# EPIDEMIOLOGY OF *ALTERNARIA LINICOLA* ON LINSEED (*LINUM USITATISSIMUM* L.)

by

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**ABSTRACT**

Conidia of *A. linicola* germinated over a wide range of temperatures (5 - 25°C) on both agar and leaves. Germination started within 2 h after inoculation at temperatures between 10°C and 25°C, either on agar or on leaves. At 5°C, there were lag periods of 2 and 4 h before the initiation of germination on agar and on leaves, respectively. Germinating *A. linicola* conidia were very sensitive to drying between 2 and 6 h after inoculation. In the presence of leaf wetness, light applied before the initiation of germination delayed the germination process and decreased the length of the germ tubes. Light applied after the onset of germination decreased both the percentage of conidia which germinated and the length of the germ tubes. In the absence of leaf wetness, light applied before or after the initiation of germination stopped the germination process or decreased the percentage of conidia which germinated, respectively. Conidia of *A. linicola* germinated by producing germ tubes and occasionally by producing secondary conidia. Formation of appressoria was inhibited at 5°C. Penetration of the leaf tissues started 12 h after inoculation at 15°C and occurred mainly directly through the epidermal cells and occasionally through stomata.

*A. linicola* is a "diurnal sporulator". *In vitro* most isolates sporulated only after exposure to diurnal NUV-light. However, for some isolates exposure to diurnal NUV-light did not seem to induce sporulation unless the mycelium was wounded and grown on a medium rich in $CaCO_3$ (S-medium) at high relative humidity. *In vivo* sporulation of *A. linicola* was increased after induction by light. The greatest numbers of conidia were produced under continuous leaf wetness and

alternating dark/light periods (12 h each). Under these conditions the number of conidia produced increased with increasing temperature from 10°C to 20°C. Alternating 15°C/10°C or 20°C/15°C day/night temperatures decreased the number of conidia produced compared with the constant temperatures 15°C and 20°C, respectively.

In controlled environment studies, infection of linseed plants by *A. linicola* and development of symptoms was affected by the leaf wetness period, its interaction with temperature and by the light conditions. Eight hours of leaf wetness were sufficient to initiate the disease at 25°C but not at 15°C when a longer period of 10 h was needed. Infection of linseed plants by *A. linicola* occurred under interrupted leaf wetness periods at 15°C, but the incidence and severity of the disease was lower than that under continuous leaf wetness. The disease incidence on stems and the disease severity on leaves was negatively correlated with the length of the light period applied immediately after inoculation. Disease incidence and severity increased with increasing inoculum concentration from $1 \times 10^3$ to $1 \times 10^5$ conidia $ml^{-1}$. The cotyledons appeared to be more susceptible to *A. linicola* infection than the leaves when the same inoculum density was used.

*A. linicola* was detected on 12 of the 20 seed samples tested and on six of them at a high incidence (> 50%). Seed seems to be the main source of primary inoculum as the pathogen was effectively transmitted from infected seeds to the emerging seedlings. Infected linseed stem debris, volunteer linseed plants and the weed *Veronica agrestis* were also sources of primary inoculum for the infection of linseed crops by *A. linicola*. Structures resembling chlamydospores formed in

the mycelium and conidia of *A. linicola* seem to be involved in the survival of the pathogen in stem debris.

Conidia of *A. linicola* were mainly dispersed by the wind (air-borne conidia) and their dispersal followed seasonal and diurnal periodicities, which were influenced by the weather conditions and the incidence of the disease in the crop. The greatest numbers of *A. linicola* conidia were collected by the Burkard spore sampler on the first dry day following periods of rain, between 12:00 h and 13:00 h and during the period between flowering and harvest of the crop (July - September). Bait plants were more efficient than the Burkard spore sampler in detecting *A. linicola* conidia present in the crop early in the growing season. The number of *A. linicola* conidia dispersed within a linseed crop decreased with increasing height above ground, but some conidia were collected 80 cm above the crop canopy. The number of *A. linicola* conidia dispersed downwind from a line inoculum source decreased with increasing distance from the source and by the end of the growing season conidia were collected by up to 40 m from the source.

When the *A. linicola* disease gradients were studied from point or line inoculum sources, the disease incidence decreased with increasing distance from the inoculum source. By the end of the growing season, the disease was detected 20 or 60 m from the point or line inoculum sources, respectively.

Multiple applications of iprodione or prochloraz sprays to control *A. linicola* infection in the crop, especially the seed-borne phase of the pathogen, and to increase crop yield gave variable results depending on the weather conditions and the incidence of the disease in the crop. Multiple applications of benomyl or

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# CHAPTER I. GENERAL INTRODUCTION AND LITERATURE REVIEW

## 1.1. RATIONALE

*Alternaria linicola* Groves & Skolko is one of the most important seed-borne pathogens of linseed (*Linum usitatissimum* L.) in the UK. The fungus attacks the young seedlings as they emerge, causing damping-off symptoms (Mercer *et al.*, 1991a). *A. linicola* can decrease emergence and yield by up to 50 and 35%, respectively and can also affect the oil quantity and quality (Mercer et al., 1991a). In the last few years, *A. linicola* infection has been the main reason for the failure of the linseed seed to reach the UK certification standards which require that less than 5% of the seed in total is infected by seed-borne pathogens (Mercer *et al.*, 1991a).

