European Journal of Plant Pathology **105:** 585–595, 1999.

Infection of linseed by *Alternaria linicola*; effects of inoculum density, temperature, leaf wetness and light regime

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Accepted 12 June 1999

Key words: Alternaria linicola, continuous leaf wetness period, infection, interrupted leaf wetness period, *Linum usitatissimum*, symptom development

Abstract

Controlled environment studies were conducted to determine the effects of inoculum density, temperature, leaf wetness and light regime on the infection of linseed by *Alternaria linicola*. The % cotyledons and leaves with symptoms, and the disease severity (% leaf area with symptoms) increased linearly when the inoculum density increased from 1×10^3 to 1×10^5 conidia ml^{-1}. The first symptoms appeared on cotyledons and leaves 4 and 6 days after inoculation, respectively. Eight hours of leaf wetness were sufficient to initiate the disease at 25 °C but not at 15 °C, when 10-h periods of leaf wetness were required. % leaf area with symptoms was lower at 15 °C than that at 25 °C irrespective of the leaf wetness periods tested. Interruption of a continuous leaf wetness period by a 12-h dry period, occurring at any time between 1 and 18 h after inoculation, decreased the % cotyledons with symptoms and the disease severity, with the greatest reductions (60% and 100%, respectively) being observed when the dry period began 6 h after inoculation. *A. linicola* conidia were able to exploit successive 12-h periods of leaf wetness cumulatively to infect linseed plants. Disease incidence and severity were positively correlated with the dark period following inoculation, but they were negatively related to the length of the initial light period. Our findings suggest that infection of linseed by *A. linicola* and further development of symptoms can occur under unfavourable environmental conditions.

Introduction

Alternaria linicola Groves & Skolko is a commonly occurring seed-borne pathogen of linseed (*Linum usitatissimum* L.) in north-western Europe. The pathogen causes pre- and post-emergence death of seedlings, decreasing establishment by up to 50% and seed yield by up to 35%. It can also affect oil quantity and quality (Mercer et al., 1991). Field observations have suggested that prolonged wet periods and relatively high temperatures between flowering and harvest favour the development of the disease (Fitt et al., 1991; Mercer et al., 1991). However, there are no published detailed studies on the effects of environmental variables, such as temperature, leaf wetness (continuous or interrupted) and light on infection by *A. linicola*, and symptom development on linseed plants. Moreover, symptom development and the severity of disease may be affected, as for other host–pathogen systems, by the amount of inoculum present on plant tissues (Eisensmith et al., 1982; Jeger et al., 1985; Makowski, 1993). Determination of the optimal environmental conditions for infection of linseed plants by *A. linicola* should help in understanding the development of epidemics in linseed crops, and in improving strategies for management of the disease. This paper describes

experiments in controlled environments to investigate the effects of inoculum density, temperature, leaf wetness (continuous or interrupted) and light regime on infection of linseed by *A. linicola*.

Materials and methods

Plant production

All experiments were done on linseed plants (cv. Antares) grown from seed treated with prochloraz (4 g a.i. kg^{-1} seed, Prelude 20LF, Agrichem) with no detectable *A. linicola* infection. Plants were grown in plastic pots (13 cm in diameter) containing a mixture of soil-less compost with a slow release fertilizer [Croxden compost produced by Nursery Trades (Lea Valley) Ltd.], placed in controlled environment cabinets set at 18 °C/13 °C day/night temperatures (unless otherwise stated). The daylength was 16 h (from 24:00 h to 16:00 h) and light (350–700 nm with a peak at 580 nm) was provided by 18 fluorescent lamps 70 cm above the plants. The light intensity at plant level was 120–160 µEinsteins m^{-2} s^{-1} and the relative humidity 60–75%.

Preparation of inoculum

All single-spore isolates of *A. linicola* used were from linseed plants (cv. Antares) naturally infected by *A. linicola* during the period 1989–1991. Stock cultures were maintained in McCartney bottles containing a sterile mixture of loam : compost : sand (1 : 2 : 1) at 4 °C. For the production of inoculum, the method of Shahin and Shepard (1979), slightly modified by Vloutoglou (1994), was used. Cultures of the isolates were grown initially in 9-cm diameter Petri plates containing 20 ml of V-8 agar medium. After 4 days in darkness at 20 °C, before the appearance of the aerial mycelium, the agar containing the developing colony was cut with a sterile scapel into small blocks (3 mm^2) under sterile conditions. The blocks were transferred to the surface of the Shahin and Shepard's sporulation medium (S-medium), and 2 ml of sterile distilled water were added to each plate to partially cover the blocks. The plates were sealed with Parafilm and incubated under diurnal NUV-light (12 h NUV-light/12 h darkness) at 20 °C. After 3 days, 10 ml of sterile distilled water (containing 0.01 ml of 0.01% Tween 80) were added and conidia were dislodged by gentle rubbing of the agar surface with a sterile bent glass rod. The conidial suspension was filtered through two layers of sterile muslin. The concentration of conidia of each individual isolate was adjusted to 3×10^4 conidia ml^{-1} (unless otherwise stated). The final inoculum was prepared by mixing together 300 ml of conidial suspension of each isolate. The viability of conidia (% conidia germinating) used for artificial inoculations ranged from 97% to 100%.

Plant inoculation

Unless otherwise stated, linseed plants at growth stage (GS) 5–6 (16–18 true leaves) (Turner, 1987) were artificially inoculated (sprayed until run-off) with the mixed conidial suspension of *A. linicola* isolates. Approximately 20 ml of the conidial suspension were sprayed onto the plants in each pot (10 plants per pot). The inoculation procedure lasted 30 min and at the end of this period no conidial germination was observed. Plants were inoculated at the beginning of a dark period (approximately 16:00 h). All experiments were arranged in randomized block designs with five blocks, with one pot for each block and treatment (total of five replicates per treatment).

