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Factors affecting the incubation period of dark leaf and pod spot (*Alternaria brassicae*) on oilseed rape (*Brassica napus*)

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Abstract

Experiments to investigate the factors affecting the incubation period of dark leaf and pod spot (*Alternaria brassicae*) on leaves and pods of oilseed rape (*Brassica napus*) were done in controlled environment (constant temperatures) and glasshouse conditions (fluctuating temperatures). The length of the incubation period of dark leaf and pod spot decreased as infection and incubation temperatures increased from 6 to 20 °C. The incubation period decreased as wetness period increased from 2 to 12 h, as inoculum concentration increased from 80 to 2×10^3 spores ml^{-1} and as leaf age increased from 4 to 10 days. Asymptotes of leaf age and inoculum concentration, above which the length of the incubation period did not decrease, were 10 days and 2×10^3 spores ml^{-1}, respectively. The shortest and longest incubation periods were 1 and 11 days. The mechanism by which the infection conditions influenced the incubation period of dark leaf and pod spot on oilseed rape seemed to be linked to lesion density. Usually, the length of the incubation period decreased greatly with increasing lesion density.

Introduction

Dark leaf and pod spot, caused by *Alternaria brassicae* (Berk.) Sacc., is a serious problem on oilseed rape in India (Saharan and Kadian, 1983), the UK (Hardwick et al., 1991), France (Marchegay et al., 1990) and Canada (Degenhardt et al., 1982). Many studies have been done on infection (Degenhardt et al., 1982; Humpherson-Jones and Hocart, 1983; Mridha and Wheeler, 1993), sporulation (Humpherson-Jones and Phelps, 1989) and spore dispersal (Sharma et al., 1983; Marchegay et al., 1990), but little is known about the incubation period of this disease. Degenhardt et al. (1982) demonstrated that *A. brassicae* can infect and cause necrosis on detached leaves of oilseed rape (*B. napus* L. subsp. *oleifera* (Metzger) Sink., cv. Zephyr) within 24 h in experiments at controlled temperatures between 11 and 29 °C. In India, the length of the incubation period of dark leaf spot was observed as 6 to 10 days on plants of eight turnip oilseed rape (*B. campestris* L.) or mustard (*B. juncea* L.) cultivars under field conditions (Saharan and Kadian, 1983).

Incubation period, defined as the time elapsed between inoculation and the appearance of the first disease symptoms (Van der Plank, 1963), is an important component of plant disease epidemics. The length of the incubation period has been widely used as a criterion for evaluation of disease resistance of hosts (Saharan and Kadian, 1983) and monitoring of fungicide resistance of pathogens (Gaunt et al., 1994). This paper describes experiments to investigate the effects of both infection and incubation conditions on the incubation period of dark leaf and pod spot on oilseed rape (*Brassica napus* L.).

Materials and methods

Five experiments were done in controlled environment or glasshouse conditions. The factors tested and the tissues and cultivars used in each experiment are listed in Table 1. For experiments on leaves, there were four pots per treatment with two plants (expts 1 and 2) or one plant (expt 3) per pot. In experiments 1 and 2 there

were two of these pots in each of two trays for each treatment, whereas in experiment 3 pots were arranged individually. For the incubation period measurements, replicates were the two trays in experiment 1, whereas only one measurement per treatment was made in experiment 2. For experiments on pods, there were three replicate flasks with four racemes per flask for each treatment (expts 4 and 5). Wetness was maintained only during the infection period and temperatures were the same for the infection (wetness period) and subsequent incubation periods, except in experiment 2. All pots or flasks were arranged in completely randomized designs in each experiment or each cabinet (temperature treatment).

Plant material

Winter oilseed rape seeds (*B. napus* cvs Envol or Bienvenu) were sown in 12.5 cm diameter pots containing a mixture of soil-less compost and a slow-release fertilizer (Croxden compost, Nursery Trades [Lee Valley] Ltd.) in experiments 1, 2 and 3. Plants were grown in a controlled environment cabinet at 18 °C with a daylength of 16 h. Light intensity at plant level was 200 μE m^{-2} s^{-1}. Four-week-old plants with 6–7 leaves each were used. The leaves of plants were marked for sampling before inoculation.

For experiments 4 and 5, fresh spring oilseed rape (cv. Starlight) racemes, at the beginning of seed development (growth stage 6.3), were collected from the Rothamsted farm on 27 July and 4 August 1994, respectively. The racemes were prepared by removing the underdeveloped pods and pods damaged by insects to leave 13–38 (mean 25.6) pods per raceme in experiment 4 and 16 pods per raceme in experiment 5. Four racemes were inserted into each flask filled with water.