*A. linicola* was not considered to be an aggressive pathogen of either flax or linseed when it was first recorded in Canada (Groves & Skolko, 1944) and in the UK (Moore, 1946; Muskett & Colhoun, 1947). The increasing importance of the disease caused by *A. linicola* on linseed crops in the UK is possibly due to the increase in the area sown with linseed in the last few years. Linseed was one of the first crops to be supported by an "Area Aid Payment". This support scheme was introduced in 1976 with the objective of encouraging the production of a commodity for which the EC was substantially in deficit. As a result the area sown with linseed in the UK has risen from 4,452 ha in 1946 to 150,000 ha in 1992 and the UK has become one of the main producers of linseed among the EC countries.

Moreover, linseed products (oil, cake, straw) are used for many different purposes. Another possible reason for the increasing importance of *A. linicola* as a pathogen of linseed in the UK is that the application of fungicide sprays to the crop is often considered to be uneconomic as linseed is a low input break crop.

Little is known about the disease caused by *A. linicola* on linseed. Current knowledge is based on observations of the disease in linseed crops during the growing season (Mercer *et al.*, 1991a; Fitt *et al.*, 1991a; 1991b; 1991c; Fitt & Vloutoglou, 1992), on the effects of fungicides as seed treatments or sprays on the seed-borne phase of the pathogen (Mercer *et al.*, 1985; 1989; 1990; 1991b; Mercer & McGimpsey, 1987; Hardwick & Mercer, 1989; Fitt & Ferguson, 1990) and on the pathogenicity of the fungus under controlled environment conditions (Fitt & Coskun, 1991; Fitt *et al.*, 1991a; Davis & Fitt, 1992). Therefore, more information is required on the life cycle of the pathogen and the epidemiology of the disease in order to develop disease management strategies which will be both effective and economic.

## 1.2. THE HOST

### 1.2.1. Origin and morphological types

*Linum usitatissimum* L. is one of the oldest known cultivated plants (Durrant, 1976). It appears to have originated from the Indian sub-continent where it has been in cultivation for more than 5,000 years. From there it is believed to have spread northwards and westwards through Afghanistan and Asia Minor. Linen was worn in ancient Egypt well before 1,000 B.C. and the Egyptians used linen cloth

to wrap their mummies and linseed oil as part of the embalming process. References to the crop occur in the Old Testament and in ancient Greek and Roman writings. In Europe, the cultivation of the plant also dates back a long way. In Germany, linen robes were used before the Roman invasion. In England, Scotland and Ireland the crop was cultivated during the Bronze age. The plant was introduced into North America by the earliest settlers as a fibre crop (Durrant, 1976).

*Linum* is a genus of nearly 200 species spread over the temperate and warm temperate zones of the northern hemisphere, most abundantly in Europe and Asia with about 50 species in America. The cultivated species *Linum usitatissimum* L. is one of the well-defined group of North African and Eurasian species which include *L. africanum* L., *L. corymbiferum* L., *L. decumbens* L., *L. nevrosum* L., *L. pallescens* L. and *L. angustifolium* L. (Durrant, 1976). Most of these species are annual or perennial and many of them have been brought into gardens. The following wild or ornamental species can be found in the UK : *L. anglicum, L. bienne, L. catharticum, L. flavum, L. grandiflorum, L. narbonense* and *L. perenne* (Turner, 1987). Linnaeus (1857) was the first to give the botanical name *Linum usitatissimum* to the cultivated species. Although this species is distributed throughout the world, it has not been found in the wild state under natural conditions. Archaeological evidence suggests that *L. angustifolium*, which is native in the Mediterranean area and Western Europe, is closely related to *L. usitatissimum* and therefore may have played some part in the development of the cultivated species (Durrant, 1976).

As the cultivation of the plant spread, two distinct morphological types developed within the species *L. usitatissimum* : flax (fibre flax or textile flax), grown

for its fibres which are used in the manufacture of linen, and linseed (flax seed or oil flax), grown for the oil and protein content of its seed (Gill, 1987). Both of these morphological types are mainly self-pollinated but a low percentage of out-crossing (0.3-2%) may occur with insects as the main pollination agents. Linseed is generally shorter than fibre flax with thicker stems, more branches, more capsules and larger seeds. Flax is harvested earlier than linseed (soon after the petals have fallen) by pulling so that the full length of the stem is secured. Linseed is combine harvested after the capsules and seeds have matured fully.

