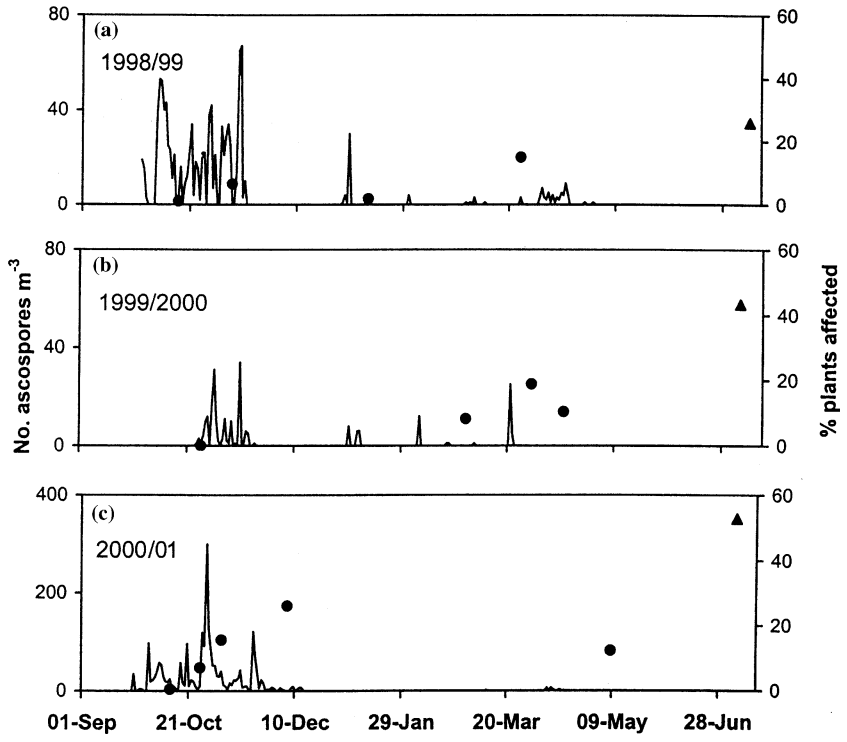

Figure 6. Changes with time in the numbers of air-borne ascospores of *Leptosphaeria biglobosa* in relation to incidence (% plants affected) of phoma leaf spot (●) and phoma upper stem lesions (▲) in untreated winter oilseed rape sown at Poznan from late August onwards in 1998/99 (a), 1999/2000 (b), and 2000/01 (c).

detected during the long, cold winter and few ascospores were detected in spring (Figure 6).

Diurnal periodicity of ascospore release in England and Poland

In England, on days without rain (dry days), there was a diurnal periodicity in release of ascospores, although numbers of spores released were small (Figure 7a). On 12 dry days between October and December in 2000 and 2001, the hourly numbers of ascospores showed a marked diurnal periodicity. Most ascospores were released in the morning between 03:00 and 11:00 h (GMT). Few spores were released between 12:00 and 24:00 h. On days with