Inoculation

Potted plants and detached racemes were inoculated by spraying with *A. brassicae* conidial suspensions. The inoculum concentration was 7×10^3 spores ml^{-1} in experiments 1 and 4, 6×10^4 spores ml^{-1} in experiment 2, and inoculum concentration was a treatment in experiments 3 and 5. After inoculation of plants, pots were immediately placed in plastic trays filled with water and covered with polyethylene bags in experiments 1 and 2. They were then moved to cabinets for wetness periods of 4, 6, 8, 12 or 24 h at temperatures of 6, 10, 15, 20 or 25 °C. After wetness periods, all the bags were removed and the plants were left to complete the incubation period at the same temperatures as for the wetness periods (expt 1) or all moved to a cabinet at a constant temperature of 20 °C for incubation (expt 2). In experiment 3, the plants were inoculated with suspensions of 80, 220 or 660 spores ml^{-1}, and pots were placed in a polyethylene tent in a glasshouse for wetness periods of 16, 20 or 24 h at c. 17 °C, then left for incubation, when temperatures fluctuated from 17 to 25 °C in the glasshouse.

In experiment 4, flasks of inoculated racemes were covered with polyethylene bags, which were then tied with tape. These racemes were kept in cabinets for wetness periods of 2, 4, 6, 8, 12 or 24 h at temperatures of 10, 15 or 20 °C. Bags were then removed and racemes were kept at the same temperatures as during wetness periods to complete the incubation period. In experiment 5, racemes were inoculated with suspensions of 80, 4×10^2, 2×10^3, 10^4 or 5×10^4 spores ml^{-1} and kept in a cabinet for a wetness period of 24 h at 18 °C. Polyethylene bags were then removed and racemes remained at 18 °C for incubation. Because racemes had been collected from oilseed rape crops exposed to natural inoculum, control treatments sprayed with sterile distilled water were included in experiments 4 and 5.

Disease assessments and analysis

Disease assessments were started 1 day (2 days in experiment 3) after inoculation. The number of lesions on each of the leaves in experiments 1 and 3 and the number of diseased pods on each of the racemes in experiments 4 and 5 were recorded daily until maximum numbers were reached. In experiment 2, the leaves were examined daily for the presence of dark leaf spot but numbers of lesions were not recorded. At the end of experiments on pods, the maximum number of lesions was recorded on each of 27 (expt 4) and 9 pods (expt 5) selected randomly from different replicates of each treatment. At the time of inoculation, the age of each of five leaves from the third to seventh from the base of the plant, measured from the day the leaf had first unfurled, was noted in experiment 3. Then the mean leaf age was calculated for each leaf position on the plants.

The incubation period was recorded as the time (days) from inoculation until the appearance of the first lesions on each leaf for dark leaf spot in experiments 1 and 3 or until the appearance of the first lesions on each raceme for dark pod spot in experiments 4 and 5. In experiment 2, the incubation period

Table 1. Treatment factors and levels, tissues and cultivars used in each of five experiments to investigate the effects of infection conditions on the incubation period (*ip*) of dark leaf and pod spot (*Alternaria brassicae*) on oilseed rape in controlled environment and glasshouse conditions

Expt	Infection condition treatments*	Incubation (°C)	Tissue	Cultivar	No. tmts	No. reps of *ip*
1	Temp : 6, 10, 15, 20, 25 WP : 4, 6, 8, 12, 24	As for infection	Leaves (1–6#)	Envol	22+	2
2	Temp : 6, 10, 15, 20, 25 WP : 4, 6, 8, 12, 24	20	Leaves (3)	Envol	25	1
3§	Leaf age : 3.9, 7.5, 10.3, 14.0, 16.9 WP : 16, 20, 24 Concn : 80, 220, 660	17–25	Leaves (3–7)	Bienvenu	45	4
4	Temp : 10, 15, 20 WP : 2, 4, 6, 8, 12, 24	As for infection	Pods	Starlight	18+3†	3
5	Concn : 80, 4×10^2, 2×10^3, 10^4, 5×10^4	18	Pods	Starlight	5+1¶	3

* Temp, temperature(°C); WP, wetness period (h); Concn, inoculum concentration (spores ml^{-1}); leaf age (days); all WP and Concn treatments were randomized but Temp and Leaf age could not be randomized.
# Leaf position from the base of plant.
\+ Combinations 6 °C × (4 or 6 h) and 25 °C × 24 h were not tested.
§ Done under glasshouse conditions (fluctuating temperatures).
† There was one control for each temperature.
¶ There was one control.

was measured as the time until the appearance of the first lesions only for each treatment. Before analysis, leaves which did not develop symptoms were excluded and mean incubation periods were calculated for each of two replicate trays (up to 24 leaves) in experiment 1 (Table 2). Incubation periods were recorded on single leaves for each leaf age/wetness period/inoculum concentration on four replicate leaves, none excluded, in experiment 3. Before analysis, racemes which did not develop lesions were excluded and mean incubation periods were calculated for each of three replicate flasks (up to four racemes) in experiments 4 and 5.