**1.2.2. Description - Cultivation**

Linseed is an annual, dicotyledonous plant that grows to a height of 60-80cm, depending on the cultivar and the environmental conditions (Gill, 1987). The plant has a short tap-root system with fibrous branches which may extend to a depth of 90-120 cm in light soil. Linseed has one main stem, although the growth of two or more branches (tillers) can be stimulated by low plant density, high soil nitrogen, frost or pest, chemical or mechanical damage. The stems of the linseed types contain fibres but they are fewer, shorter and of inferior quality to those of the flax types. The leaves are simple, linear, 3-4 cm long, smooth on the upper surface with fairly prominent veins on the lower surface. The basal leaves are in alternate pairs and those above the fourth node are arranged spirally. A mature plant has approximately 60 leaves. The main stem and the branches give rise to a terminal multi-branched inflorescence called a panicle or cyme, which bears the flowers. These are 2-3 cm in diameter and pale blue to purple-blue in most of the commercial cultivars (Fig. 1.1). The flowers are hermaphrodite and hypogynous, with 5 sepals, 5 petals, 5 stamens and a compound pistil of 5 carpels. Flowering occurs from mid-June to

**Figure 1.1.** Linseed flowers (cv. Antares).

mid-July in the UK. Each flower lasts less than a day; the petals open shortly after sunrise and are shed in the early afternoon. The mature fruit of a linseed plant is a capsule. Depending on the cultivar and the plant population, an average of 10-15 capsules are formed on each main stem. Each capsule has 5 segments which are separated by a wall or septum. Each segment produces two seeds separated by a partition called a "false septum". As many as 10 seeds can develop in a capsule but most commercial cultivars produce 6-8 seeds per capsule. The seeds are flat, oval and pointed at the end, 4-6 mm long and 2-3 mm wide, ranging from yellow to dark reddish brown in colour. The mature seed is shiny, slippery and covered with a mucilaginous coating which makes the seed sticky when wet. The main stem development of *L. usitatissimum* can be separated into twelve growth stages (Fig. 1.2 & Table 1.1) (Turner, 1987).

Linseed is one of the few crops that grows throughout the world under different agricultural systems and environments. In the UK it is grown as a break crop in cereal rotations and although it originates from warm and humid climates, modern cultivars are well suited to the moist and moderate climate of the UK (Gill, 1987). The crop is drilled in spring from mid-March to mid-April at a seed rate of 250-500 seeds $m^{-2}$. For best establishment, the crop requires warm moist spring weather. Under these conditions emergence can take place in 7 to 10 days. Linseed can grow on most soil types, from light loams to clays, provided seedbed conditions are good and weeds are controlled, as it is not competitive with fast-growing weed species (Gill, 1987). The growing season lasts approximately 150 days and the crop is combine harvested from mid-August to mid-September, depending on the cultivar and the weather conditions during the growing season. Yields in the UK range from

**Figure 1.2.** Linseed main stem growth stages. A description of the different growth stages is given in Table 1.1 (Turner, 1987).

**Table 1.1.** Description of the linseed main stem growth stages (Turner, 1987).

| Growth Stage (GS) | Description |
|---|---|
| 1 | Cotyledon |
| 2 | Growing point emerged |
| 3 | First pair of true leaves unfolded |
| 4 | Third pair of true leaves unfolded<br>Start of leaf spiral |
| 5 | Stem extension |
| 6 | Buds visible |
| 7 | First flower<br>Early branching |
| 8 | Full flower<br>Capsules start forming<br>Continuation of branching |
| 9 | Late flower<br>Most branches and capsules formed |
| 10 | Green capsule<br>Seed white - lower leaves yellow |
| 11 | Brown capsule<br>Seeds light brown<br>Branches, stem and upper leaves green/yellow. Middle leaves partly senescent and lower leaves shrivelled or dropped |
| 12 | Seed ripe<br>Seeds brown and rattle in capsules<br>Branches and upper leaves senescent but stem still green/yellow |

0.07 to 3.3 t $ha^{-1}$ with an average of 1.9 t $ha^{-1}$ (Gilbertson, 1990). Chemical desiccation may be used before harvest to reduce time from maturity to harvest and to facilitate combining harvest. Diquat is the most frequently used desiccant on linseed although glyphosate may also kill the stems but more slowly. The desiccant is applied when 95% of the capsules are ripe, brown and the seeds rattle inside the capsules.

**1.2.3. Linseed products and uses**

Linseed is grown primarily for the oil contained in the seed. Oil content ranges from 40 to 45%, depending on the cultivar and the growing conditions (Gill, 1987). Linseed oil is a high quality "drying oil" which forms a durable film when exposed to the air. Linseed oil is used in the manufacture of paints, stains, lacquers and varnishes for its drying properties. It can also be used in soaps, putty, linoleum, oilcloth, printing ink, patent leather and as a curing agent for concrete surfaces including those of highways and bridges (Gill, 1987). Linseed oil is very rich in unsaturated fatty acids such as oleic, linoleic and linolenic acid. The greater the proportion of highly unsaturated fatty acids, the better the drying quality of the linseed oil. The high linolenic content in linseed oil (45-60%) makes it unsuitable for human consumption. Green (1986) identified a genotype of *L. usitatissimum* which can be used in future for the production of edible oil as it contains a very low level of linolenic acid ($<2\%$) in its seeds. Small quantities of whole linseed seed are considered beneficial in the human diet due to their high mucilage and fibre content. Linseed seed can be found in Canadian markets in products such as bread and cooked or dry cereals (Anonymous, 1986). Linseed seed also contains natural toxicants such as linamarin and linatine; linatine is an antagonist of vitamin $B_6$ and it can be poisonous to humans or animals if a large quantity of seed is consumed.

