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Effects of temperature and wetness duration on infection of oilseed rape leaves by ascospores of *Leptosphaeria maculans* (stem canker)

J.E. Biddulph[1], B.D.L. Fitt[1], P.K. Leech[1], S.J. Welham[1] and P. Gladders[2]
[1]*IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK (Fax: +44 1582 760981);* [2]*ADAS Boxworth, Cambridge CB3 8NN, UK*

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Abstract

In controlled environment experiments, ascospores of *Leptosphaeria maculans* (stem canker) infected oilseed rape (cv. Nickel) leaves and caused phoma leaf spots at temperatures from 8 °C to 24 °C and leaf wetness durations from 8 h to 72 h. The conditions that produced the greatest numbers of leaf spot lesions were a leaf wetness duration of 48 h at 20 °C; numbers of lesions decreased with decreasing leaf wetness duration and increasing or decreasing temperature. At 20 °C with 48 h of leaf wetness, it was estimated that one out of four spores infected leaves to cause a lesion whereas with 8 h of leaf wetness only one out of 300 spores caused a lesion. As temperature increased from 8 °C to 20 °C, the time from inoculation to the appearance of the first lesions (a measure of the incubation period) decreased from 15 to 5 days but leaf wetness duration affected the length of the incubation period only at sub-optimal temperatures. Analyses suggested that, within the optimal ranges, there was little effect of temperature or wetness duration on incubation period expressed as degree-days; the time until appearance of 50% of the lesions was *ca*. 145 degree-days. A linear regression of % leaves with lesions (P_l) (square-root transformed) on % plants with lesions (P_p) accounted for 93% of the variance: $\sqrt{P_l} = 1.31 + 0.061\ P_p$. This relationship was also investigated in winter oilseed rape field experiments in unsprayed plots from October to April in 1995/96 (cv. Envol), 1996/97 (cv. Envol), 1997/98 (cvs Bristol and Capitol) and 1998/99 (cvs Apex, Bristol and Capitol) seasons. The linear regression of % leaves with lesions (square-root transformed) on % plants with lesions accounted for 90% of the variance and had a similar slope to the controlled environment relationship: $\sqrt{P_l} = 0.81 + 0.051\ P_p$. These results were used to examine relationships between the development of phoma leaf spot on plants in winter oilseed rape crops, the incubation period of *L. maculans* and the occurrence of infection criteria (temperature, rainfall) in the autumns of 1996, 1997 and 1998.

Introduction

Stem canker (blackleg), caused by *Leptosphaeria maculans* (Desm.) Ces & de Not. (anamorph *Phoma lingam* (Tode ex Schw.) Desm.) is a damaging disease of oilseed rape (canola) worldwide. In the UK, stem canker has been estimated to have caused losses of over £30M in winter oilseed rape (*Brassica napus* L. subsp. *oleifera* Metzg. & Sinsk.) in each season from 1993 to 1996 (Fitt et al., 1997). However, winter oilseed rape disease surveys show that the severity of epidemics differs between seasons and between regions, with the most severe attacks generally occurring in eastern England (Gladders and Symonds, 1995). The disease decreases yield substantially when stem base lesions (stem canker) girdle oilseed rape stems in the spring and summer. However, these severe stem base lesions are initiated in the autumn by air-borne ascospores which infect leaves (Gladders and Musa, 1980). Detailed experiments showed that from phoma leaf spot lesions

on leaves the pathogen grows systemically down the petioles into stems to cause stem lesions (Hammond et al., 1985). Severe basal stem canker lesions originate from infections on leaves at the rosette stage of crop growth in the autumn, whereas lesions higher up the stem originate from infections on later leaves.

Observations of disease gradients in crops suggest that the source of ascospores in autumn is stem debris from the previous season and that the disease is monocyclic, with little or no secondary spread by splash-borne conidiospores produced on primary leaf lesions during a season (Hammond and Lewis, 1986). Experiments with fungicides applied at different timings suggest that stem canker can be controlled only early in its development before the pathogen reaches the stem and therefore fungicides have frequently failed to control the disease (Gladders et al., 1998). To optimise fungicide use by predicting the occurrence of severe stem canker epidemics, there is a need to determine the conditions when ascospores of *L. maculans* can infect leaves of winter oilseed rape crops in the autumn.

Both disease surveys and observations on stem canker development in individual crops suggest that many of the differences in severity of epidemics between seasons and between sites might be attributed to differences in the occurrence of weather favourable for infection of crops by *L. maculans* ascospores, especially to differences in rainfall and temperature (Gladders and Musa, 1980; Hammond and Lewis, 1986; Gladders and Symonds, 1995). However, there has been little detailed work on the effects of temperature and wetness duration on the infection of oilseed rape leaves by ascospores of *L. maculans*. Few experiments on the stem canker pathogen have used ascospores as inoculum for infecting oilseed rape leaves. In most experiments (e.g. those of Badawy et al., 1991), conidiospores have been used to inoculate leaves, which have had to be wounded beforehand because conidiospores cannot usually infect healthy leaves (Hammond and Lewis, 1987). In some experiments, leaves have been inoculated with single ascospores (Hammond and Lewis, 1987) or with ascospore suspensions (Gladders and Musa, 1980) but infection conditions were not examined. This paper describes the effects of temperature and wetness duration on infection of oilseed rape leaves by ascospores of *L. maculans* and the subsequent development of phoma leaf spot lesions.

Materials and methods

Controlled environment experiments

Five experiments were done in controlled environment cabinets. The factors tested are listed in Table 1. Two cabinets used were Fisons 600H cabinets (dimensions $1.2 \times 0.6 \times 0.8$ m) with fluorescent lighting giving a light intensity of 240 $\mu E\, m^{-2}\, s^{-1}$ at plant canopy level and a relative humidity of 55–90%; the other three cabinets used were designed at Rothamsted (dimensions $2.5 \times 1.0 \times 1.35$ m) with a light intensity of 190 $\mu E\, m^{-2}\, s^{-1}$ and 80–85% r.h. There were six pots per wetness duration treatment (experiments 2, 3, 4 and 5) or three pots per treatment (preliminary experiment 1), with each pot containing one plant. The pots for a wetness duration treatment were all placed in one seed tray (23×36 cm). The same temperatures were maintained for the wetness period and subsequent period of incubation. Seed trays (wetness duration treatment), containing either three or six pots, were arranged in completely randomised designs in each growth cabinet (temperature treatment). The series of experiments was arranged in a split plot design with cabinets at different temperatures as the main plots replicated in time (usually four replicates, experiments 2, 3, 4 and 5) and wetness duration treatments as sub-plots. Cabinets for temperature treatments were allocated randomly wherever possible, but some cabinets could not operate at the lower temperatures. During experiments a daylength of 12 h was maintained.