These data were analyzed by analysis of variance using Genstat (Payne et al., 1993). For the data sets where some leaves or pods did not become diseased, tray or flask means were calculated for the diseased leaves or pods and the number of observations per tray or flask was used as a weighting factor in the analysis. Since untransformed incubation period data showed increasing variance as incubation period increased, a $\log_{10}$ (data + 1) transformation was used to stabilise the variance. Analyses were done on both untransformed and $\log_{10}$-transformed incubation period data but only the analyses on $\log_{10}$-transformed data are presented.

At the end of experiments, leaf area was measured using a leaf area meter (Delta-T Devices Ltd., Burwell, Cambridge) in experiments 1 and 3 and the length and diameter of pods were measured using a micrometer in experiments 4 and 5. Then lesion density was calculated as the number of lesions cm^{-2} by dividing the maximum recorded number of lesions on a leaf or pod by the leaf area or pod surface area, respectively. Regression analyses of the incubation period (untransformed and $\log_{10}$-transformed) on the lesion density

Table 2. Statistical analyses used to test the effects of infection conditions on incubation period (days, $\log_{10}$-transformed)

Expt	Values for analysis	Type of analysis	
1	Mean per tray (≤24 leaves)	ANOVA*	Weighted means†
2	One per treatment	ANOVA	Interaction used as error
3	Single leaf value	ANOVA	Multi-factor
4	Mean per flask (≤4 racemes)	ANOVA	Multi-factor
5	Mean per flask (≤4 racemes)	ANOVA	Single factor

* Analysis of variance.
† Means weighted according to number of leaves diseased in each tray.

were done for these dark leaf spot and dark pod spot data. Analyses of position and parallelism were done to determine whether the relationships were fitted best by separate parallel or non-parallel lines for different treatments or by single lines for all treatments. Only the analyses on $\log_{10}$-transformed data are presented.

Results

Effects of temperature

Expt 1. On leaves of potted plants inoculated with an *A. brassicae* conidial suspension of 7×10^3 spores ml^{-1}, and exposed to the same temperatures throughout the infection and subsequent incubation periods, the length of the incubation period decreased significantly ($P < 0.001$) as temperature increased from 6 to 25 °C for all wetness periods (4, 6, 8, 12 or 24 h). However, the extent of the decrease in the incubation period decreased as temperature increased from 15 to 25 °C (Figure 1a, Table 3). In experiment 1, in which treatments with 4 or 6 h wetness at 6 °C and 24 h wetness at 25 °C were not included, the shortest and longest incubation periods were 1 day at 20 °C with 24 h of wetness and 11 days at 10 °C with 4 h of wetness, respectively.

Expt 2. No lesions were observed in treatments with 4 h wetness at 6 or 10 °C. The length of the incubation period of dark leaf spot on leaves of potted plants inoculated with an *A. brassicae* suspension of 6×10^4 spores ml^{-1} decreased significantly ($P < 0.001$) as infection temperature increased from 6 to 20 °C when the subsequent incubation temperature was 20 °C for all treatments (Figure 1b, Table 3). The lengths of the incubation period were shortest at an infection temperature of 20 °C; 4 days with 4 h of wetness and 1 day for treatments with wetness periods of ≥6 h. However, at 25 °C the incubation periods were longer than at 20 °C for wetness periods of 4 h (11 days) or 6 h (2 days), respectively.