The cake that remains after the extraction of oil from the seeds contains 3% oil and 34 to 42% protein and it is used as a protein supplement in feed for livestock (Gill, 1987). Fibres from linseed stems are short and for this reason they are not used in the textile industry. However, the linseed straw can be used for the production of cigarette-paper and other fine bond papers. Although the protein content of the linseed straw is very low (3-5%), the fibre content is very high and for this reason linseed straw can be used as a supplement feed to livestock. Only mature linseed straw should be used for this purpose as the green straw contains prussic acid which is poisonous to animals.

## 1.3. THE PATHOGEN

### 1.3.1. Taxonomy

*Alternaria linicola* Groves & Skolko belongs to the Fungi Imperfecti, class *Moniliales* (or *Hyphomycetales*) and to the family *Dematiaceae* (Neergaard, 1945).

### 1.3.2. Morphology - Differentiation from other *Alternaria* species

*A. linicola* was first described by Groves & Skolko (1944) and later by David (1991). The hyphae are septate, branched, hyaline to olive-brown, (2-)3-6(-7) μm wide. The conidiophores are single or in bundles, pale olive-brown, septate, unbranched, erect, often geniculate with 1-2 or more scars, variable in length, (5-)6-8(-9) μm in diameter. The conidia are single, smooth-walled, olive-brown, paler on the host than in culture, elongated conical to ellipsoid or obclavate, muriform, with (4-)7-11(-16) transverse septa and occasionally 1-4 longitudinal septa, not or only slightly constricted at the septa, gradually tapering towards the beak, 20 - 130 x (7-) 17-24 (-30) μm

(Fig.1.3). Secondary conidiophores may be produced from cells of the conidium body. The conidium beak is long, filiform, often branched (more often in culture), septate, occasionally swollen at the tip when producing a secondary conidiophore (more often in culture), 16-230 x 3-4.5 μm.

The appearance of the fungus in culture depends on the medium. On malt agar (MA) the aerial mycelium is cottony, white, "deep olive-grey" to "dark olive-grey" paler at the centre to "pale olive-grey". The submerged mycelium is dense, mostly radiating, "olive-brown". The colony reaches about 6-7 cm in diameter after 10 days. On potato dextrose agar (PDA) the aerial mycelium is dense, cottony, rough, white to "leaf-green". The submerged mycelium is radiating, white to "dark greyish olive" or "olivaceous black". On most media the areas where the sporulation is concentrated appear darker and under conditions that induce sporulation (alternating light and darkness) light and dark concentric zones are formed on cultures (Neergaard, 1945).

*A. linicola* is closely related to *A. porri* (Ellis) Cif., *A. dauci* (Kuhn) Groves & Skolko and *A. brassicae* (Berk.) Sacc but it differs in having smaller, more delicate conidia, different cultural characters and hosts (Neergaard, 1945). Two other *Alternaria* species, *A. alternata* (Fr.) Keissler and the *Alternaria* state of *Lewia infectoria* (Fuckel) Barr & Simmons, often isolated from linseed plants in the UK (Fig. 1.4), and *A. lini*, described by Dey (1933) on linseed plants in India, form conidia which are always in chains and are quite different in morphology from *A. linicola* conidia. *A. alternata* conidia are dark brown or "olivaceous-brown", are produced in simple or branched chains of 2-7 and vary in shape and dimensions (average 37 x 13 μm, including the beak when present).