Plant material

Winter oilseed rape seeds of the stem canker susceptible cultivar Nickel were sown in Jiffy pots containing peat-based compost (1.5 kg PG mix per m^3) and a slow release fertiliser (2 kg Osmocote per m^3) (Petersfield Products, Cosby, Leicester). Plants were initially grown in a glasshouse and, after 17 days (experiments 1, 2, 3 and 4) or 13 days (experiment 5) from sowing, the plants were transplanted into 9 cm diameter pots. On the day prior to inoculation, six pots (experiments 2, 3, 4 and 5) or three pots (experiment 1) were transferred to seed trays lined with absorbent paper and placed in plastic saucers which were kept filled with water throughout the experiments. These trays were then transferred to the controlled environment cabinets for each temperature treatment. Each

Table 1. Treatment factors, tissues and controlled temperature cabinets used in each of five experiments to investigate the effects of infection conditions on development of phoma leaf spot (*L. maculans*) lesions on oilseed rape leaves

Experiment	Infection condition treatments			
	Temperature (°C)	Wetness duration (h)	Tissue	Cabinets
1	5, 10, 15, 20[2]	24, 48, 72	Cotyledon[1] + leaf 1	A, B, C, D[2]
2	8, 12, 16, 20	8, 16, 20, 24, 30, 48	Leaves 1–4	B, A, D, C
3	8, 12, 16, 20	8, 16, 20, 24, 30, 48	Leaves 1–4	A, B, C, D
4	20, 24	8, 16, 20, 24, 30, 48	Leaves 1–4	C, D
5	8, 12, 16, 20	24, 48, 72	Leaves 1–4	B, E, C, D

[1]In experiment 1, leaves were inoculated by placing on each leaf a 20 µl droplet containing approximately 50 ascospores. In other experiments leaves were sprayed with spore suspensions containing 10^3 ascospores ml^{-1}.
[2]Cabinets and temperature treatments are in order of increasing temperature; cabinets were allocated randomly to temperature treatments whenever it was possible, but cabinets C and D could not operate at temperatures <15°C. A and B were Fisons 600H cabinets and C, D and E were Rothamsted-designed cabinets.

plant had two true leaves fully expanded with third and fourth leaves just starting to expand. Each leaf was numbered using a non-phytotoxic marker pen.

Preparation of ascospore inoculum

Oilseed rape stem bases with stem canker lesions were collected after harvest from fields in the UK and stored dry at approximately 4 °C until required. A number of stem bases were removed from storage and soaked in distilled water, then kept moist until asci could be seen protruding from pseudothecia. These mature pseudothecia were excised from the stems using a scalpel and transferred to a small volume of distilled water. Pseudothecia were kept frozen for up to one month prior to inoculation. Prior to inoculation the suspension of pseudothecia was allowed to thaw and the pseudothecia were crushed to release the ascospores. The concentration of spores in the suspension was adjusted using a haemocytometer slide.

Inoculation

In the preliminary experiment (experiment 1), a single 20 µl drop of spore suspension containing approximately 50 ascospores ml^{-1} was applied to one cotyledon and to the first true leaf of each plant. In the other experiments, potted plants were inoculated by spraying using an aerosol pressurised sprayer (Humbrol Air Brush, Humbrol Ltd., Marfleet, Hull) with suspensions of 10^3 *L. maculans* ascospores ml^{-1}. After inoculation, the plants were immediately covered with plastic seed tray covers and these were then covered with polyethylene bags. In the preliminary experiment, plants were given wetness periods of 24 h, 48 h or 72 h each at temperatures of 5 °C, 10 °C, 15 °C or 20 °C. In experiments 2 and 3, the wetness periods were 8 h, 16 h, 20 h, 24 h, 30 h or 48 h each at temperatures of 8 °C, 12 °C, 16°C or 20°C. In experiment 4, the wetness periods were 8 h, 16 h, 20 h, 24 h, 30 h or 48 h each at temperatures of 20 °C or 24 °C. In experiment 5, the wetness periods were 24 h, 48 h or 72 h each at temperatures of 8 °C, 12 °C, 16 °C or 20 °C. After wetness periods, the plastic tray covers and polyethylene bags were removed.

Disease assessments

In the preliminary experiment, the numbers of phoma leaf spots on inoculated tissues were counted 8, 10, 13, 23 and 27 days after inoculation. In the other experiments, the numbers of phoma leaf spots on each of the four leaves were counted almost daily until either the leaves died (at temperatures of 16 °C, 20 °C and 24 °C) or no new leaf spots appeared (at temperatures of 8 °C and 12 °C) up to 32 days after inoculation. The numbers of lesions per plant and the proportions (%) of leaves and of plants with lesions were calculated for each date of assessment. The incubation period was estimated as the time from inoculation until the first lesions were observed (calculated as the mean of the last time when no lesions were present and the first time when lesions were present), the time until 50% of

the lesions were observed or the time until 20% of the leaves had lesions.

Infection efficiency experiment

In a final experiment under near-optimal conditions (temperature 20 °C, leaf wetness duration after inoculation 48 h) in cabinet D, twelve plants were sprayed with an ascospore suspension (ca. 10^3 spores/ml). The numbers of lesions which developed on the first two true leaves of each plant were counted 9, 10 and 11 days after inoculation, after which no new lesions were observed. At the same time, six other plants were sprayed with the same ascospore suspension. Immediately after spraying, the spore suspension was transferred from each of the first two true leaves onto separate microscope slides and the number of spores deposited was counted. The areas of these twelve leaves were also measured. The mean number of spores deposited per leaf and the mean number of lesions which developed per unit area were calculated.

Statistical analyses of controlled environment data

Data for the maximum numbers of lesions per plant in experiments 2, 3 and 5 were $\log_{10}$-transformed before analysis to improve the homogeneity of variance. The effects of temperature and wetness duration and the interaction between them on the maximum number of lesions were analysed by restricted maximum likelihood estimation (REML) (Payne et al., 1993). REML was used because it allowed for estimation of variability due to plants, trays and incubators within the unbalanced structure of the experiment (unequal replication of treatments over time). Wald statistics were used to assess the importance of treatment effects. Mean values for transformed data and back-transformed means were calculated. Data for % plants with phoma leaf spots and % leaves with phoma leaf spots were logit-transformed before analysis to improve the homogeneity of variance. Effects of temperature, wetness duration and their interactions were analysed using a generalised linear mixed model (GLMM) (Welham, 1992) with a logit-link function and a binomial distribution to estimate the probability that a plant or leaf had symptoms, and Wald statistics were calculated. Tables of logit-transformed and back-transformed means were prepared. Incubation period data for the three different measures of incubation period were analysed by REML and Wald statistics were calculated to estimate the effects of temperature, wetness duration and their interaction.

Relationship between % leaves with lesions and % plants with lesions

This relationship was assessed by linear regression, using data from the controlled environment and field experiments at Rothamsted. To increase the range of values for % plants with lesions in controlled environment experiments (units of assessment in these experiments were groups of six plants, with four leaves assessed on each), data for the replicates were combined for each treatment /date (to give units of up to 24 plants and 96 leaves). Field experiment data were from samples of plants collected from unsprayed plots (10 plants per plot, 3–12 leaves per plant) in winter oilseed rape experiments at Rothamsted from October to April in 1995/96 (cv. Envol), 1996/97 (cv. Envol), 1997/98 (cvs Bristol and Capitol) and 1998/99 (cvs Apex, Bristol and Capitol) seasons. To increase the range of values of % plants with lesions, data for replicate plots sampled on a particular date in these field experiments were combined (to give units of 60–300 plants and 240–1500 leaves, except for cv. Apex in 1998/99 with 25 plants and ca. 150 leaves). Different transformations of these variates to linearise the relationship between them were tested. Values for 0% and 100% plants with lesions were excluded from the analysis before linear regression of % leaves with lesions on % plants with lesions.