Effects of wetness period

Expts 1 and 2. As wetness period increased, the length of the incubation period of dark leaf spot on leaves of potted plants decreased significantly ($P < 0.001$) at all temperatures. The minimum incubation period recorded was 1 day. This was recorded at only one temperature (20 °C) when the infection and subsequent incubation temperatures were the same in experiment

Table 3. Effects of temperature and wetness period on incubation period ($\log_{10}$-transformed) of dark leaf spot on oilseed rape inoculated with spore suspensions of *Alternaria brassicae*: (a) 7×10^3 spores ml^{-1}, plants incubated at infection temperature, experiment 1; (b) 6×10^4 spores ml^{-1}, plants transferred to 20 °C after infection period, experiment 2

Incubation period (days) ($\log_{10}$-transformed)					
(a) Temperature (°C)	6	10	15	20	25
Wetness period (h)					
4	*	1.10	1.02	0.81	0.79
6	*	0.94	0.78	0.83	0.75
8	1.01	0.99	0.69	0.72	0.68
12	0.92	0.88	0.65	0.60	0.52
24	0.96	0.67	0.50	0.34	*
S.E.D. (21 D.F.)			0.063		
(b) Temperature (°C)	6	10	15	20	25
	1.89	1.68	0.89	0.17	0.51
S.E.D. (14 D.F.)			0.243		
Wetness period (h)	4	6	8	12	24
	2.30	1.12	0.88	0.75	0.29
S.E.D. (14 D.F.)			0.287		

* not tested.

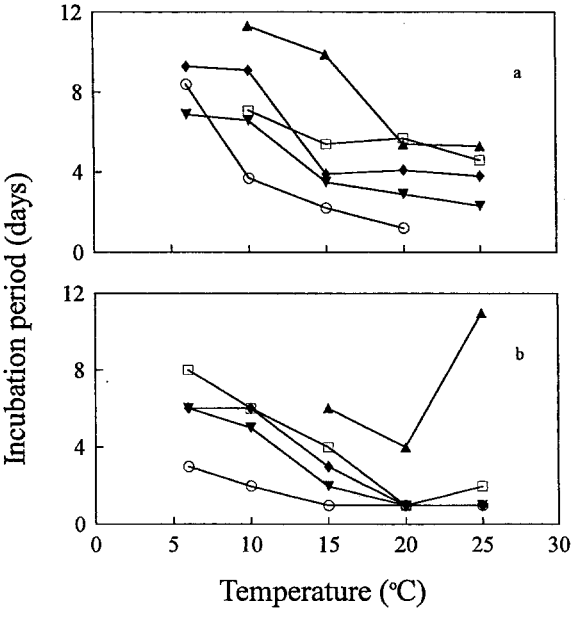

Figure 1. Effects of infection temperature on the incubation period of dark leaf spot on oilseed rape plants (cv. Envol) inoculated with spore suspensions of *Alternaria brassicae* and exposed to wetness periods of 4 (▲), 6 (□), 8 (♦), 12 (▼) or 24 h (○): (a) 7×10^3 spores ml^{-1}, plants incubated at infection temperature, experiment 1; (b) 6×10^4 spores ml^{-1}, plants transferred to 20 °C after infection period (no lesions observed with 4 h wetness at 6 or 10 °C, experiment 2).

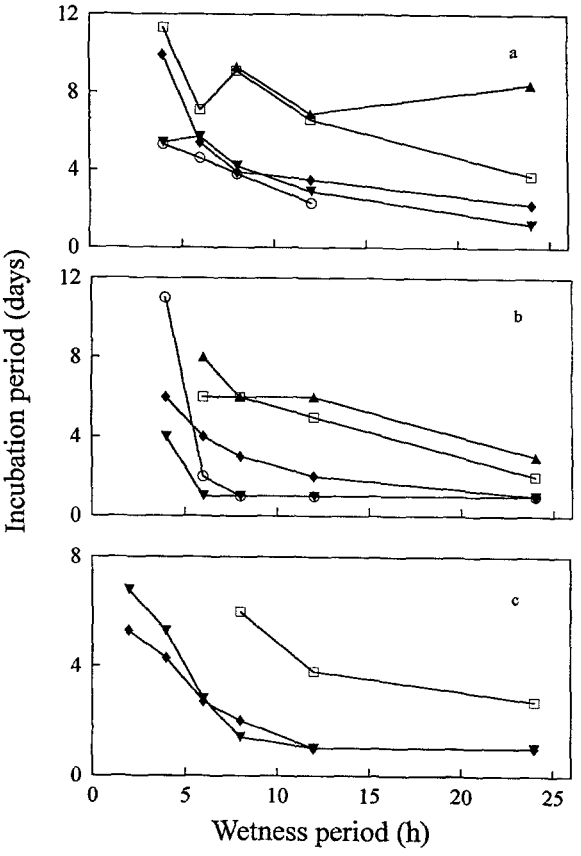

Figure 2. Effects of wetness period on the incubation period of dark leaf and pod spot (*Alternaria brassicae*) on oilseed rape: (a) plants (cv. Envol) inoculated with 7×10^3 spores ml^{-1} and exposed to temperatures of 6 (▲), 10 (□), 15 (♦), 20 (▼) or 25 °C (○), experiment 1; (b) plants (cv. Envol) inoculated with a suspension of 6×10^4 spores ml^{-1} and exposed to infection temperatures of 6 (▲), 10 (□), 15 (♦), 20 (▼) or 25 °C (○), then incubated at 20 °C (no lesions observed with 4 h wetness at 6 or 10 °C), experiment 2; (c) racemes (cv. Starlight) inoculated with 7×10^3 spores ml^{-1} and exposed to temperatures of 10 (□), 15 (♦) or 20 °C (▼) (no lesions observed with ≤6 h wetness at 10 °C, experiment 4).