Effects of inoculum density on infection and symptom development

Linseed plants were inoculated with a mixture of three single-spore isolates of *A. linicola* (Al 1, Al 5, Al 15). Conidial suspensions contained 1×10^3, 1×10^4, 5×10^4 and 1×10^5 conidia ml^{-1}. Inoculated plants were covered for 72 h with polyethylene bags sprayed inside with water (100% r.h.) to provide a water-saturated atmosphere favourable for infection.

Disease assessments. The % cotyledons with symptoms was assessed 4, 7, 9, 13 and 18 days after inoculation. All the cotyledons were included in the assessments (20 cotyledons per replicate, 100 cotyledons per treatment). The % leaves with symptoms was assessed 7, 9, 13 and 18 days after inoculation. All leaves present on each plant at the time of inoculation (average of 17 leaves) were included in the assessments (170 leaves per replicate, 850 leaves per treatment). The % stems or hypocotyls with lesions or cankers was assessed 18 days after inoculation (total of 10 stems or hypocotyls per replicate, 50 stems or hypocotyls per treatment). Disease severity (% area with symptoms) was assessed, on the cotyledons only, 4, 7, 9, 13

and 18 days after inoculation using an arbitrary 0–6 scale: 0 = no symptoms, 1 = 1–10%, 2 = 11–20%, 3 = 21–40%, 4 = 41–60%, 5 = 61–80% and 6 = 81–100%. A disease index (DI) was calculated as:

$$\mathrm{DI} = [(0 \times A) + (1 \times B) + (2 \times C) + (3 \times D) + (4 \times E) + (5 \times F) + (6 \times G)]/100 \quad (1)$$

in which A, B, C, D, E, F and G are the mean % area of the cotyledons with symptoms in each of the scale categories (0, 1, 2, 3, 4, 5 and 6, respectively). In all cases linear regressions were used to describe the relationships between inoculum concentration and disease variables.

Effects of temperature and leaf wetness duration on infection and symptom development

Linseed plants were grown at 18 °C/13 °C day/night temperatures. Twenty-four hours before inoculation the two cabinets were set at 15 °C and 25 °C, respectively. Plants were inoculated with a conidial suspension containing 3×10^4 conidia $ml^{-1}$of a mixture of four single-spore isolates of *A. linicola* (Al 10, Al 15, Al 23, Al 24). Only the third, fourth, fifth and sixth leaf of each plant, counting from the base of the stem, were inoculated. The inoculated plants were exposed to periods of 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48 and 72 h leaf wetness immediately after inoculation. During the wet periods, the plants were covered with polyethylene bags sprayed inside with water and kept in darkness. The temperature inside the bags deviated from the temperature set (15 °C or 25 °C) by less than ±2 °C. At the end of each leaf wetness period, the plants were uncovered, dried immediately by blowing air (at ambient temperature) gently over them with a hair-dryer and transferred to a controlled environment room set at 18 °C/13 °C day/night temperatures with a 16-h photoperiod (light intensity at plant level 120–160 µEinsteins m^{-2} s^{-1}) until symptoms developed. It took approximately 5 min for the plants in five pots (one replicate) to dry, and therefore this time was not included in the recorded wetness period. There were three different controls: (a) plants which were inoculated and covered for 72 h with polyethylene bags sprayed inside with water (72 h leaf wetness), (b) plants which were inoculated, dried immediately after inoculation and left uncovered for 72 h (0 h leaf wetness), and (c) plants which were sprayed with sterile distilled water containing 0.01% Tween 80 and covered for 72 h with polyethylene bags sprayed inside with water (uninoculated plants).

Disease assessments. The % plants with symptoms and % leaves with symptoms were assessed 4 days after inoculation. The % leaves with symptoms was based on the number of leaves with symptoms out of the four leaves per plant which were inoculated (total of 40 leaves per pot or replicate, 200 leaves per treatment).

Effects of interrupted leaf wetness period on infection and symptom development

Pre- and post-inoculation treatments. Linseed plants (cv. Antares) grown at 15 °C were inoculated at GS 4 (6–8 true leaves) with a conidial suspension of four single-spore isolates of *A. linicola* (Al 10, Al 24, Al 36, Al 38). During the wet periods, the plants were covered with polyethylene bags sprayed inside with water. For the dry periods, the plants were uncovered and dried immediately with a hair-dryer. For producing a wet period after a dry period, the plants were rewetted by spraying them with a fine spray of water droplets and covered with polyethylene bags sprayed inside with water. The temperature inside the bags deviated from the temperature set by less than ±2 °C. All plants were kept in dark after inoculation until symptoms developed.

Interruption by one dry period. After inoculation the plants were given an initial period of leaf wetness of 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18 or 24 h, followed by a 12-h dry period and a final period of leaf wetness of sufficient length to complete a leaf wetness period of 36 h in total (Table 1). There were three different controls: (a) plants which were inoculated and covered for 36 h with polyethylene bags sprayed inside with water (36 h leaf wetness), (b) plants which were inoculated, dried immediately after inoculation and left uncovered for 36 h (0 h leaf wetness), and (c) plants which were sprayed with sterile distilled water containing 0.01% Tween 80 and covered for 36 h with polyethylene bags sprayed inside with water (uninoculated plants). At the end of the 36-h incubation time, the plants were uncovered and incubated in dark until symptoms developed.