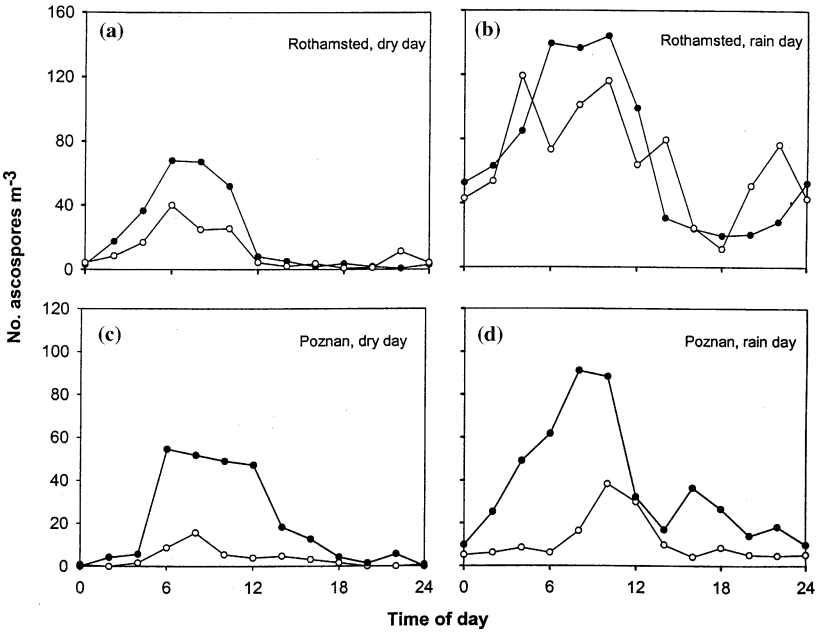

Figure 7. Numbers of ascospores of *Leptosphaeria maculans* (a, b) or *L. biglobosa* (c, d) collected at different times of day by a Burkard sampler on dry days (days without rain) (a, c) and rain days (days with rain ≥ 0.5 mm) (b, d). *L. maculans* data were from Rothamsted, England for 12 dry days (a) or 12 rain days (b) in October/ November 2000 (●) or 2002 (○). *L. biglobosa* data were from Poznan, Poland for 10 dry days (c) or 10 rain days (d) in October/November in 1998 (●) or for 14 dry days (c) or 22 rain days (d) in October/ November 2003 (○). Local times are used; UK local time is GMT and Polish local time is GMT plus 1 h.

≥0.5 mm rain (rain days), a larger number of ascospores were collected but there was still a diurnal periodicity in release of ascospores (Figure 7b). On days with rain between October and December in both 2000 and 2001, ascospores were collected throughout the day, but most ascospores were detected in the morning between 03:00 and 13:00 h. Ascospore release generally commenced within 1 h of the start of rain and usually continued for 4–6 h. Generally, there was only one main period of ascospore release within a 24 h period even if there was continuous rain. In Poland, on days without rain, there was also a diurnal periodicity in release of ascospores (Figure 7c). On dry days in October and November, the hourly numbers of ascospores collected showed a marked diurnal periodicity. Most ascospores were released between 05:00 and 15:00 h (GMT + 1) and few spores were released between 15:00 and 24:00 h. On days with rain, ascospores were released throughout the day but the majority were released between 03:00 and 13:00 h (Figure 7d).

Patterns of phoma leaf spotting and stem base canker development in England

Patterns of phoma leaf spotting epidemics differed between the five seasons investigated, but not between Rothamsted and Boxworth. In 2000/01 at Rothamsted (Figure 3c), the epidemic was early and severe, with 76% plants affected in late October and 100% plants affected in late November. In 1999/2000 (Figure 3b; no significant difference observed between cvs Lipton and Capitol; 1998/99 and 1999/2000 data presented as the mean of both cultivars) and 2001/02 (Figure 3d), the epidemics were early but less severe, with 45% and 56% plants affected in mid-October, but the maximum incidences were only 89% and 70%, respectively. In 1998/1999 (Figure 3a) and 2002/03 (Figure 3e), the epidemics started later, but the epidemic still became severe in 2002/03, with the incidence of plants affected reaching 100% in January (cf. an incidence of 100% plants affected in November in 2000/01).

At Boxworth, the phoma leaf spotting epidemic was early and severe in 1999/2000 (Figure 4b) and 2001/02 (Figure 4c). In 1999/2000, 48% of plants were affected in early October and the incidence of leaf spotting rapidly increased to 100% in mid-October. In 2001/02, 52% of plants were affected in mid-October and 100% of plants affected by late November. In 1999/98 (Figure 4a), the epidemic was late and less severe; only 18.8% of plants were affected in late October and incidence of affected plants did not reach a maximum (93%) until the following spring. In 2002/03 (Figure 4d), the epidemic was late but severe; the phoma leaf spotting epidemic did not start until early November (with 8.8% plants affected), but the incidence of plants affected increased rapidly to 100% by mid-December.

At Rothamsted although the times when the first phoma leaf spotting was observed differed between seasons, the incubation period from the first release of ascospores to the first observed leaf spots on plants in untreated plots was 8–20 days (105–234 degree-days) (Figure 3; Table 2). In seasons with early release of ascospores, the incidence of phoma leaf spots increased rapidly. For example, at Rothamsted ascospores were first detected on 14 September 1999, which was 37 days earlier than in 2002; the first leaf spotting was observed on 30 September 1999, which was 31 days earlier than in 2002. In 1999/2000 (Figure 3b), the incidence of leaf spotting increased rapidly to a maximum (89%) by 12 October, which was 29 days after the first release of ascospores. In contrast, in 2002/03 (Figure 3e), the incidence of leaf spotting increased slowly to a maximum (93%) on 16 January 2003, which was 87 days after the first release of ascospores.