1 (Figure 2a, Table 3) but at infection temperatures of 15, 20 or 25 °C when all inoculated plants were incubated at 20 °C after wetness periods in experiment 2 (Figure 2b, Table 3).

Expt 3. At fluctuating temperatures of 17–25 °C in the glasshouse, the time required for development of dark leaf spot on leaves of plants also decreased significantly ($P < 0.001$) as wetness period increased from 16 to 24 h, regardless of either inoculum concentration or leaf age (Figure 3, Table 4).

Expt 4. No dark pod spot lesions were observed on pods on detached racemes sprayed with sterile distilled water with a 24 h wetness period at 10, 15 or 20 °C or on pods inoculated with a suspension of 7×10^3 spores ml^{-1} with a wetness period ≤6 h at 10 °C. The length of the incubation period of dark pod spot decreased significantly ($P < 0.001$) as the wetness period increased from 2 to 12 h for temperatures of 10, 15 or 20 °C throughout the infection and subsequent incubation periods (Figure 2c, Table 5). The incubation period of dark pod spot on pods on detached racemes was only 1 day at 20 or 15 °C with 12 or 24 h wetness periods and 7 days at 20 °C with a 2 h wetness period.

Table 4. Effects of leaf age, wetness period and inoculum concentration on incubation period (log_{10}-transformed) of dark leaf spot on oilseed rape inoculated with spores suspensions of *Alternaria brassicae* (experiment 3)

Incubation period (days) (log_{10}-transformed)						
Inoculum concentration (spores/ml)	Wetness period (h)	Leaf age (days) 3.9	7.5	10.3	14.0	16.9
80	16	1.55	1.69	1.50	1.65	1.50
	20	1.23	1.29	1.70	1.46	1.41
	24	1.00	0.92	1.09	1.34	1.56
220	16	1.09	1.30	1.24	1.57	1.79
	20	1.09	1.00	1.00	1.21	1.50
	24	0.92	0.92	0.92	1.16	1.55
660	16	1.00	0.92	1.00	1.38	1.66
	20	1.00	0.92	0.92	1.11	1.44
	24	0.92	0.92	0.92	0.92	0.92
S.E.D. (98 D.F.)			0.166			

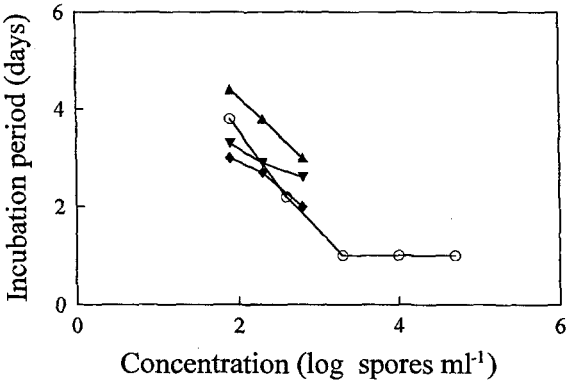

Figure 3. Effect of inoculum concentration (log_{10}-transformed) on the incubation period of dark leaf and pod spot (*Alternaria brassicae*) on oilseed rape leaves (cv. Bienvenu) with wetness periods of 16 (▲), 20 (▼) or 24 h (♦) at fluctuating temperatures (17–25 °C) (experiment 3) or on pods (cv. Starlight) with a wetness period of 24 h at 18 °C (○) (experiment 5).

Table 5. Effects of temperature, wetness period (a, experiment 4) and inoculum concentration (b, experiment 5) on incubation period (log_{10}-transformed) of dark pod spot on oilseed rape inoculated with spore suspensions of *Alternaria brassicae*

Incubation period (days) (log_{10}-transformed)					
(a) Temperature (°C)		10		15	20
Wetness period (h)					
2		*		0.55	0.60
4		*		0.57	0.54
6		*		0.54	0.50
8		0.48		0.51	0.48
12		0.52		0.43	0.37
24		0.53		0.37	0.32
S.E.D. (27 D.F.)			0.016		
(b) Concentration (spores/ml)	80	4×10^2	2×10^3	10^4	5×10^4
	0.66	0.50	0.30	0.30	0.30
S.E.D. (10 D.F.)		0.056			

* no lesions were observed.