Interruption by several dry periods. Plants were exposed to 12-, 24-, 36- or 48-h periods of continuous leaf wetness immediately after inoculation or to 24-, 36- or 48-h periods of leaf wetness interrupted 12 h after

Table 1. Treatments used to study the effect of interrupting a continuous leaf wetness period by a 12-h dry period at various times after inoculation on the development of symptoms by *A. linicola* on linseed plants (cv. Antares)

Treatment	Initial wet period (h)	Dry period (h)	Final wet period (h)	Total period of leaf wetness (h)
1	1	12	35	36
2	2	12	34	36
3	3	12	33	36
4	4	12	32	36
5	5	12	31	36
6	6	12	30	36
7	7	12	29	36
8	8	12	28	36
9	10	12	26	36
10	12	12	24	36
11	18	12	18	36
12	24	12	12	36

Control 1: Inoculated, dried, left uncovered for 36 h.
Control 2: Inoculated, dried, covered with polyethylene bags for 36 h.

inoculation by one, two or three dry periods (12 h each), respectively (Table 2). There were three different controls: (a) plants which were inoculated and covered for 48 h with polyethylene bags sprayed inside with water (48 h leaf wetness), (b) plants which were inoculated, dried immediately after inoculation and left uncovered for 48 h (0 h leaf wetness), and (c) plants which were sprayed with sterile distilled water containing 0.01% Tween 80 and covered for 48 h with polyethylene bags sprayed inside with water (uninoculated plants). At the end of the 48-h incubation time all the plants were uncovered and incubated in the cabinets in dark until symptoms developed.

Disease assessments. The % cotyledons with symptoms and % leaf area with symptoms were assessed 6 days after inoculation. For the disease severity (% leaf area with symptoms) on the cotyledons, the arbitrary 0–6 scale was used and the DI in Equation (1) was calculated.

Effects of light regime on infection and symptom development

Linseed plants (cv. Antares) at GS 5–6 (16–18 true leaves) were inoculated with a conidial suspension of four single-spore isolates of *A. linicola* (Al 10, Al 15, Al 23, Al 24). At the end of the inoculation procedure,

Table 2. Treatments used to study the effects of interrupted or continuous leaf wetness period on the development of symptoms by *A. linicola* on linseed plants (cv. Antares)

Treatments	Total period of leaf wetness (h)
A. Interrupted leaf wetness	
12 W–12 D–12 W[a]	24
12 W–12 D–12 W–12 D–12 W	36
12 W–12 D–12 W–12 D–12 W–12 D–12 W	48
B. Continuous leaf wetness	
12 W	12
24 W	24
36 W	36
48 W	48
C. Controls	
0 W (Control 1)	0
48 W (Control 2)	48
Uninoculated (Control 3)	48

[a]Hours of the wet (W) or dry (D) period.

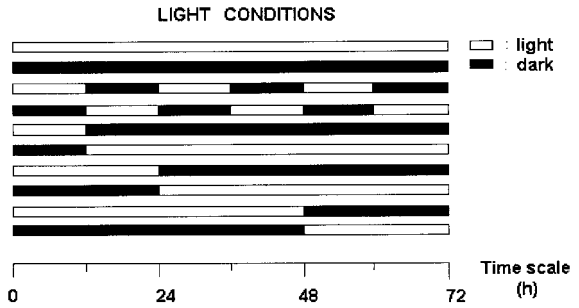

Figure 1. Treatments used to study the effects of light regime on the development of symptoms on linseed plants (cv. Antares) inoculated with *A. linicola* conidia.

the cabinet was set to give a continuous light regime. Immediately after inoculation, the plants were covered for 72 h with polyethylene bags sprayed inside with water and were exposed to the light regimes shown in Figure 1. During the dark periods, the plants, which were already covered with clear polyethylene bags, were wrapped with aluminium foil to exclude light. Controls were plants which were inoculated, covered immediately after inoculation with polyethylene bags sprayed inside with water and either wrapped with aluminium foil (72 h in dark) or exposed to light for 72 h. The temperature inside the bags and the aluminium foil deviated from the temperature set (15 °C) by less than ±2 °C.

Disease assessments. The % cotyledons with symptoms and % stems with symptoms were assessed 3 days after inoculation. The disease severity on leaves (% leaf area with symptoms) was also assessed 3 days after inoculation by using an arbitrary 0–5 scale: 0 = no symptoms, 0.1 = <1%, 1 = 1–5%, 2 = 6–10%, 3 = 11–30%, 4 = 31–50% and 5 = 51–70%. A DI was calculated as:

$$\mathrm{DI} = [(0 \times A) + (0.1 \times B) + (1 \times C) + (2 \times D) + (3 \times E) + (4 \times F) + (5 \times G)]/100 \quad (2)$$

in which A, B, C, D, E, F and G are the mean % leaf area with symptoms in each of the scale categories (0, 0.1, 1, 2, 3, 4 and 5, respectively).

For analysing the data on the incidence of the disease on stems under light or dark periods of different durations, the linear regressions used were

$$y = a - bx \quad \text{(for the light period)}, \quad (3)$$

$$y = c + dz \quad \text{(for the dark period)}, \quad (4)$$

in which y is the % stems with symptoms, x, z are the durations of the light and dark periods, respectively, a, c are the intercepts on the y-axis in Equations (3) and (4), respectively, and b, d are the slopes of the lines for Equations (3) and (4), respectively. For analysing the data on the severity of the disease on leaves under light or dark periods of different durations, the linear regressions used were

$$w = f - gx \quad \text{(for the light period)}, \quad (5)$$

$$w = k + mz \quad \text{(for the dark period)} \quad (6)$$

in which w is the disease severity on leaves (% leaf area with symptoms), x, z are the durations of the light or dark periods, respectively, f, k are the intercepts on the y-axis in Equations (5) and (6), respectively and g, m are the slopes of the lines for Equations (5) and (6), respectively.