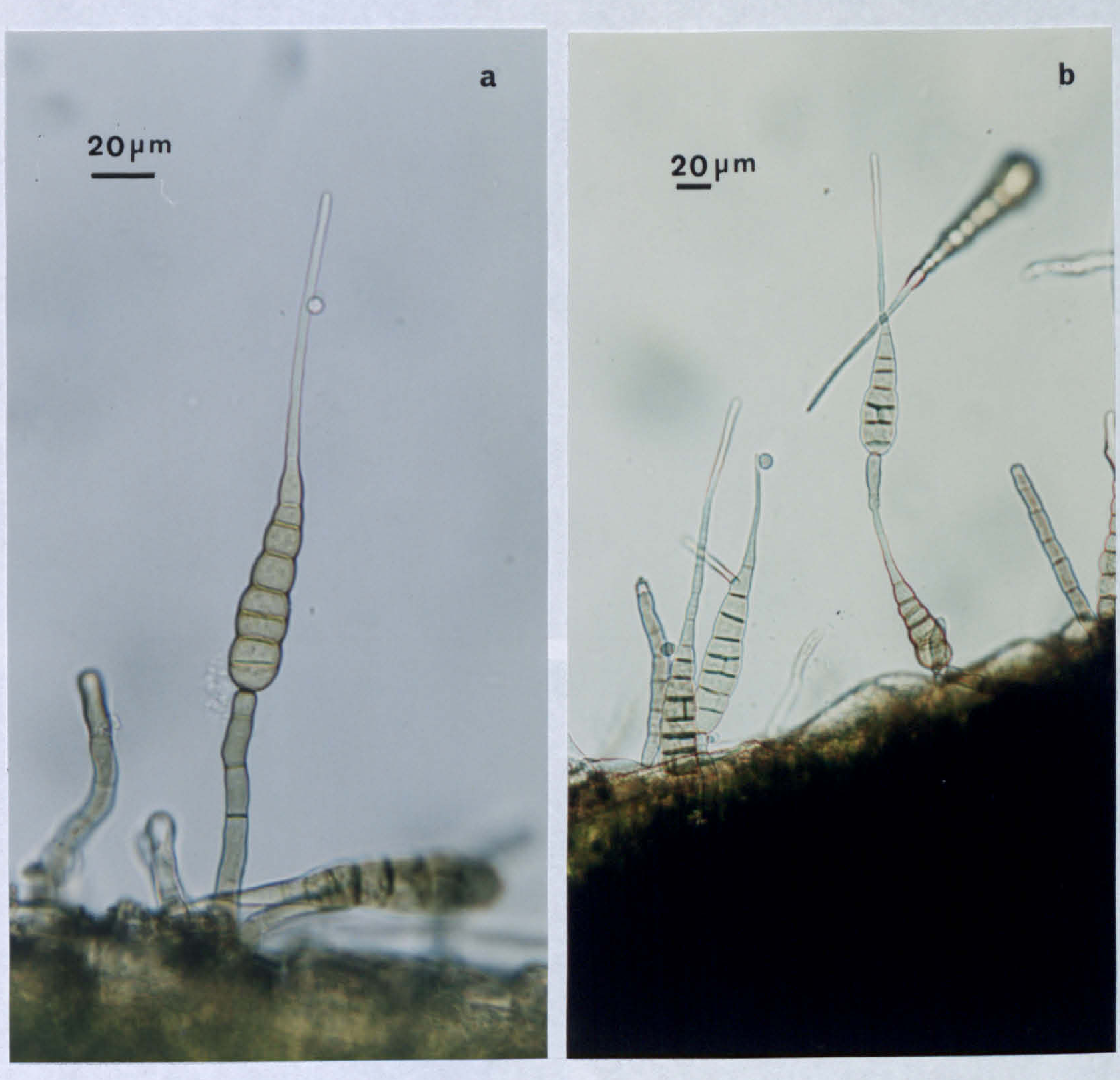



**Figure 1.3.** Conidia of *A. linicola* produced singly (a) or rarely in chains of two (b) on conidiophores.

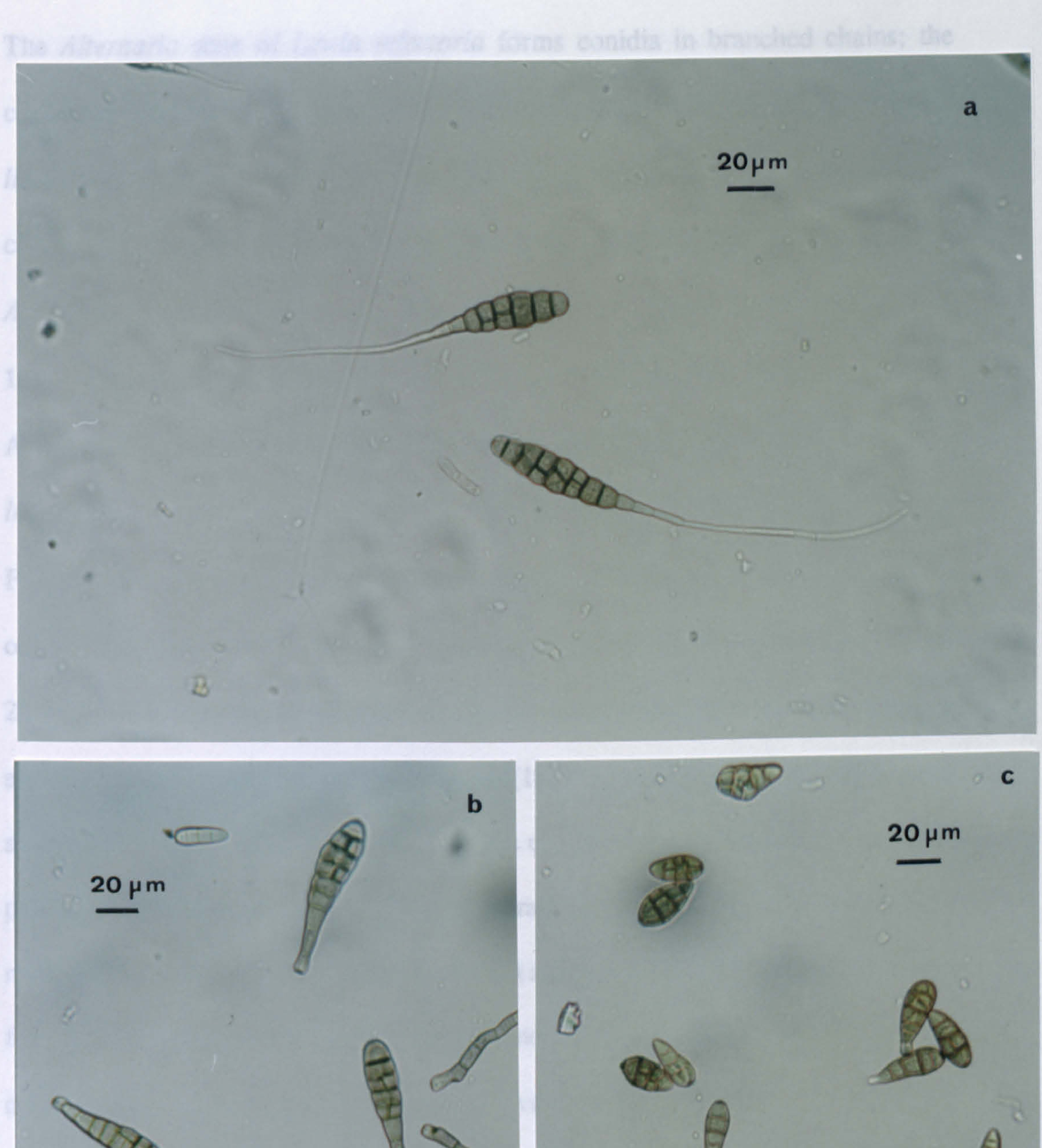



**Figure 1.4.** Conidia of *A. linicola* (a), *A. infectoria* (b) and *A. alternata* (c).

The *Alternaria* state of *Lewia infectoria* forms conidia in branched chains; the conidia are smooth-walled, bigger than *A. alternata* conidia but smaller than *A. linicola* conidia (average 50 x 14 μm, including the beak) (Ellis, 1971). *A. lini* conidia are also formed in chains, but are flask-shaped and smaller than those of *A. linicola* (average 24 x 7 μm, including the beak) (Dey, 1933).

### 1.3.3. Sporulation

Although it is well known that most *Alternaria* species do not sporulate abundantly *in vitro*, there is little information on the factors affecting sporulation of *A. linicola*. Fitt & Coskun (1991) reported that cultures of *A. linicola* sporulated abundantly on V-8 juice agar under alternating NUV-light (12h NUV-light/12h darkness) at 20°C. The literature on the effects of light and temperature on sporulation of *Alternaria* species is voluminous. According to Leach (1967), *Alternaria* species are "diurnal sporulators" which have two distinct phases of photosporogenesis: an "inductive phase" which when stimulated by ultra-violet radiation at relatively high temperatures results in the formation of conidiophores and a "terminal phase" which leads to the formation of conidia and is inhibited by light and high temperatures (>25°C). Neither conidiophores nor conidia of *A. solani* developed in complete darkness at any temperature (Vakalounakis, 1982). Waggoner & Horsfall (1969), Leach (1967) and Zimmer & McKeen (1969) also mentioned the inhibitory