Phoma stem base canker first appeared between late March and early May. At Rothamsted in 2000/01, a high incidence of phoma leaf spots (100%) in winter was associated with a high incidence of stem base canker (100%) in spring/summer (Figure 3c). In 2001/02, following appearance of leaf spotting earlier than in 2000/01, stem base canker development started 16 days earlier. In 1998/99, following the late release of ascospores and late leaf spotting epidemic, stem base canker development started later, and stem base canker incidence and severity (0–4 scale) were lower before harvest (77% and 2.2) than in 1999/2000 (95% and 2.9). The incubation period from first leaf spotting to first stem base canker was 174–198 days (1044–1326 degree-days) (Table 2). In the five seasons investigated at Rothamsted, later release of ascospores (e.g. in 2002/03) was associated with lower stem base canker severity (2.2 in 1998/99, 2.9 in 1999/2000, 2.9 in 2000/01, 2.2 in 2001/02, 2.0 in 2002/03).

At Boxworth, severe stem base canker epidemics were observed in seasons with early phoma leaf spotting epidemics (Figure 4). In 1999/2000, a high incidence of phoma leaf spots (100%) in early winter was associated with a high incidence (99%) and severity (2.8) of stem base canker before harvest. In 1998/99, when appearance of leaf spotting was later than in 1999/2000, stem base canker development started 17 days later. Before harvest, both the incidence (76%) and severity (1.1) of stem base canker were smaller than in 1999/2000. In 2002/03, although the phoma leaf spotting epidemic started very late (5 November), the high incidence of phoma leaf spotting (100%) was associated with a high incidence of stem base canker (90%). The leaf spot incubation period and stem base canker incubation period were similar to those at Rothamsted, with 11–20 days (137–280 degree-days) from the first release of ascospores to the first leaf spotting, and 162–179 days (1056–1196 degree-days) from the first leaf spotting to appearance of the first stem base cankers (Table 2).

Patterns of phoma leaf spotting and upper stem lesion development in Poland

In Poland at Poznan, differences in patterns of ascospore release between the three seasons investigated were associated with differences in phoma leaf spotting epidemics between the seasons (Figure 6). In 1999/2000, few phoma leaf spots developed in the autumn, particularly as mean temperatures remained ≤ 0°C after 11 November 1999. However, the incidence of phoma leaf spotting increased in late winter/early spring, reaching 19% plants affected by late March. In 1998, phoma leaf spots appeared (7% plants affected) by 9 November, over 1 month after spores were first detected. The incidence of affected plants decreased during the winter due to the shedding of affected leaves. However, the incidence of plants with phoma leaf spots increased to 15% by March 1999. In 2000/01, there was an early appearance of

phoma leaf spots (7% plants affected) by 26 October and leaf spotting increased to a maximum of 26% plants affected by 6 December. In the three seasons investigated, the time from the first release of ascospores to the first leaf spotting varied much more in days (31–130 days) than in degree-days (278–387 degree-days). In Poland, phoma upper stem lesions (phoma stem base canker was observed very rarely) appeared from early June onwards in the three seasons. There were differences in incidence and severity of upper stem lesions between the three seasons investigated. In 1998/99, the incidence of upper stem lesions (26%) was lower than in 1999/2000 (43%) and 2000/01 (53%), but the severity (0–9 scale) before harvest was higher (3.4) than in the other two seasons (3.1 for 1999/2000, 0.9 for 2000/01).

Discussion

These results suggest that differences in the development of phoma stem canker epidemics between England and Poland relate to differences in weather patterns between the two countries rather than differences in their pathogen populations. For example, the main difference in the seasonal pattern of ascospore release between England (*L. maculans*) and Poland (*L. biglobosa*) was that the duration of ascospore release was longer in England than Poland. The sub-zero temperatures and snow cover during winter in Poland (Figure 1) restricted ascospore release, whereas in England ascospore release continued throughout the wet, mild winter. In both England and Poland, differences between seasons in the date of first ascospore release were related to rainfall in August/September, since dry weather delays maturation of ascospores of both *L. maculans* and *L. biglobosa* (Toscano-Underwood et al., 2003). Furthermore, because the winter is colder in Poland than England, leaves infected early in autumn in Poland may fall off before the pathogen reaches the stem base, so that the plants generally escape development of stem base canker. Previous studies suggest that the low temperatures not only decrease the germination of ascospores and penetration of leaf surfaces, but also slow down the growth of the fungus in plants and delay the development of symptoms (Hammond et al., 1985; Toscano-Underwood et al., 2001; Huang et al., 2001, 2003). This may also explain why stem base canker is rarely observed in Poland but is common in England. It is unlikely that this difference was related to the differences in the pathogen populations between the two countries, since both *L. maculans* and *L. biglobosa* were isolated from stem base cankers in England, although some differences in their distribution in stem base tissues have been observed (West et al., 2002a).