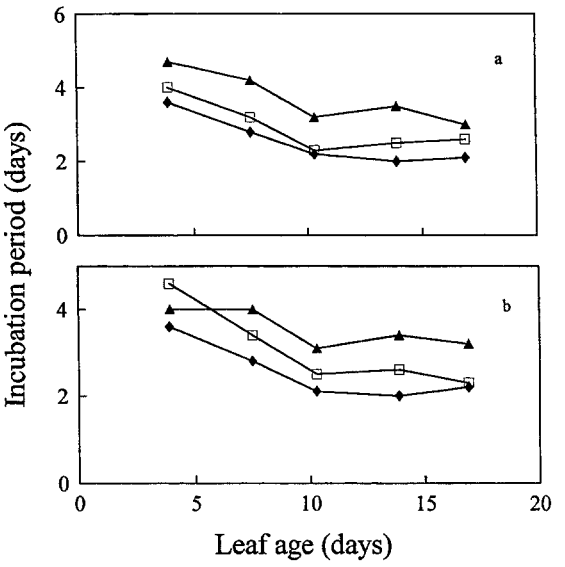

Figure 4. Incubation periods of dark leaf spot (*Alternaria brassicae*) on leaves of different ages on oilseed rape plants (cv. Bienvenu) (a) exposed to wetness periods of 16 (▲), 20 (□) or 24 h (♦) or (b) inoculated with *A.brassicae* suspensions of 80 (▲), 220 (□) or 660 spores ml^{-1} (♦), experiment 3.

Effects of inoculum concentration

Expt 3. The length of the incubation period of dark leaf spot on leaves of potted plants decreased significantly ($P < 0.001$) as inoculum concentration increased from 80 to 660 spores ml^{-1} for wetness periods of 16, 20 or 24 h at fluctuating temperatures of 17–25 °C (Figure 3, Table 4) in the glasshouse. Under these conditions the incubation period of dark leaf spot was 2 and 4 days on the potted plants inoculated with suspensions of 660 spores ml^{-1} with 24 h of wetness and 80 spores ml^{-1} with 16 h of wetness, respectively.

Expt 5. The incubation period of dark pod spot on detached racemes decreased ($P < 0.001$) as inoculum concentration increased from 80 to 2×10^3 spores ml^{-1} at 18 °C with 24 h of wetness (Figure 3, Table 5). Increasing inoculum concentration above 2×10^3 spores ml^{-1} did not further decrease the incubation period. The incubation period of dark pod spot was 1 and 4 days on pods of racemes inoculated with suspensions of $\geq$2000 and 80 spores ml^{-1}, respectively. No lesions were observed on control racemes.

Effects of leaf age

Expt 3. The length of incubation period of dark leaf spot on leaves of potted plants decreased significantly ($P < 0.001$) as mean leaf age increased from 3.9 to 10.3 days, regardless of wetness period (16, 20 or 24 h) (Figure 4a, Table 4) or inoculum concentration (80, 220 or 660 spores ml^{-1}) (Figure 4b, Table 4). However, no significant differences in the incubation period were observed between leaves which were more than 10 days old.

Interactions between infection conditions

The interactions between effects of temperature and wetness period on the incubation period were significant ($P = 0.007$) when the infection and subsequent incubation temperatures were the same in experiment 1 (Table 3). The effects of unfavourable temperatures were, to some extent, overcome by more favourable (longer) wetness periods (Figure 1a) and more favourable temperature also compensated for shorter wetness periods (Figure 2a). Similar interactions between effects of infection temperature and wetness period on the incubation period were observed for dark pod spot (Figure 2c, Table 5).

No interactions were observed between effects of inoculum concentration and wetness period, between effects of inoculum concentration and leaf age or between effects of wetness period and leaf age on the incubation period of dark leaf spot. However, interactions between effects of inoculum concentration, wetness period and leaf age on the incubation period of dark leaf spot were significant ($P < 0.001$, Table 4).