effect of high temperatures (>25°C) under continuous illumination on sporulation of *A. solani* and *A. dauci*. Lukens (1964) reported that light favours formation of conidiophores and inhibits the development of conidia of *A. solani*. Wavelengths stimulating the process of photosporogenesis are in the ultra-violet (UV) region of the spectrum (230-360 nm) (Leach, 1964). Numerous workers have applied different techniques to induce the

sporulation of *Alternaria* species *in vitro*. These methods have included exposure to ultra-violet light (McCallan & Chan, 1943; Charlton, 1953), mutilation of the mycelium (Rands, 1917; McCallan & Chan, 1943; Charlton, 1953; Lukens, 1960; Ludwig *et al.*, 1962; Barksdale, 1968), exposure to sunlight (Rands, 1917; McCallan & Chan, 1943), use of different culture media (Aragaki, 1963; Shahin & Shepard, 1979; Vakalounakis, 1982; Miles & Wilcoxson, 1984; Senior *et al.*, 1987) or dehydration of the medium (Rands, 1917; McCallan & Chan, 1943; Charlton, 1953; Ludwig *et al.*, 1962; Barksdale, 1968).

Most studies on the environmental factors affecting sporulation of *Alternaria* species were done *in vitro*. However, sporulation patterns *in vivo* may be different. Although *Alternaria porri* f. sp. *solani* sporulates abundantly on potato leaves exposed to light, few spores are produced under continuous darkness (Aragaki, 1964; Rotem & Bashi, 1969). Sporulation of *A. brassicae in vivo* was inhibited by white light (Humpherson-Jones & Phelps, 1989). In other cases, light may affect sporulation through interactions with temperature and moisture. Bashi & Rotem (1975a) found that *A. porri* f. sp. *solani* sporulates better under several wet periods interrupted by dry periods than under periods of continuous wetness.