Development of phoma leaf spot lesions in winter oilseed rape crops

Changes with time in the percentage of plants with phoma leaf spots were assessed in unsprayed plots of winter oilseed rape or unsprayed areas (minimum 40 m^2) of commercial crops at ADAS Boxworth in autumn 1996, at three sites (ADAS Boxworth, ADAS High Mowthorpe and ADAS Rosemaund) in autumn 1997 and two sites (ADAS Boxworth and Rothamsted) in autumn 1998. The cultivars were Rocket, sown on 31 August, at ADAS Boxworth in 1996, Alpine (26 August) at ADAS Boxworth, Apex (4 September) at ADAS High Mowthorpe, Apex (6 September) at ADAS Rosemaund in 1997 and Apex at ADAS Boxworth (27 August) and Rothamsted (27 August) in 1998. At each site, samples of five to ten plants were taken from three to five plots/areas of crop, to give a total

of 25 plants for assessment at regular intervals (often weekly) from mid-September to late-December. Each plant was assessed for phoma leaf spot and the percentage of plants with lesions in each sample was calculated. Temperature (°C) and rainfall (mm) were recorded by automatic weather stations at each site in each season. The development of phoma leaf spot at each site in relation to temperature and rainfall was then assessed. This was done by using observed increases in incidence (% plants) of leaf spot and the length of the incubation period in degree-days (calculated from weather data) and the infection criteria (estimated from controlled environment experiments) to estimate when infections might have occurred.

Results

Development of phoma leaf spot lesions (controlled environment)

Ascospores of *L. maculans* were able to infect leaves of oilseed rape and cause phoma leaf spot lesions at temperatures from 8 °C to 24 °C (Figure 1) and leaf wetness durations from 8 h to 72 h (Figure 2). Effects of temperature and leaf wetness duration on the pattern of lesion development were similar in experiments 2 (Figures 1a and 2a), 3 (Figures 1b and 2b), 4 (Figures 1c and 2c) and 5 (Figures 1d and 2d), although the total numbers of lesions which developed were greater in experiments 4 and 5 than in experiments 2 and 3, possibly because of differences in the efficiency of the natural inoculum used. With a leaf wetness duration of 48 h at a temperature of 20 °C, the greatest number of lesions was produced, the time from inoculation to the appearance of the first lesions (a measure of the incubation period) was shortest and the period of lesion production (before the number of lesions reached its maximum under the conditions tested) was one of the longest. These observations confirmed those made in the preliminary experiment (1), when at 20 °C 19 lesions had formed after 8 days with a wetness duration of 48 h and numbers of lesions increased with time at all wetness durations. In contrast, only seven lesions were observed at 5 °C 27 days after inoculation and then only when wetness duration was 48 h or 72 h.

At a leaf wetness duration of 48 h, the proportions of plants with lesions (Figure 3b) and of leaves with lesions (Figure 3a) increased from 0 to their maximum values most rapidly at 20 °C and 24 °C, and less quickly at 16 °C, 12 °C and 8 °C. At a temperature of 20 °C, the proportion of plants with lesions increased from 0% to 100% in a period from 3 to 7 days after inoculation at leaf wetness durations from 20 h to 72 h, from 3 to 13 days at a leaf wetness duration of 16 h and did not reach more than 60% at 8 h or more than 15% at 4 h of leaf wetness duration (Figure 4b). The proportion of leaves with lesions increased from 0 to a maximum from 3 to 10 days after inoculation at all leaf wetness durations (Figure 4a).

Maximum number of lesions

Both temperature (Wald statistic 23.0, 4 df) and leaf wetness duration (443.1, 7 df) affected the maximum number of lesions which developed ($P < 0.001$) but the interaction between temperature and leaf wetness duration was not significant (30.6, 25 df) (Table 2). Most lesions developed at 20 °C with a 48 h leaf wetness duration (>60 lesions per plant). Numbers of lesions were less at 24 °C than at 20 °C and decreased with decreasing temperature from 20 °C to 8 °C. Numbers of lesions decreased as leaf wetness duration decreased from 48 h to 4 h; with a leaf wetness of 4 h very few lesions developed, mostly at 16 °C. As leaf wetness duration increased from 48 h to 72 h, the maximum number of lesions increased at 8 °C and 12 °C but did not increase at 16 °C and 20 °C.

Maximum proportions of leaves or plants with lesions

The maximum proportion of leaves with lesions was affected ($P < 0.001$) by both temperature (Wald statistic 33.5, 4 df) and leaf wetness duration (126.8, 7 df) but the interaction between temperature and leaf wetness duration was not significant (18.8, 25 df) (Table 3). The proportion of leaves with lesions increased as temperature increased from 8 °C to 16 °C at all wetness durations. However, as temperature increased from 16 °C to 24 °C the proportion of leaves with lesions decreased at wetness durations of 4 h or 8 h and did not increase greatly at wetness durations of 16–72 h. The proportion of leaves with lesions generally increased as leaf wetness duration increased from 4 h to 48 h at all temperatures but did not increase greatly as wetness duration increased from 48 h to 72 h. In these controlled environment experiments, 100% of plants developed lesions in all the temperature and wetness treatments which were most favourable for infection (Table 4). The leaf wetness duration required for