Results

Effects of inoculum density on infection and symptom development

The first symptoms were observed on the cotyledons 4 days after inoculation at all the inoculum densities tested (Figure 2a). The % cotyledons with symptoms increased linearly ($R^2 \geq 0.78$) with increasing inoculum density and increasing time. However, the rate of increase in the incidence of the disease on cotyledons was slower at the lower inoculum density (1×10^3 conidia ml^{-1}) than that at the higher inoculum densities (1×10^4, 5×10^4 or 1×10^5 conidia ml^{-1}). Four days after inoculation, the % cotyledons with symptoms was 10% on plants inoculated with the lowest inoculum density and >70% on plants inoculated with higher inoculum concentrations. When the highest inoculum density (1×10^5 conidia ml^{-1}) was used, 100% of the cotyledons had developed symptoms 6 days after inoculation (data not shown).

The DI on cotyledons increased linearly ($R^2 \geq 0.78$) with increasing inoculum concentration and increasing time (Figure 2b). Moreover, the rate of increase in the

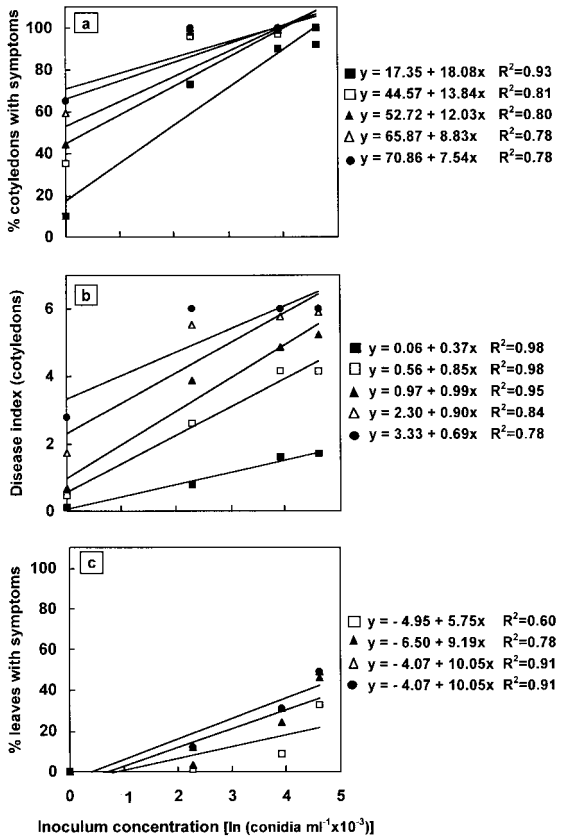

Figure 2. Regression lines fitted to describe the relationships between inoculum concentrations of *A. linicola* conidia and % cotyledons with symptoms (a), % cotyledon area with symptoms (DI) (b) or % leaves with symptoms (c) on linseed plants (cv. Antares). The DI was calculated by using Equation (1). Assessments were made (■): 4, (□): 7, (▲): 9, (△): 13 and (●): 18 days after inoculation.

disease severity was less rapid on plants inoculated with the lowest inoculum density than that on plants inoculated with higher inoculum concentrations. The first symptoms appeared on leaves 6 days after inoculation and 2 days later than on the cotyledons (data not shown). However, no symptoms developed on leaves of plants inoculated with the lowest inoculum density (1×10^3 conidia ml^{-1}), even 18 days after inoculation (Figure 2c). Although the incidence of the disease on leaves increased linearly ($R^2 \geq 0.60$) with increasing inoculum concentration and with time, it reached no more than 50% 18 days after inoculation, even when the highest inoculum density was used (1×10^5 conidia ml^{-1}).

No lesions or cankers formed either on the hypocotyls or on the stems of plants inoculated with the lowest inoculum concentration (1×10^3 conidia ml^{-1}) (Figure 3). The linear regressions fitted the data well ($R^2 \geq 0.75$). The % stems and % hypocotyls with lesions or cankers increased with increasing inoculum density and a greater % plants formed lesions on the stems than on the hypocotyls.

Effects of temperature and leaf wetness duration on infection and symptom development

No symptoms developed on plants exposed to leaf wetness periods of up to 8 h at 15 °C and up to 6 h at 25 °C (Figure 4a). However, at 15 °C the % plants with symptoms increased rapidly from 0% to 96%, when the leaf wetness period increased from 8 to 10 h. Similarly, the % plants with symptoms at 25 °C increased rapidly from 0% to 100%, when the leaf wetness duration

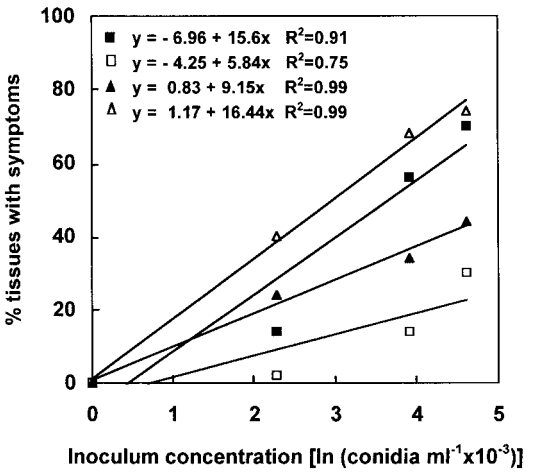

Figure 3. Effect of inoculum concentration on the % stems (■, □) or hypocotyls (▲, △) with lesions (■, ▲) or cankers (□, △) on linseed plants (cv. Antares) inoculated with 1×10^3, 1×10^4, 5×10^4 and 1×10^5 conidia ml^{-1} of *A. linicola* and assessed 18 days after inoculation. Relationships were assessed by linear regression of ln(conidial concentration) on % tissues affected.