For facultative (necrotrophic) parasites like *Alternaria* species, leaf senescence may also have an important effect on sporulation. Conidial production of *A. solani* on tomato leaves (Bashi & Rotem, 1975a) and of *A. porri* on onions (Everts & Lacy, 1990) increased as leaves became senescent.

**1.3.4. Germination - Penetration**

Although there is no information on the conidial germination and mode of penetration of *A. linicola*, these processes have been studied for other *Alternaria*

species. Conidia of most *Alternaria* species germinate by producing germ tubes. All the cells of the conidium can germinate and several germ tubes can be produced by a single cell (Fahim & El-Shehedi, 1966; Tsuneda & Skoropad, 1978; Allen *et al.*, 1983). However, conidia of *A. brassicae* (Tsuneda & Skoropad, 1978), *A. helianthi* (Allen *et al.*, 1983) and *A. porri* f.sp. *solani* (Rotem & Bashi, 1969) germinate by producing either germ tubes or secondary conidia. Germination of *Alternaria* species occurs over a wide range of temperatures (5°C to 35°C with optima close to 25°C) (Lacey, 1992). Under optimum temperatures and high relative humidity (100%) or in the presence of wetness on the plant tissues, conidial germination of many *Alternaria* species starts within 3 h after inoculation (Waggoner & Horsfall, 1969; Strandberg, 1987). Conidia of most *Alternaria* species germinate only when the relative humidity is greater than 85% (Dickinson & Bottomley, 1980).

Not only conidial germination but also host penetration can be a very rapid process for many *Alternaria* species. On oilseed rape leaves, conidia of *A. brassicae* germinated after a short incubation period (3 h) and penetration of the host was evident after 12 h (Tsuneda & Skoropad, 1978). Host penetration by *Alternaria* species can occur with or without the formation of appressoria, either directly through the epidermal cells or indirectly through stomata, depending on the species and the host (Riley, 1949; Changsri & Weber, 1963; Fahim & El-Shehedi, 1966; Tsuneda & Skoropad, 1978; Allen *et al.*, 1983; Strandberg, 1983). However, there are contradictory reports on the mode of penetration by the same *Alternaria* species. Angell (1929) reported penetration of onion leaves by *A. porri* only through stomata, whereas Walker (1952) observed penetration by the same fungus through stomata

and wounds.

### 1.3.5. Dispersal

The only information available on the dispersal of *A. linicola* is that from field studies at Rothamsted Experimental Station (Fitt & Vloutoglou, 1992). These studies suggest that *A. linicola* conidia are mainly dispersed by the wind (air-borne conidia) and that their dispersal follows a seasonal periodicity related to rainfall. Maximum numbers of spores were observed in July and August, with increases in conidial concentration occurring after rain or on dry windy days. The seasonal periodicity of *A. linicola* conidia was similar to that of conidia of other *Alternaria* species collected above a linseed crop (Fitt & Vloutoglou, 1992).

In general, conidia of many *Alternaria* species are dispersed by the wind although splash dispersal is occasionally important for some of these species (Rotem, 1964). The dispersal of air-borne *Alternaria* conidia follows a seasonal periodicity which is influenced by the prevailing weather conditions. The greatest numbers of air-borne *Alternaria* conidia above crops were observed after a period of rain or prolonged leaf wetness (Meredith, 1966; Schenk, 1968; Strandberg, 1977; Datar & Mayee, 1982; Allen *et al.*, 1983; Mortensen *et al.*, 1983). Unlike the biotrophic pathogens (e.g. downy mildew) which sporulate on living plant tissues and reach maximum dispersal early in the growing season, conidia of *Alternaria* species are produced in greatest numbers on senescent plant tissues and therefore the peak in their dispersal occurs late in the growing season (Cohen & Rotem, 1987).

Dispersal of *Alternaria* species follows a diurnal periodicity associated with the warmest and driest part of the day (Gregory, 1973). Hirst (1953) reported that the air-borne concentration of conidia of *Alternaria* spp. reached a well-defined

maximum at approximately 13:00 h in the UK. Maximum concentrations of *A. porri* f. sp. *solani* and *A. alternata* conidia occurred at 11:00 h in Israel (Rotem, 1964). Concentrations of air-borne *A. porri* conidia above onion crops increased after rainfall or irrigation, during windy days and between 08:00 and 14:00 h (Meredith, 1966). Numbers of *A. dauci* conidia dispersed above carrot crops increased after 08:00h as the leaves were drying, the relative humidity was decreasing and the temperature and wind speed were increasing, and reached a maximum at about 13:00 h (Langenberg *et al.*, 1977). The maximum numbers of air-borne *A. brassicicola* conidia above an oilseed rape crop were collected between 13:00 and 15:00 h, when the relative humidity decreased and temperature increased (Humpherson-Jones & Maude, 1982a). Hirst (1953) observed that diurnal periodicities of conidia of *Alternaria* spp. could be modified by changes in wind velocity and direction, temperature, humidity, sunshine, rainfall and dew.