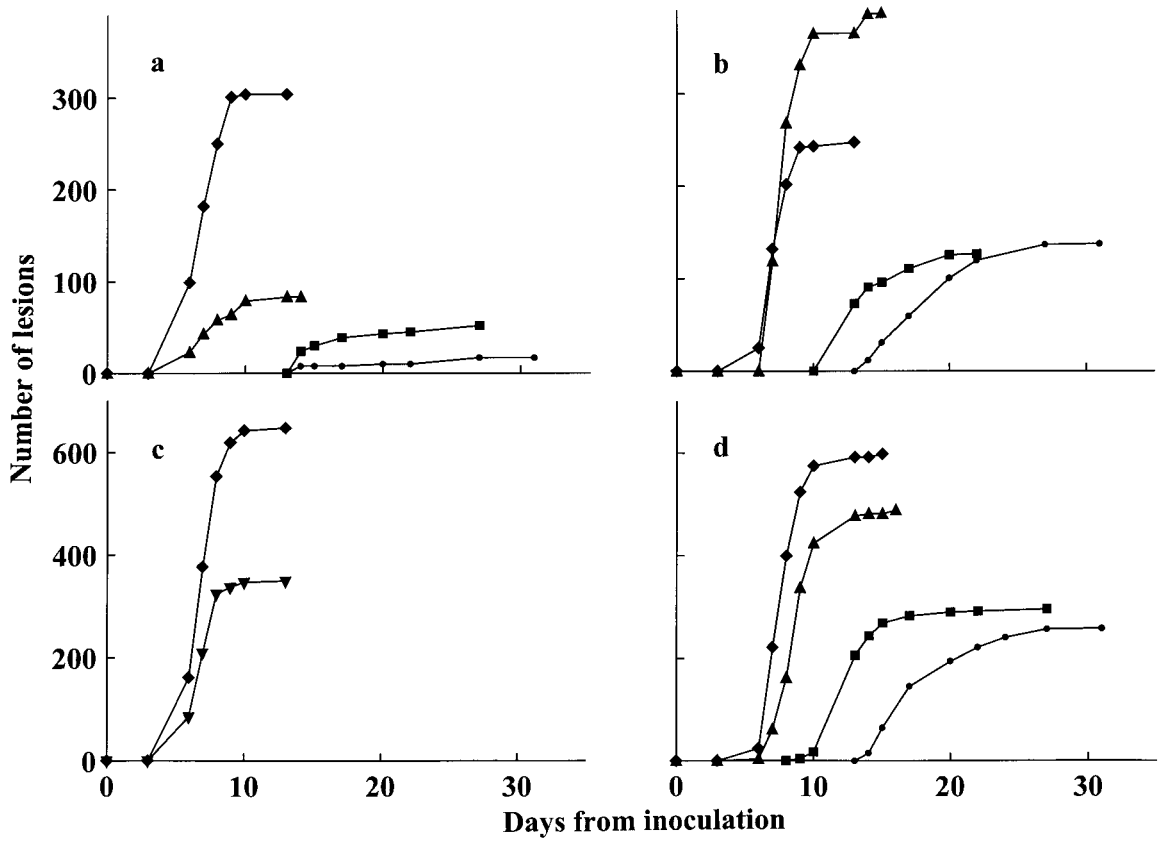

Figure 1. Changes with time in the number of phoma leaf spot lesions produced on oilseed rape plants inoculated with ascospores of *L. maculans* in four experiments with a wetness duration of 48 h at temperatures of 8 °C(●), 12 °C(■), 16 °C(▲), 20 °C(♦) or 24 °C(▼). Lesions were assessed on four leaves per plant on six plants per treatment in experiments 2(a), 3(b), 4(c) and 5(d).

L. maculans to produce phoma leaf spots on 100% of plants was 16 h at 20 °C or 24 °C, and increased as temperature decreased until a 72 h leaf wetness duration was required at 8 °C. There were, however, effects of wetness duration (Wald statistic 17.2, 7 df, $P < 0.05$) and temperature (3.5, 4 df, $P > 0.05$) on the proportion of plants with lesions, with increasing proportions of plants affected as temperature increased from 8 °C to 16 °C and wetness duration increased from 4 h to 48 h.

Infection efficiency

In the experiment with near-optimal conditions for infection at 20 °C with a 48 h leaf wetness duration, the mean leaf area was 9.5 cm^2. The estimated mean number of spores per leaf was 111.6 (11.8 spores cm^{-2}). The mean number of lesions per leaf was 28.8 (3.0 lesions cm^{-2}). Thus the estimated efficiency of infection was 25.8% (i.e. about one out of four spores on the leaf surface caused a lesion under optimal conditions). As temperature decreased or leaf wetness decreased below the optimum, the efficiency of infection by spores decreased. Thus the results in Table 2 suggest that only about one in 300 spores caused a lesion with a leaf wetness duration of 8 h.

Incubation period

Both temperature (Wald statistic 141.3, 4 df) and wetness duration (80.0, 7 df) affected the length of the incubation period ($P < 0.001$), expressed as the time from inoculation to the appearance of the first lesions (t_1), and the interaction between effects of temperature and wetness duration was also significant (55.9, 24 df, $P < 0.005$). As temperature increased, the time to the appearance of the first lesion decreased greatly

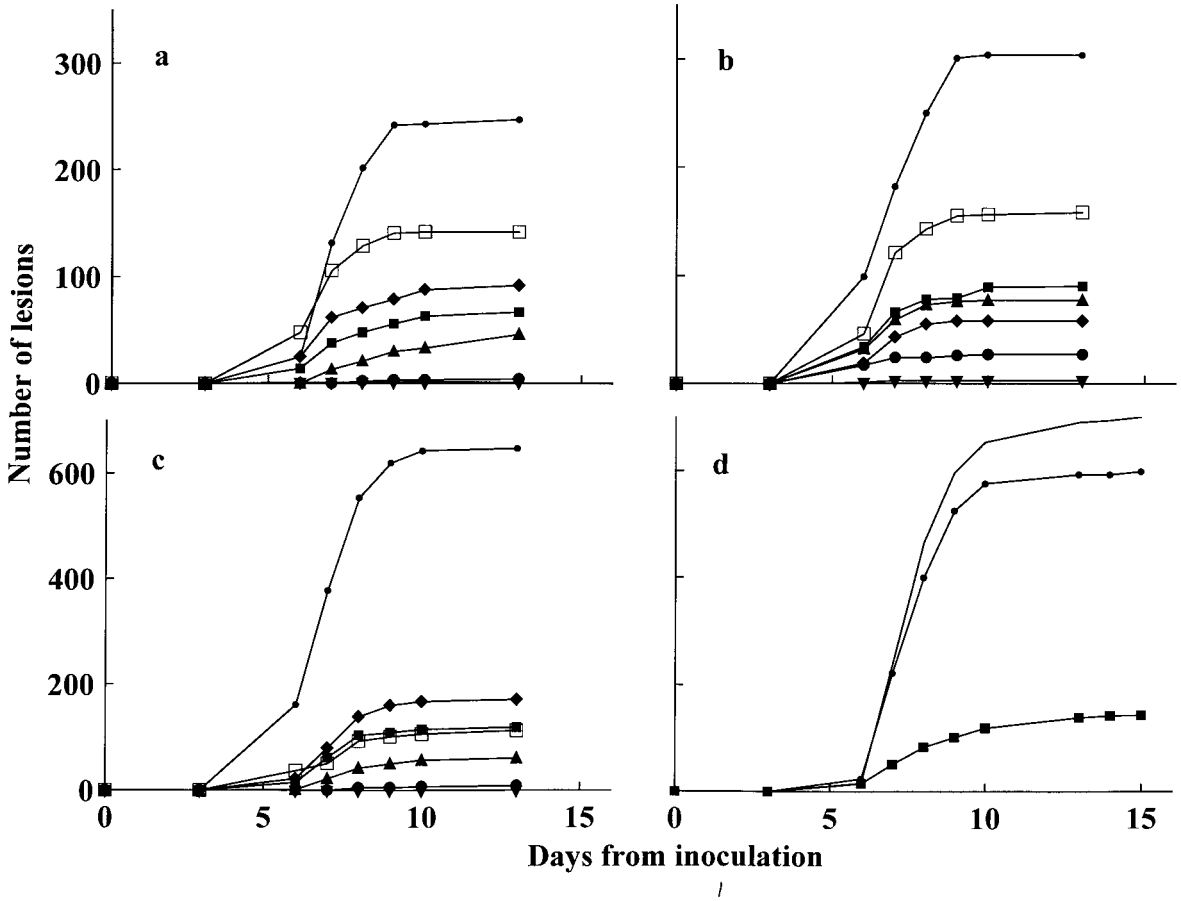

Figure 2. Changes with time in the number of phoma leaf spot lesions produced on oilseed rape plants inoculated with ascospores of *L. maculans* in four experiments at a temperature of 20 °C with wetness durations of 4 h (▼), 8 h (●), 16 h (▲), 20 h (♦), 24 h (■), 30 h (□), 48 h (●) or 72 h (-). Lesions were assessed on four leaves per plant on six plants per treatment in experiments 2(a), 3(b), 4(c) and 5(d).