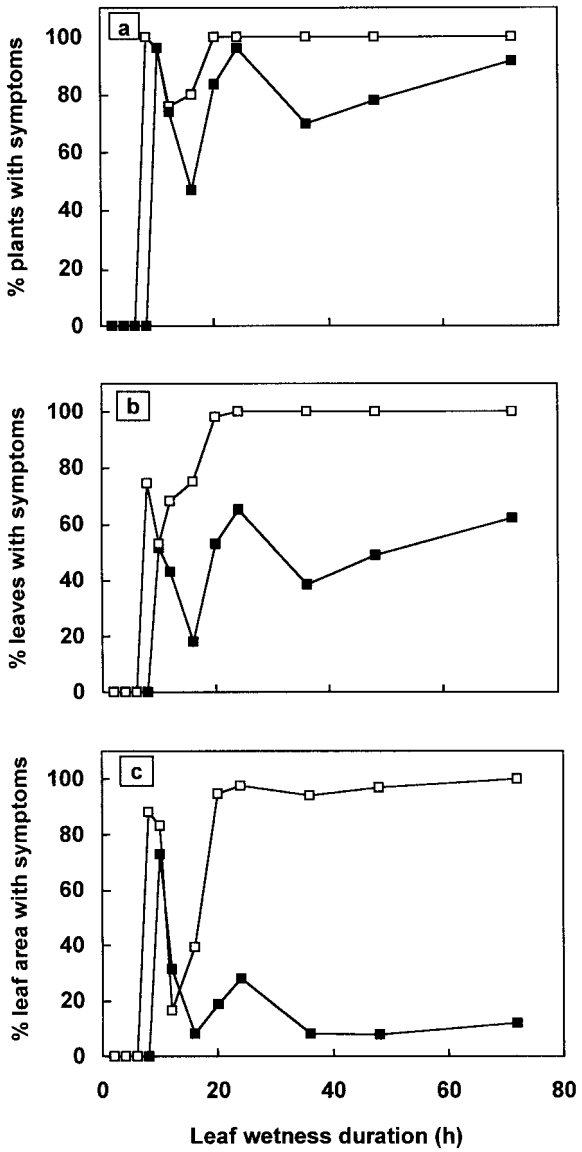

Figure 4. Effects of leaf wetness duration on the % plants with symptoms (a), the % leaves with symptoms (b) and the % leaf area with symptoms (c) on linseed plants (cv. Antares) at 15 °C (■) or 25 °C (□). Plants were inoculated with *A. linicola* conidia (3×10^4 conidia ml^{-1}) and assessed 4 days after inoculation. % plants with symptoms SED (56 df) = 9.7; % leaves with symptoms SED (56 df) = 12; % leaf area with symptoms SED (56 df) = 7.9.

increased from 6 to 8 h. Although the % plants with symptoms decreased as leaf wetness periods increased from 10 to 16 h at 15 °C and from 8 to 12 h at 25 °C, it increased again as leaf wetness periods increased above 16 h at 15 °C and above 12 h at 25 °C. The % leaves with symptoms was affected by the length of the leaf wetness period in a similar way to the % plants with symptoms at both temperatures tested (Figure 4b). However, the % leaves with symptoms was lower at 15 °C than that at 25 °C. The severity of the disease was also less at 15 °C than that at 25 °C for all leaf wetness periods tested (Figure 4c). At 15 °C, the % leaf area with symptoms was only 8% even after 72 h of leaf wetness; whereas at 25 °C, approximately 100% of the leaf area developed symptoms after 20 h of exposure to leaf wetness. When the control plants were examined for symptoms, only the plants exposed to 72 h of leaf wetness had developed symptoms at 15 °C and 25 °C (the incidence of the disease on the cotyledons was 100%). No symptoms developed on the plants in the absence of leaf wetness (24 h dry period) at either of the temperatures tested.

Effects of interrupted leaf wetness on infection and symptom development

Interruption by one dry period. A 12-h dry period, applied 1 h after inoculation, decreased the % cotyledons which developed symptoms by 20% compared with that on plants (100%) incubated for 24 h under continuous leaf wetness (control plants) (Figure 5a). A dry period applied 2 h or ≥18 h after inoculation did not significantly affect the development of symptoms. However, a 12-h dry period applied at any time between 3 and 12 h after inoculation decreased the % cotyledons which developed symptoms, with the maximum decrease occurred 6 h after inoculation. Moreover, a dry period applied at any time between 1 and 24 h after inoculation decreased the severity of the disease on the cotyledons, with the greatest decrease (approximately 100%) being observed when the dry period was applied 6 h after inoculation (Figure 5b). No symptoms were observed on plants in the absence of leaf wetness (24-h dry period for control plants).