Differences in numbers of ascospores released between days following rainfall and periods of dry weather suggest that wetness provided by rainfall is essential for release of ascospores of both *L. maculans* in England and *L. biglobosa* in Poland. In the absence of rain, dew appeared to be sufficient to stimulate release of only small numbers of ascospores. After a period of dry weather, the first rain was most important, since ascospore release increased rapidly after rainfall and did not increase with long periods of rain. These results agree studies in Australia and Canada, where release of *L. maculans aspospores* was associated with the occurrence of rainfall (Petrie, 1995; Salam et al., 2003). The experiments provide no evidence that temperature affected release of ascospores of either *L. maculans* or *L. biglobosa* over the range 5–20 °C. This could explain the long period of ascospore release in England. At Rothamsted, the average winter temperature is usually >4°C (Figure 1). By contrast, at Poznan in Poland, mean temperatures in December, January, and February are generally about 4°C lower than at Rothamsted; the average temperature is <5°C in November and <0°C in December/January, with snow cover on many days during winter. The situation in Poland may be intermediate between that in England and that in Canada, where there are generally sub-zero temperatures and a long period of snow cover in winter, and no *L. maculans* ascospores were detected before spring, 9–10 months after harvest (Petrie, 1995).

These results indicate that diurnal periodicity of ascospore release of *L. maculans* in England and *L. biglobosa* in Poland in autumn is similar. In both countries, although a small number of ascospores were released on days without rain, ascospores were mainly released on days with rain. In England most ascospores were released between 03:00 and 13:00 h and in Poland most ascospores were released between 03:00 and 15:00 h. The

diurnal patterns of ascospore release of *L. maculans* in England and *L. biglobosa* in Poland differ from those in Canada, where *L. maculans* ascospores were mainly released at night between 22:00 and 4:00 h (Guo and Fernando, 2003). However, ascospores of *L. maculans* are released mainly in summer (June/July) from one-year old debris in Canada, whereas in Europe ascospores of *L. maculans* or *L. biglobosa* are released mainly in autumn (October/November) from 3-month old debris (Petrie, 1995; West et al., 2001).

The experiments provide evidence that ascospore release is essential for initiation of phoma stem canker epidemics in both England and Poland and that the timing of release determines the severity of stem base cankers and upper stem lesions in the following summer. Data presented also suggest that autumn ascospore release may be more important in England than in Poland. In England, early release of ascospores led to early phoma leaf spots in autumn, which led to severe stem base canker before harvest (Zhou et al., 1999; Sun et al., 2001). This is reflected by the observation that, over the five seasons investigated, thermal times (degree-days) for the two incubation periods (i.e. from first release of ascospores to first leaf spotting; from first phoma leaf spotting to first appearance of stem base canker) were both within narrow ranges. These results confirm those obtained in controlled environments and field experiments in England (Biddulph et al., 1999; Sun et al., 2001). In Poland, ascospore release in spring appears to be more important than in England. The winter is colder in Poland than in England, so many leaves infected early in autumn may fall off before the pathogen reaches the stem. Since the springs and summers are hotter in Poland than England, the pathogen may develop rapidly in the leaves infected late in spring to reach upper stems and cause severe upper stem lesions. This may explain why upper stem lesions are common in Poland, since the severity of upper stem lesions increases linearly with accumulated degree-days (Sun et al., 2001).

Results from these experiments suggest that it may be easier to develop schemes to forecast severity of stem canker epidemics in England than in Poland, since no evidence that early autumn leaf spotting was associated with severe upper stem lesion epidemics was found in Poland. Weather-based forecasting systems to predict ascospore release have been introduced to guide decisions about autumn fungicide application in France (Pérès and Poisson, 1997) and to guide decisions about sowing date in Australia (Salam et al., 2003). The good relationship between the date of the first release of ascospores and rainfall (total rainfall or rain days) in August/September in these experiments suggests that it is possible to develop a weather-based forecasting system to predict the first major release of ascospores in England. However, unlike in France and Australia, ascospore release in England tends to be spread over a long period in autumn and winter. Furthermore, timing of ascospore release is not the only factor that influences the severity of phoma stem base canker epidemics. Environmental factors (temperature and rainfall), cultivar resistance, and the vigour and size of plants also influence onset of phoma leaf spots after release of ascospores and subsequent development of stem canker epidemics (West et al., 2001).

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