from ca. 14–17 days at 8 °C to ca. 5–6 days at 20 °C; however, there was little difference between 20 °C and 24 °C (Table 5a). There was no evidence that increasing wetness duration above 20 h affected the time to the appearance of the first lesions at temperatures of 16 °C, 20 °C or 24 °C. However, there was some indication that, when conditions were less favourable for infection and few lesions developed, decreasing wetness duration increased the length of the time to the first lesion.

The effect of temperature (Wald statistic 189.8, 8 df) on incubation period, estimated as the time from inoculation to the appearance of 50% of the lesions (t_{50}), was significant ($P < 0.001$) but effects of wetness duration (3.5, 5 df) and the interaction between temperature and wetness duration (8.9, 11 df) were not (Table 5b). It was necessary to exclude sub-optimal temperature/wetness duration treatments from these analyses because too few lesions developed to estimate the time to the appearance of 50% of the lesions. The times to appearance of 50% of the lesions (t_{50}) were only slightly longer than those to the appearance of the first lesions (t_1), decreasing from ca. 17 days at 8 °C to ca. 7 days at 20 °C. Furthermore, when $1/t_{50}$ was regressed on temperature (T):

$$1/t_{50} = 0.012 - 0.0063T \quad (1)$$

the linear regression accounted for 92% of the variance, suggesting that use of accumulated temperature (degree-days) to estimate the length of the incubation period was a valid approximation over this temperature range (Figueroa et al., 1995). The REML analysis suggested that there were no effects of temperature

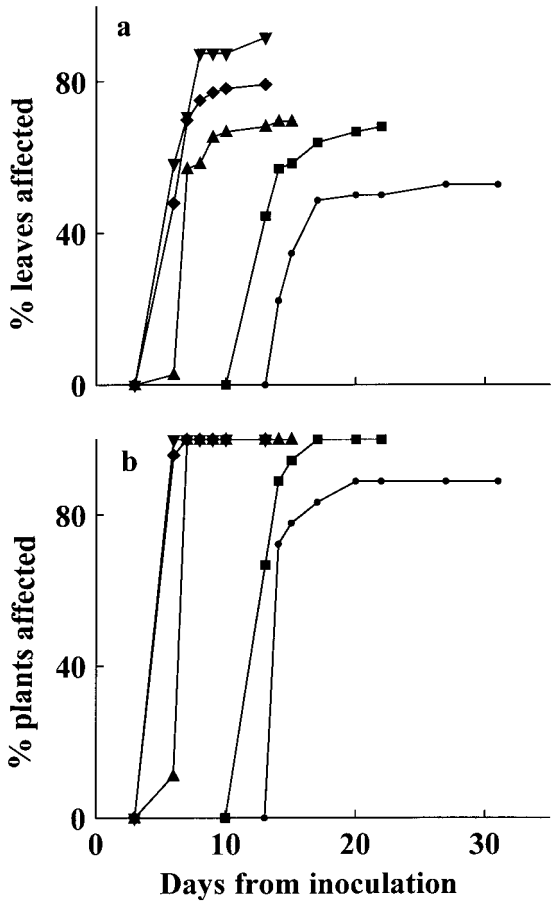

Figure 3. Changes with time in the proportions (%) of oilseed rape leaves (a) or plants (b) with phoma leaf spot lesions for plants inoculated with ascospores of *L. maculans* in experiments (2, 3, 4 and 5) with a wetness duration of 48 h at temperatures of 8 °C(●), 12 °C(■), 16 °C(▲), 20 °C(♦) or 24 °C(▼). Lesions were assessed on four leaves per plant on six plants per treatment; mean values for the four experiments are presented.

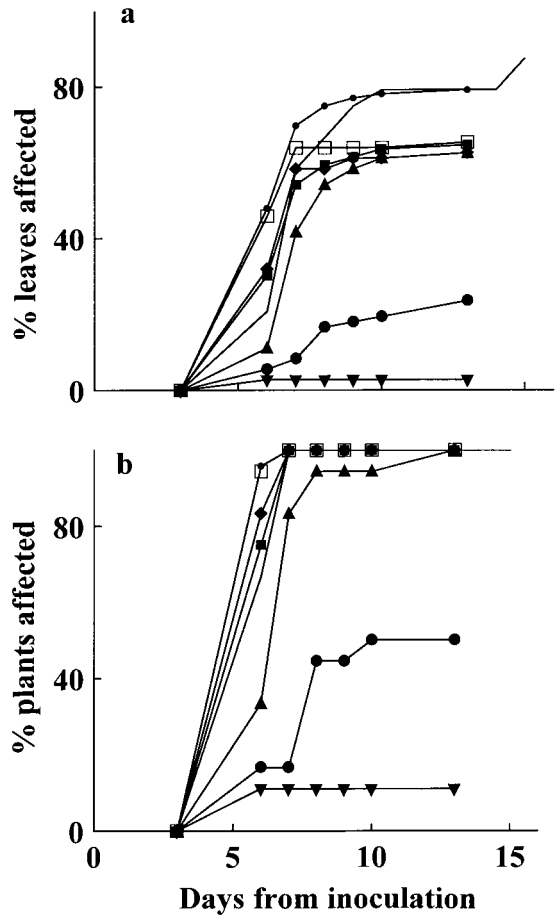

Figure 4. Changes with time in the proportions (%) of oilseed rape leaves (a) or plants (b) with phoma leaf spot lesions for plants inoculated with ascospores of *L. maculans* in experiments (2, 3, 4 and 5) at 20 °C with wetness durations of 4(▼), 8(●), 16(▲), 20(♦), 24(■), 30(□), 48(●) or 72 h(-). Lesions were assessed on four leaves per plant on six plants per treatment; mean values for four experiments are presented.

or wetness duration on incubation period (t_{50}) estimated in degree-days, which ranged from 130 to 160 degree-days. When data for sub-optimal treatments were included in estimating accumulated temperature to the appearance of the first lesion (t_1), the range was much greater, from 90 to 200 degree-days.