Interruption by several dry periods. The % cotyledons with symptoms on plants exposed to two, three or four 12-h periods of leaf wetness was greater than that on plants exposed only to one 12-h period of leaf wetness (Figure 6a). When a 24-h period of leaf wetness was interrupted 12 h after inoculation by a

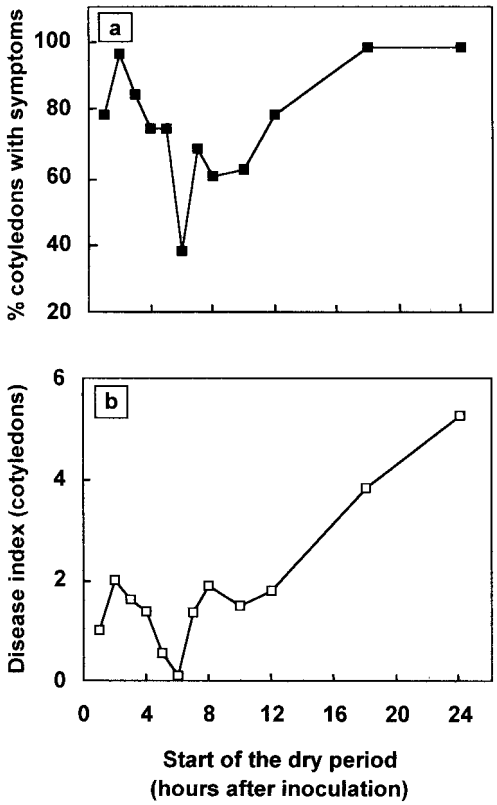

Figure 5. Effects of interrupting a continuous leaf wetness period by a 12-h dry period at various times after inoculation on the % cotyledons with symptoms (a) and the severity of symptoms (DI) (b) on linseed plants (cv. Antares) at 15 °C. Plants were inoculated with *A. linicola* conidia (3×10^4 conidia ml^{-1}) and assessed 4 days after inoculation. The DI was calculated by using Equation (1). % cotyledons with symptoms SED (44 df) = 12.4; DI SED (44 df) = 0.4.

12-h dry period, it resulted in a 30% decrease in the % cotyledons with symptoms. This decrease was less (approximately 5%) when longer (36 or 48 h) leaf wetness periods were interrupted by dry periods. However, the DI on leaves was the same on plants exposed to one, two, three or four 12-h periods of leaf wetness and it was much less than that in the equivalent of continuous leaf wetness treatments (Figure 6b).

Effects of light regime on infection and symptom development

There were no significant differences in the % cotyledons with symptoms between plants exposed for 72 h to different light treatments immediately after inoculation (Table 3). However, the % cotyledons which developed

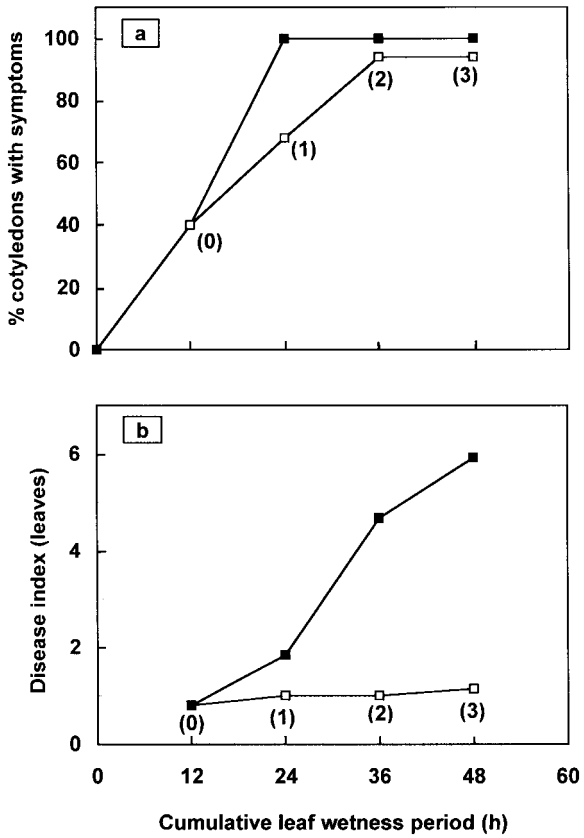

Figure 6. Effects of continuous (■) or interrupted (□) leaf wetness periods on the % cotyledons with symptoms (a) and the severity of symptoms (DI) (b) on linseed plants (cv. Antares) at 15 °C. Plants were inoculated with *A. linicola* conidia (3×10^4 conidia ml^{-1}) and assessed 6 days after inoculation. The DI was calculated by using Equation (1). Figures in parentheses are the numbers of dry periods (12 h each) interrupting the leaf wetness period. % cotyledons with symptoms SED (12 df) = 7.9; DI SED (12 df) = 5.8.

symptoms on plants exposed to continuous light for 72 h was slightly smaller than on plants exposed to all other treatments. The % stems with symptoms was positively correlated ($R^2 = 0.93$) with the length of the initial period in darkness (Figure 7a), but it was negatively correlated ($R^2 = 0.91$) with the length of the initial period in light. Only 4% of the stems developed symptoms when the plants were exposed to continuous light for 72 h, whereas 92% of the stems developed symptoms on plants exposed to continuous darkness for 72 h (Table 3). The DI on leaves was also positively related ($R^2 = 0.99$) to the length of the initial dark period but it was negatively related ($R^2 = 0.94$) to the length of the initial light period (Figure 7b). The smallest DI was

Table 3. Effects of light regime on disease incidence (%) on cotyledons and stems and on disease severity on leaves (% leaf area with symptoms) of linseed plants (cv. Antares) inoculated with *A. linicola* conidia (3×10^4 conidia ml^{-1}) under controlled environment conditions