The incubation period in relation to lesion density

For all experiments in which the numbers of lesions were recorded, the time needed for the development of dark leaf spot symptoms on leaves of potted plants (Figure 5) or dark pod spot symptoms on pods of detached racemes (Figure 6) decreased with increasing lesion density. The extent by which the incubation period decreased with increasing lesion density increased with increasing temperature during the infection and subsequent incubation periods in experiments 1 (Figure 5c) and 4 (Figure 6a), and with increasing wetness period in experiments 3 (Figure 5a) and 4 (Figure 6a). The regression analyses of position and parallelism (Table 6) suggested that the relationships between incubation period and lesion density were fitted best by three parallel lines for three wetness periods in experiment 3 (Figure 5a), and by five parallel lines for five temperatures in experiment 1 (Figure 5c). The analyses of parallelism also indicated that inoculum concentration did not greatly affect the relationship between incubation period and lesion density and data were fitted best by single lines in experiments 3 (Figure 5b) and 5 (Figure 6b). In experiment 4 both temperature and wetness period significantly affected the relationship between incubation period and lesion density (Figure 6a).

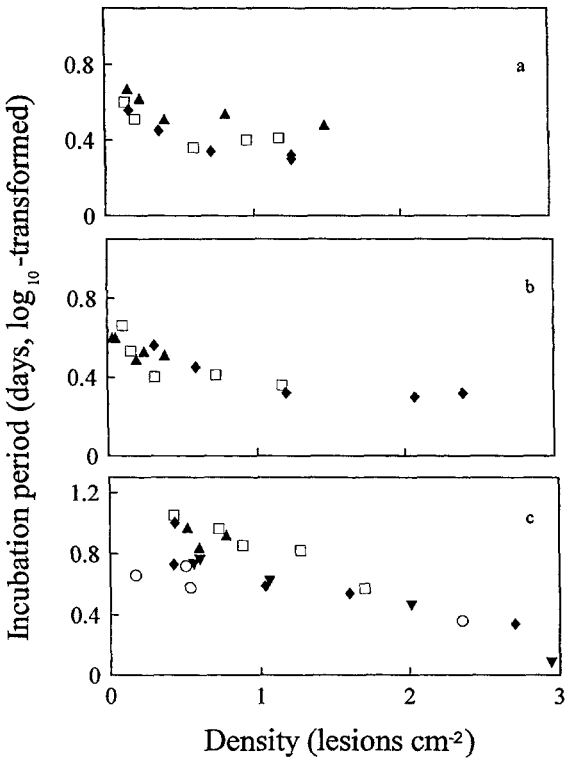

Figure 5. Incubation period of dark leaf spot (*Alternaria brassicae*) (log_{10}-transformed) in relation to the density of lesions: (a) on leaves of different ages inoculated with suspensions and exposed to wetness periods of 16 (▲), 20 (□) or 24 h (♦) at c. 17 °C for infection and then incubated at fluctuating temperatures (17–25 °C); (b) on leaves of different ages inoculated with suspensions of 80 (▲), 220 (□) or 660 spores ml^{-1} (♦) and exposed to wetness periods of ≥16 h at c. 17 °C for infection and then incubated at fluctuating temperatures (17–25 °C); (c) on leaves of potted oilseed rape plants inoculated with a suspension of 7×10^3 spores ml^{-1} and then exposed to wetness periods of 4, 6, 8, 12 or 24 h at temperatures of 6 (▲), 10 (□), 15 (♦), 20 (▼) or 25 °C (○).

Discussion

These results suggest that the length of the incubation period of dark leaf and pod spot on oilseed rape, ranging from 1 to 11 days, is greatly influenced by infection temperature, wetness period, inoculum concentration and leaf age and by subsequent incubation temperature. It was demonstrated that the incubation period of dark leaf spot on leaves of potted plants decreased as both infection and incubation temperatures increased from 6 to 20 °C (Figures 1a & b). However, it is interesting that increasing the temperature from 20 to 25 °C only during the infection period increased the incubation period if wetness period was ≤6 h (Figure 1b), whereas when the incubation temperature was also increased from 20 to 25 °C the incubation period did not increase (Figure 1a). It appeared that a higher incubation temperature (25 °C) compensated for sub-optimal infection conditions (a wetness period of only 4 h) to allow development of dark leaf spot (Rotem, 1978). These results suggest that wetness periods of ≤12 h at 25 °C would fulfil infection criteria and favour development of dark leaf and pod spot on oilseed rape in the UK. It is surprising that the optimal temperature for the incubation of dark leaf and pod spot on oilseed rape may be higher than that for infection.