If incubation period was expressed as the time from inoculation until 20% of the leaves had developed lesions (t_{20}) (Table 5c), the pattern of effects of temperature (Wald statistic 48.1, 4 df) and wetness duration (43.5, 7 df) ($P < 0.001$) was similar to that for time to the appearance of the first lesions (t_1). The differences in the values of t_1 and t_{20} ranged from <1 day at temperatures >16 °C and wetness durations >16 h to ca. 4 days at the most sub-optimal conditions when few lesions developed. Estimates of t_{20} in degree-days, including these sub-optimal conditions, ranged from 90 to 240 degree-days.

Relationship between % leaves with lesions and % plants with lesions

A linear regression of square root (% leaves with lesions (P_l)) on % plants with lesions (P_p) in controlled

Table 2. Effects of temperature and wetness duration on maximum number of phoma leaf spot lesions produced on leaves of oilseed rape inoculated with ascospores of *L. maculans*

	Temperature (°C)				
Wetness duration (h)	8	12	16	20	24
Number of lesions (log_{10}-transformed)[1]					
4	*[2]	*	0.32	0.13	0.0
8	0.30	0.29	0.44	0.40	0.18
16	0.35	0.41	1.00	1.06	0.97
20	0.60	0.80	1.20	1.27	0.99
24	0.66	0.66	1.26	1.22	1.23
30	0.70	0.82	1.60	1.40	1.32
48	1.13	1.30	1.60	1.83	1.74
72	1.31	1.51	1.46	1.84	*
SED (max)[3]			0.332		
Number of lesions (back-transformed means)					
4	*	*	1.08	0.34	0.0
8	1.01	0.93	1.78	1.52	0.51
16	1.25	1.58	9.02	10.41	8.27
20	3.00	5.36	14.68	17.48	8.75
24	3.60	3.63	17.27	15.33	15.83
30	4.07	5.70	38.45	24.06	20.12
48	12.37	18.58	39.29	66.11	54.46
72	19.58	31.57	28.11	68.37	*

[1]Number of lesions per plant (four leaves); mean of data from experiments 2, 3, 4 and 5.
[2]These treatment combinations were not tested.
[3]Estimated by REML; it was not possible to give df.

Table 3. Effects of temperature and wetness duration on the maximum proportion (%) of oilseed rape leaves which developed phoma leaf spot lesions after inoculation with ascospores of *L. maculans*

	Temperature (°C)				
Wetness duration (h)	8	12	16	20	24
% leaves affected (logit-transformed)[1]					
4	*[2]	*	−1.83	−3.45	†[3]
8	−1.91	−2.17	−0.90	−1.07	−1.64
16	−1.53	−1.56	0.13	0.63	0.85
20	−0.61	0.13	0.56	0.72	0.48
24	−0.52	−0.49	0.85	0.80	0.85
30	−0.65	−0.13	1.11	0.76	1.30
48	0.11	0.79	0.93	1.43	2.36
72	0.04	0.67	2.00	1.59	*
SED (max)[4]			1.206		
% leaves affected (back-transformed means)					
4	*	*	14.0	3.1	0.01
8	12.9	10.5	29.2	25.8	16.2
16	18.0	17.6	53.5	65.6	70.1
20	35.4	53.5	63.8	66.1	61.7
24	37.4	38.1	70.2	65.9	70.1
30	34.6	47.1	75.4	68.4	78.6
48	52.8	68.7	71.8	80.7	91.4
72	50.7	65.6	87.8	82.5	*

[1]Mean of data from experiments 2, 3, 4 and 5.
[2]These treatment combinations were not tested.
[3]This treatment was not included in the analysis.
[4]SED for values not 0 or 100% estimated by REML; it was not possible to give df.

environment experiments (Figure 5a):

$$\sqrt{P_l} = 1.31 + 0.061 P_p \quad (2)$$

accounted for 93% of the variance and suggested that, in these experiments, when 20% of the plants had symptoms, about 6% of the leaves had symptoms and when 50% of plants had symptoms, about 20% of leaves had symptoms. However, there was insufficient data to directly estimate incubation period as the time from inoculation until 50% of plants had developed lesions. A linear regression of square root (% leaves with lesions) on % plants with lesions, for plants sampled from field experiments at Rothamsted (Figure 5b):

$$\sqrt{P_l} = 0.81 + 0.051 P_p \quad (3)$$

accounted for 90% of the variance. This regression suggested that when 20% of plants had symptoms about 4% of leaves had symptoms and, when 50% of plants had symptoms, about 12% of leaves had symptoms.

Development of phoma leaf spot lesions (winter oilseed rape crops)

The week that the first plants with phoma leaf spot were observed in unsprayed plots or crops of winter oilseed rape ranged from mid-October (Boxworth 1996, Figure 6a; Boxworth 1998, Figure 6e), late October (Rothamsted 1998, Figure 6f; High Mowthorpe 1997, Figure 6b), early November (Rosemaund 1997, Figure 6d) to mid-November (Boxworth 1997, Figure 6c). There was usually a rapid increase in the % plants with phoma leaf spots, so that the estimated dates when 20% of plants had leaf spots were 17 October (Boxworth 1998), 23 October (Boxworth 1996), 5 November (Rosemaund 1997), 6 November (Rothamsted 1998), 8 November (Boxworth 1997) and 20 November (High Mowthorpe 1997). At five out of six sites, the maximum % plants with phoma leaf spot was >80% but at High Mowthorpe in 1997

Table 4. Effects of temperature and wetness duration on the maximum proportion (%) of oilseed rape plants which developed phoma leaf spot lesions after inoculation with ascospores of *L. maculans*

	Temperature (°C)				
Wetness duration (h)	8	12	16	20	24
% plants affected (logit-transformed)[1]					
4	*[2]	*	−0.40	−1.82	−3.62
8	−0.25	−1.17	0.72	0.31	−0.53
16	0.11	1.07	3.05	†[3]	†
20	1.95	2.57	†	†	†
24	1.43	1.59	†	†	†
30	1.95	2.57	†	†	†
48	2.65	†	†	†	†
72	†	†	†	†	*
SED (max)[4]			3.810		
% plants affected (back-transformed means)					
4	*	*	40.1	13.9	2.6
8	43.8	23.6	67.2	57.7	37.1
16	52.7	74.5	95.5	100	100
20	87.6	92.9	100	100	100
24	80.7	83.1	100	100	100
30	87.6	92.9	100	100	100
48	93.4	100	100	100	100
72	100	100	100	100	*

[1]Mean of data from experiments 2, 3, 4 and 5.
[2]These treatment combinations were not tested.
[3]These treatments were not included in the analysis.
[4]SED for values not 0 or 100% estimated by REML; it was not possible to give df.