Light regimes	Cotyledons with symptoms (%)	Stems with symptoms (%)	Leaf disease index[a]
72 L[b] (continuous)	73.0[c]	4	0.41
72 D (continuous)	91.0	92	3.54
12 L/12 D (alternating)	94.5	28	1.15
12 D/12 L (alternating)	83.5	18	0.79
12 L + 60 D	97.5	80	2.86
12 D + 60 L	82.0	18	0.66
24 L + 48 D	98.5	88	3.16
24 D + 48 L	95.5	50	1.44
48 L + 24 D	97.5	24	1.50
48 D + 24 L	98.5	83	2.66
SED (36 df)	5.07	16.27	0.29

[a]DI based on a 0–5 scale in which, 0 = no symptoms, 0.1 = <1%, 1 = 1–5%, 2 = 6–10%, 3 = 11–30%, 4 = 31–50%, and 5 = 51–70% and calculated by using Equation (2).
[b]Hours of the light (L) or dark (D) period.
[c]Mean of five replicates.

observed on plants exposed to continuous light for 72 h, whereas the greatest was observed on plants exposed to continuous darkness for 72 h (Table 3).

Discussion

Development of symptoms on linseed plants inoculated with *A. linicola* was affected by inoculum density, temperature, duration of the wetness period, light regime and the interaction between temperature and the wetness period. The increase in incidence of disease on cotyledons, leaves, stems and hypocotyls with increasing inoculum concentration suggests that numbers of conidia, which were retained then germinated and penetrated the tissues, probably increased with increasing inoculum density. With the lowest inoculum concentration (1×10^3 conidia ml^{-1}), symptoms developed only on cotyledons, but the incidence remained relatively low (65%) even 18 days after inoculation. With higher inoculum concentrations, all the cotyledons developed symptoms of *A. linicola* within 6 or 11 days after inoculation, depending on the conidial concentration. However, the incubation period (time between inoculation and appearance of symptoms) was not affected by the inoculum concentration tested. The first symptoms appeared on the cotyledons 4 days after inoculation,

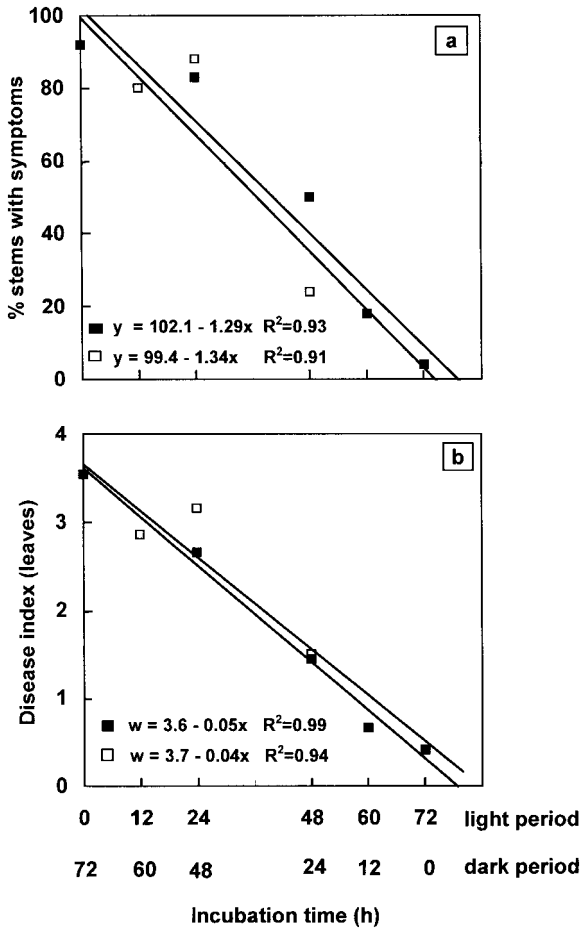

Figure 7. The relationship between the period of light (□) [or the period of darkness (■)] and the % stems with symptoms (a) and the DI (b) on leaves of linseed plants (cv. Antares) at 15 °C. Plants were inoculated with *A. linicola* conidia (3×10^4 conidia ml^{-1}) and assessed 3 days after inoculation. The DI was calculated by using Equation (2).

even when the lowest inoculum concentration (1×10^3 conidia ml^{-1}) was used.

Severity of disease also increased with increasing inoculum up to a concentration of 5×10^4 conidia ml^{-1}. A relationship between inoculum concentration and symptom development has also been shown for other fungi, including other *Alternaria* species (Singh and Chand, 1982; Jeger et al., 1985; Trapero-Casas and Kaiser, 1992; Makowski, 1993). However, the severity of the disease did not increase further as inoculum concentration increased to 1×10^5 conidia ml^{-1}. These results suggest that at high inoculum concentrations there might be some self-inhibition of germination of *A. linicola* conidia, especially when the conidia are clumped, as has been reported for other *Alternaria* species (Rotem, 1994).

Eight hours of leaf wetness were sufficient to initiate the disease at 25 °C, but not at 15 °C, when a longer leaf wetness period of 10 h was required. For most *Alternaria* species the incubation period ranges from 3 to 72 h, depending on the species and the temperature (Norse, 1973; Degenhardt et al., 1982; Rotem, 1994). Under controlled environment conditions, interruption of a continuous period of leaf wetness by a 12-h dry period may also affect the development of *A. linicola* symptoms, depending on the time during the germination and infection process at which the interruption occurs. The % cotyledons with symptoms decreased after exposure to a 12-h dry period at any time between 3 and 12 h after inoculation. However, the dry period affected the severity of symptoms rather than the incidence of symptoms. A dry period occurring at any time between 1 and 12 h after inoculation decreased the % leaf area with symptoms by more than 50%. Moreover, interrupting the leaf wetness period 1 h after inoculation decreased the incidence and severity of the disease more than interrupting it after 2 h. It seems that the germinating conidia of *A. linicola* are more susceptible to drying during the water imbibition phase (<2 h from inoculation) than during the germ-tube initiation phase (≥2 h from inoculation). Experiments on the effects of dry interruptions of a continuous leaf wetness period on conidial germination of *A. linicola* (Vloutoglou et al., 1996), suggested that a 12-h dry period applied at any time during the germination process and before the penetration of the plant tissue stopped the germination of conidia and germ-tube elongation. Moreover, these conidia did not recover, irrespective of the length of the wet period that may follow the dry period. Therefore, it seems that the inhibitory effects of the dry period on the development of symptoms on linseed plants were due to effects on conidial germination.