These results suggest that the length of the incubation period of dark leaf and pod spot decreased as wetness period increased from 2 to 24 h, regardless of infection and incubation temperatures (Figures 2a, b &c). However, the extent by which the incubation period decreased with increasing wetness period decreased with increasing temperature and with increasing wetness period, so that there was often little further change above 20 °C. Nevertheless, it is clear that the incubation period decreases with increasing infection temperature from 6 to 20 °C and with increasing wetness period. The results suggest that more favourable infection temperatures can compensate for

Table 6. Effects of infection conditions on the relationship between incubation period (days, $\log_{10}$-transformed) and lesion density# (lesions cm^{-2} leaf or pod surface area); models which fitted data best, as indicated by regression analyses of position and parallelism

Exp	Best fitting model*	% variance accounted for		*P*	Figure
1	*ip = constant + x + temp*	86.2	P_x	< 0.001	5c
			P_{temp}	< 0.001	
3	*ip = constant + x + wp*	73.9	P_x	< 0.001	5a
			P_{wp}	< 0.001	
	ip = constant + x	67.9	P_x	< 0.001	5b
4	*ip = constant + x + temp + wp*	94.4	P_x	< 0.001	6a
			P_{temp}	= 0.023	
			P_{wp}	= 0.013	
5	*ip = constant + x*	55.5	P_x	= 0.092	6b

data was insufficient to identify interactions between infection conditions (where applicable).
* *ip*, incubation period, *x*, lesion density (lesions cm^{-2}); *temp*, temperature (°C); *wp*, wetness period (h); *concn*, inoculum concentration (spores ml^{-1}).

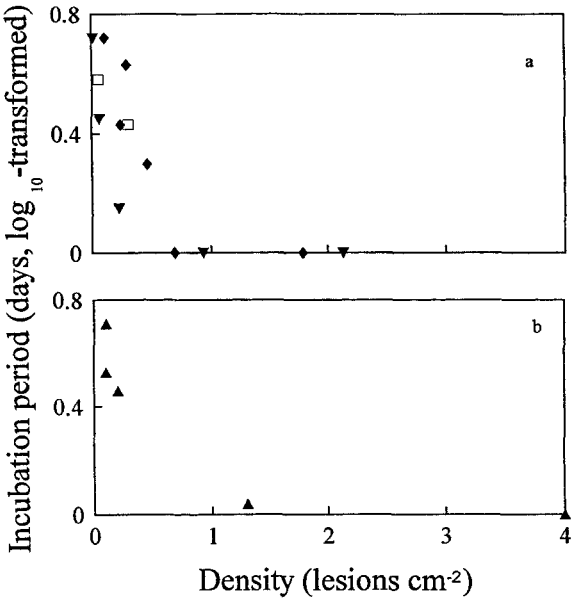

Figure 6. Incubation period of dark pod spot (*Alternaria brassicae*) ($\log_{10}$-transformed) in relation to the density of lesions on pods of detached oilseed rape racemes: (a) inoculated with a suspension of 7×10^3 spores ml^{-1} and exposed to wetness periods of 2, 4, 6, 8, 12 or 24 h at temperatures of 10 (□), 15 (♦) or 20 °C (▼); (b) inoculated with suspensions with 80, 4×10^2, 2×10^3, 10^4 or 5×10^4 spores ml^{-1} and exposed to a wetness period of 24 h at 18 °C.

less favourable wetness periods and more favourable wetness periods can compensate for less favourable infection temperatures (Rotem, 1978). The results also suggest that the time required for the development of dark leaf and pod spot symptoms is inversely related to inoculum concentration on both leaves of potted plants at fluctuating temperatures (17–25 °C), regardless of wetness period and leaf age (Figure 3), and on pods of detached racemes at a constant temperature of 18 °C. However, the first assessments of disease development in most of experiments were done 1 day after inoculation, by which time the incubation period was completed at temperatures of 20 and 25 °C and for inoculum concentrations of 2×10^3, 10^4 and 5×10^4 spores ml^{-1}. These results suggest that the length of the incubation period of dark leaf spot on leaves of potted plants shortens as leaf age increases from 4 to 10 days. However, there were no differences between leaves more than 10 days old (Figures 4a & b).

Incubation period is usually considered to be a function of temperature (Subba Rao et al., 1990) and little work has been done to investigate the effects of the infection conditions on the incubation period of a disease. However, these results suggest that the infection conditions greatly affect the length of the incubation period. Similar results were obtained for the effects of infection temperature and wetness period on soya bean brown spot (Peterson and Edwards, 1982), the effects of leaf age on potato early blight (Johnson and Teng, 1990) and the effects of inoculum concentration on banana black sigatoka disease (Jacome and Schuh, 1992). It is possible that the mechanism by which infection conditions affect the length of the incubation period of dark leaf and pod spot on oilseed rape

is related to their effects on the density of the lesions which develop, through effects of infection conditions on germination of and infection by *A. brassicae* spores.

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