Table 5. Effects of temperature and wetness duration on the incubation period, estimated as the time from inoculation to the appearance of the first phoma leaf spot lesions on winter oilseed rape leaves inoculated with ascospores of *L. maculans*, the time to the appearance of 50% of the lesions or the time to the appearance of lesions on 20% of the leaves

	Temperature (°C)				
Wetness duration (h)	8	12	16	20	24
(a) (Days to first lesions, fitted values)[1]					
4	*[2]	*	12.5	4.6	†[3]
8	15.3	15.9	8.9	6.6	6.0
16	15.0	13.9	6.9	6.2	5.3
20	17.5	13.0	6.9	4.8	4.5
24	14.9	12.9	6.3	5.0	4.5
30	14.2	11.6	6.2	4.6	4.5
48	14.0	11.8	6.3	4.6	4.5
72	13.9	10.8	5.7	5.3	*
SED (max)[4]			2.02		
(b) (Days to 50% of lesions, fitted values)					
4	*	*	†	†	†
8	†	†	†	†	†
16	†	†	9.1	7.2	6.6
20	†	†	8.5	7.1	5.7
24	†	†	8.2	7.0	6.4
30	†	†	8.1	7.0	6.7
48	17.4	13.3	8.0	7.0	6.7
72	17.0	12.7	8.4	6.6	*
SED (max)			1.39		
(c) (Days to 20% leaves affected, fitted values)					
4	*	*	14.8	†	†
8	†	20.2	11.5	7.9	†
16	11.6	16.2	7.5	5.8	4.5
20	19.2	16.7	7.0	4.4	4.5
24	16.2	16.5	6.5	5.0	4.5
30	16.7	11.2	6.5	4.4	4.5
48	15.2	11.5	6.5	4.5	4.5
72	14.7	11.1	6.5	4.7	*
SED (max)			3.85		

[1]Mean of data from experiments 2, 3, 4 and 5.
[2]These treatment combinations were not tested.
[3]These treatments were not included in the analysis.
[4]SED for values not 0 or 100% estimated by REML; it was not possible to give df.

it was not more than 30%. However, the period between the observation of the first plants with phoma leaf spot and the time when >80% of plants were affected ranged from 1 week (Boxworth 1997) to 2 months (Rothamsted 1998) but was generally about 1 month. Observed decreases in the % plants with phoma leaf spot at Boxworth and Rosemaund in 1997 and Rothamsted in 1998 were related to death and loss of leaves with symptoms. If the estimated date when 20% of plants had phoma leaf spot was taken to indicate the onset of phoma leaf spotting and the incubation period between infection and the appearance of symptoms was assumed to be 145 degree-days, then the date when these infections occurred could be estimated. These estimated infection dates were 3 October (Boxworth 1998), 10 October (Boxworth 1996), 13 October (High Mowthorpe 1997), 17 October (Rosemaund 1997), 18 October (Boxworth 1997) and 20 October (Rothamsted 1998). At all six sites, >2 mm of rain occurred on dates not more than 3 days before these estimated infection dates, although there were subsequently long periods of dry, cold weather between the estimated infection dates and the appearance of leaf spot lesions at all three sites in 1997.

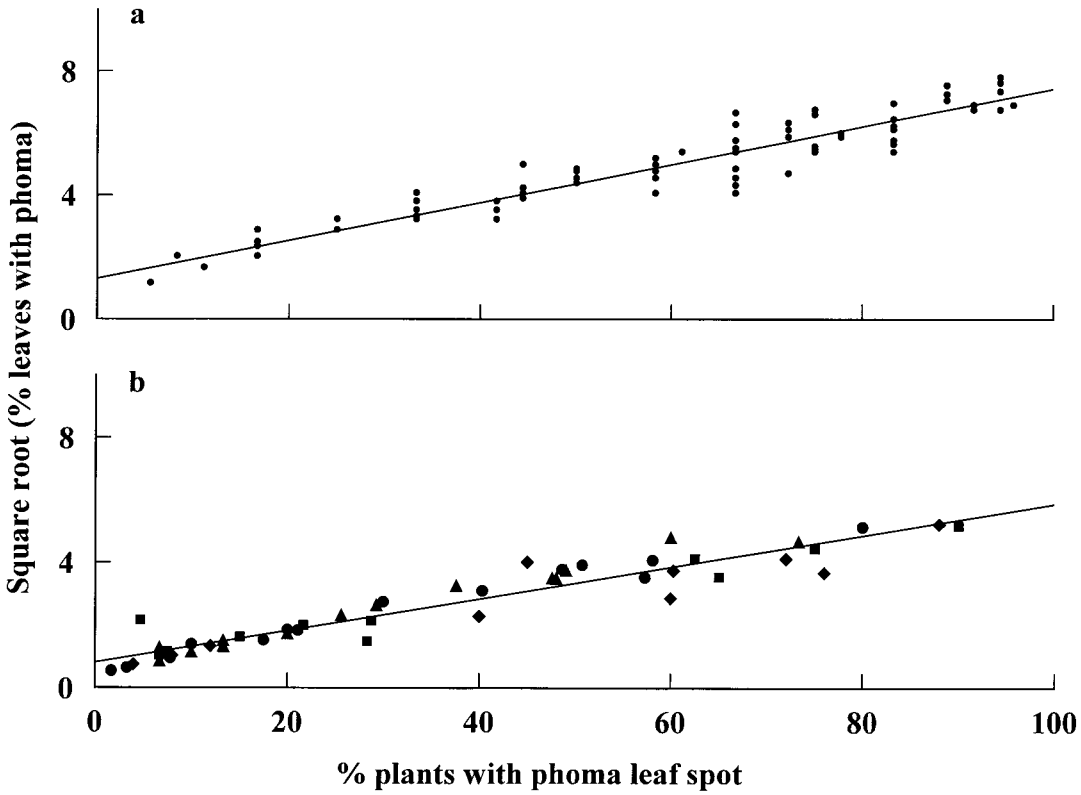

Figure 5. Relationship between the proportion (%) of leaves (P_l) which developed phoma leaf spot lesions and the proportion (%) of oilseed rape plants which developed lesions (P_p), Data for each treatment/assessment date were combined across replicates. Relationships were assessed by linear regression, when plants were inoculated with ascospores of *L. maculans* in four controlled environment experiments with different temperature and leaf wetness duration treatments (a) $\sqrt{P_l} = 1.31 + 0.061 P_p$ ($R^2 = 0.93$) and for samples of plants collected from unsprayed plots in field experiments at Rothamsted in 1995/96, 1996/97, 1997/98 and 1998/99 with cvs Envol (■), Bristol (•), Capitol (▲) and Apex (♦) (b) $P_l = 0.81 + 0.051 P_p$ ($R^2 = 0.90$).

Discussion

These controlled environment experiments suggest that ascospores of *Leptosphaeria maculans* can infect oilseed rape leaves over a wide range of temperatures (from 8 °C to 24 °C) and wetness durations (from 8 to 72 h). Previous work (Gladders and Musa, 1980; Hammond and Lewis, 1987) had already shown that ascospores can infect oilseed rape leaves, but these experiments now provide details of the range of conditions over which infection can occur. The experiments also suggest that the optimum temperature for infection is about 18 °C; at this temperature the shortest wetness period is needed for infection to occur. These experiments also suggest that at 18–20 °C the efficiency of infection is greatest, with most lesions produced, when wetness duration is at least 48 h. When infection conditions were sub-optimal, with shorter wetness duration or lower or higher temperatures than the optima, then the efficiency of infection was less, as in work with *Alternaria* species (Rotem, 1994). The weather records for the field experiments reported in this paper suggest that temperatures were sub-optimal for infection by *L. maculans* ascospores during the period in October when it was estimated that infections occurred in winter oilseed rape crops. Nevertheless, there was frequent rainfall during that period and it seems likely that the leaf wetness duration was sufficiently long for infection criteria to be fulfilled on many occasions in October.