Controlled environment studies also suggest that *A. linicola* conidia are able to exploit successive 12-h periods of leaf wetness cumulatively to infect linseed plants, although the disease incidence and severity were lower after interrupted than after continuous leaf wetness periods. Previous studies (Vloutoglou et al., 1996) have shown that at 15 °C, conidia of *A. linicola* applied as a suspension to linseed plants are very susceptible to drying, especially when the dry period is 12-h long and occurs between 1 and 12 h after inoculation. However, a 12-h dry period applied after 12 h of leaf wetness, when most of the conidia have germinated,

does not affect germination, but decreases the rate of germ-tube elongation (Vloutoglou et al., 1996). Therefore, it may delay the formation of appressoria, the penetration and subsequently the infection of plants by *A. linicola*. Although most *Alternaria* species can successfully make use of both interrupted and continuous leaf wetness periods to infect their hosts (Allen et al., 1983), it has been reported that interruption of the leaf wetness period decreases the incidence of the disease caused by *A. brassicae* or *A. brassicicola* on brassica plants (Humpherson-Jones et al., 1983).

The present results suggest that light is another environmental factor which can affect the development of symptoms caused by *A. linicola* on linseed plants, and its effects on infection can be as important as those of leaf wetness duration. Controlled environment studies showed that, although the incidence of the disease on cotyledons was slightly less on plants exposed to continuous light than on those exposed to other light regimes, the development of symptoms on the cotyledons was affected less by light conditions than the development of symptoms on stems or leaves. It is possible that the intensity of the light reaching the cotyledons was lower (although it was not measured at the level of the cotyledons) than that of light reaching the leaves or the stems. However, the length of the period during which the plants were exposed to light immediately after inoculation was negatively correlated with the incidence of the disease on stems and the severity of the disease on leaves. Previous studies (Vloutoglou et al., 1996) have shown that wet light periods of 12 or 24 h applied before a wet dark period may delay conidial germination and germ-tube elongation, but most of the conidia recover and continue to germinate during the wet dark period that follows the exposure to light. Wet light applied after a 12-h or a 24-h initial period of wet darkness has no effect on germination as most of the conidia have germinated during the wet dark period. Therefore, it is unlikely that light in this study influenced the development of symptoms on stems and leaves through effects on the germination process.

In the case of other *Alternaria* species, light inhibits the production of toxins produced by the conidia during their germination (Haggblom and Hiltunen, 1992). However, it is not known if toxins are produced by *A. linicola* and if light affects the development of symptoms on linseed plants by inhibiting toxin production.

These experiments show that the length of the incubation period and further development of symptoms differ between different plant tissues. Leaves appeared to be more resistant to *A. linicola* infection than cotyledons. This might have been related to differences (a) in tissue maturity with the cotyledons being older, more senescent and as a consequence, more susceptible than the younger and physiologically more active leaves, (b) in wax content. This may also explain the absence of symptoms on leaves, but not on the cotyledons, when the lowest inoculum concentration (1×10^3 conidia ml^{-1}) was used, if no conidia were retained on leaves. According to Conn and Tewari (1989) leaf epicuticular wax also decreased susceptibility of canola (*Brassica napus* or *B. campestris*) to *A. brassicae*, whereas Bashi et al. (1983) reported that symptoms caused by *A. macrospora* on cotton plants developed to a much greater extent on the cotyledons than on the leaves.

According to the results of the present study, at 15 °C, which is the average temperature during the period between flowering and harvest of the linseed crop in the SE of England (July–September) and in the presence of leaf wetness or high relative humidity, germination, penetration and infection of linseed plants by *A. linicola* can occur within 10 h. Under these conditions, the first symptoms appear within 4 days, and conidia can be produced on cotyledons at the end of the first wet night (Vloutoglou, 1994). Therefore, cotyledons infected early in the growing season can support the first stages of the disease epidemic. However, periods of leaf wetness on linseed crops are often shorter than those required for infection of linseed by *A. linicola* to be completed. Nevertheless, the present results showed that the pathogen was able to use successive periods of leaf wetness cumulatively to infect linseed plants, although symptoms produced under interrupted periods of leaf wetness were less severe than those produced in continuous leaf wetness periods.

The effects of leaf wetness duration and light regime on infection and symptom development of *A. linicola* on linseed plants were studied under relatively constant temperatures. However, under field conditions with fluctuating temperatures, not only the infection but also the development of symptoms might be different. Moreover, it is possible that sunlight may also affect the expression of symptoms caused by *A. linicola* on linseed plants since the symptoms which developed on cotyledons and leaves under artificial light (fluorescent lamps) were different from those observed in the field (Vloutoglou, 1994). It is necessary to confirm these results under field conditions.

Acknowledgements

This work was supported by a scholarship for I. Vloutoglou from the Greek State Scholarship Foundation, Greece. IACR receives grant-aided support from the Biological and Biotechnology Sciences Research Council of the United Kingdom. We also thank Dr. Roger Plumb for critically reading the manuscript.

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