Analyses of data from these controlled environment experiments suggest that the incubation period of *L. maculans*, between infection and appearance of leaf spot lesions, expressed in degree-days, can

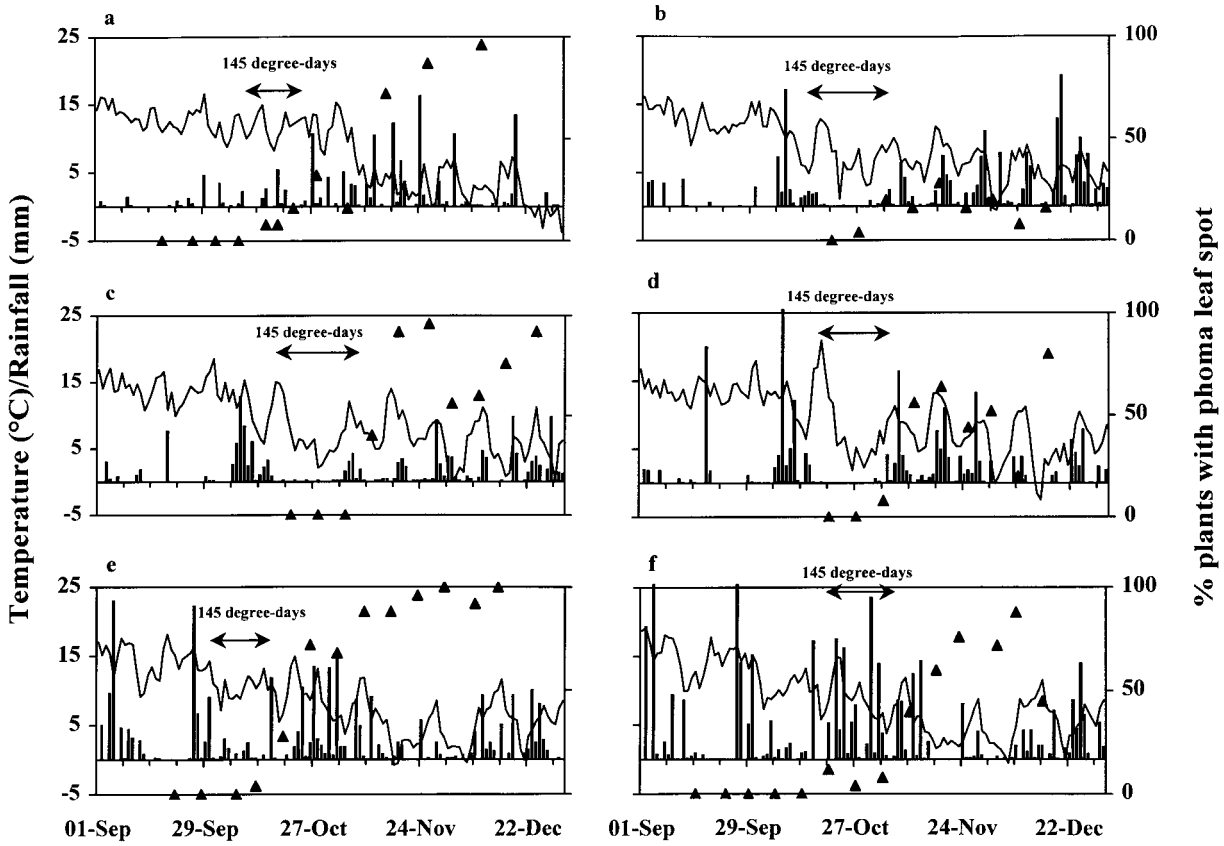

Figure 6. Development of phoma leaf spot lesions (▲) in winter oilseed rape crops in relation to daily mean temperature (°C, line) and rainfall (mm, bars) for the following sites and years: (a) ADAS Boxworth 1996; (b) ADAS High Mowthorpe 1997; (c) ADAS Boxworth 1997; (d) ADAS Rosemaund 1997; (e) ADAS Boxworth 1998; (f) Rothamsted 1998.

be considered as constant over the temperature range 8–20 °C, like the incubation period of *Pyrenopeziza brassicae* Sutton and Rawlinson on oilseed rape (Figueroa et al., 1995). The temperatures generally observed in October in the UK suggest that it is valid to use the degree-day approximation for estimating the length of the incubation period at that time; this would not necessarily be acceptable later in the autumn/winter (e.g. late December 1996, when the mean temperature was below 0 °C). Furthermore, the results of the controlled environment experiments suggest that if infection criteria are sub-optimal, the length of the incubation period may appear to be longer because the infection efficiency of the ascospores is decreased.

The similar slopes observed for regressions of % leaves with phoma leaf spot (square-root transformed) on % plants with phoma leaf spot for data from controlled environment and field experiments with different cultivars provide further evidence that results of the controlled environment experiments can be applied to disease development in winter oilseed rape crops. In the controlled environment cabinet experiments, it is difficult to use enough plants to accurately estimate effects of temperature and leaf wetness duration on the % plants with phoma leaf spot. However, in oilseed rape crops, it is considerably easier to assess the % of plants with phoma leaf spot than to assess the % leaves with phoma leaf spot or to count the number of lesions. Thus this relationship provides the link between the 'model system' and the winter oilseed rape crops. Nevertheless, the positions of the regression lines differed between controlled environment and field data. There were fewer leaves with phoma leaf spot on plants with lesions in the winter oilseed rape crops than in the controlled environment experiments, probably because the natural concentrations of air-borne

L. maculans ascospores are smaller than those concentrations applied as inoculum in the controlled environment experiments.

Results of these controlled environment and field experiments provide further support for the conclusion that epidemics of stem canker in the UK are initiated by air-borne ascospores of *L. maculans* (Gladders and Musa, 1980; Hammond and Lewis, 1986). It was possible to retrospectively explain the observed development of phoma leaf spots on plants in crops using the incubation period and infection criteria derived from the controlled environment experiments. However, these experiments also suggest that it will not be possible to use the occurrence of infection criteria alone in a weather-based system for predicting the occurrence of phoma leaf spots on winter oilseed rape to guide the timing of fungicide sprays in autumn (Gladders et al., 1998). The results suggest that infection criteria were fulfilled frequently during this period, which implies that epidemic development may have been limited instead by the availability of inoculum. Thus the relationship between August and September rainfall and the subsequent development of stem canker epidemics in eastern England (Gladders and Musa, 1980) may be mediated by effects of rainfall on the maturation and release of *L. maculans* ascospores from oilseed rape stem debris. These relationships require further investigation in order to explain the differences between sites and seasons in the development of stem canker epidemics and guide decisions about the application of fungicides to control the disease.

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