Although wind is the main means for the dispersal of *Alternaria* conidia, there is little information on how far conidia of *Alternaria* species can be transported by wind currents. *A. brassicicola* conidia were collected up to 1800m downwind from an inoculum source (Humpherson-Jones & Maude, 1982a). Air-borne *A. alternata* conidia were detected as far as 20 m from the inoculum source in a cotton crop (Bashan & Hernandez-Saavedra, 1992). *Alternaria* species may also be effectively dispersed over long distances to agro-ecosystems that contain susceptible hosts by their ability to travel in or on seeds.

**1.3.6. Survival**

*A. linicola* is a seed-borne pathogen which can survive for long periods (5 years) in infected seed as resting hyphae, which activate when seeds start to absorb water

(Mercer *et al.*, 1991a). Conidia do not appear to be an important means of dissemination of *A. linicola* between seasons (Mercer & Hardwick, 1991; Mercer, 1992c). Although seed is considered to be the main source of primary inoculum, the possibility that the pathogen survives on infected plant debris, volunteer plants or alternate hosts (weeds), like other *Alternaria* species, has not been studied. *Alternaria* species are considered to be long-lived fungi, especially when associated with the seed of their hosts (Neergaard, 1977). *A. radicina* and *A. dauci* can survive on carrot seeds stored at -20°C for 14 and 9-14 years, respectively (Hewett, 1987). The incidence of *A. brassicae* on oilseed rape seed stored at 0°C did not decrease over a period of 20 months (Rangel, 1945). However, there was a 50% reduction in the incidence of seed infection by the same fungus after 6-8 months of storage of the seed at 25°C.

Although infected seed is probably the main means for the introduction of *Alternaria* diseases in new areas, once the pathogen becomes established in the area infected debris or alternate hosts can transfer the inoculum from one growing season to another (Rotem, 1994). Survival on plant debris has been reported for *A. alternata* on tobacco (Von Ramm & Lucas, 1963), *A. helianthi* on sunflower (Jeffrey *et al.*, 1984), *A. solani* on potato and tomato (Rotem, 1968), *A. brassicae* and *A. brassicicola* on oilseed rape and cabbage (Humpherson-Jones, 1984), *A. macrospora* on cotton (Rotem, 1990), *A. porri* on onions (Pandotra, 1965) and *A. dauci* on carrots (Netzer & Kenneth, 1969). Some *Alternaria* species can infect several related or unrelated crops and weeds (Riley, 1949; Netzer & Kenneth, 1969; Soteros, 1979; Jeffrey *et al.*, 1984; Bashan, 1984; Humpherson-Jones, 1989; Bashan & Hernandez-Saavedra, 1992;

Strandberg, 1992).

Conidia and hyphae of *Alternaria* species are resistant to unfavourable environmental conditions (Patterson, 1991). However, the prolonged survival of some *Alternaria* species is attributed to the formation of chlamydospores or microsclerotia. Chlamydospores have been observed in mycelium and conidia of *A. raphani* (Atkinson, 1953), *A. porri* f.sp. *solani* (Basu, 1971) and *A. brassicae* (Tsuneda & Skoropad, 1977a). Formation of microsclerotia has been reported on rape leaves infected by *A. brassicae* after storage at $3^0$C for 2-3 weeks (Tsuneda & Skoropad, 1977a).

**1.3.7. Toxin production**

A large number of *Alternaria* metabolites with various chemical properties have been identified as antibiotics, mycotoxins and phytotoxins. Most *Alternaria* species produce general phytotoxins such as alternariol, macrosporin, alternaric acid, tentotoxin and tenuazonic acid. These toxins, which are involved in the pathogenesis of these fungi, can cause chlorosis and necrosis of plant tissues when introduced into the plants. *A. alternata* is the only known *Alternaria* species that produces host-specific toxins with the same host-specificity as the pathogen. These toxins (AM-, AC-, AK-, AF-, AT-, and AL-toxin) are produced by six pathotypes of *A. alternata* : *A. mali* (Kohmoto *et al.*, 1976), *A. citri* (Kohmoto *et al.*, 1979), *A. kikuchiana* (Nakashima *et al.*, 1982), the Strawberry pathotype (Nishimura *et al.*, 1978), *A. longipes* (Kohmoto *et al.*, 1981) and *A. alternata* f. sp. *lycopersici* (Gilchrist & Grogan, 1976), respectively.

Although the chemical structure and properties of these toxins have been described in detail, there is little information about the effects of environmental factors

on toxin production or the stage at which these toxins are released from the pathogen. The only detailed study is that on *A. kikuchiana*, the Japanese pear pathotype of *A. alternata* that releases AK-toxin (Otani *et al.*, 1975). According to this study, the toxin molecules are released from germinating conidia before penetration of the host surface occurs. The toxin is not required to kill the host cells but to suppress the general resistance mechanism in susceptible plants by causing a slight disruption of host metabolic activities. It is not known if *A. linicola* conidia produce a host-specific toxin similar to that of *A. alternata* or a general phytotoxin like that of most the *Alternaria* species for invading the host tissue, although Leduc (1958) indicated that at least some of the pathogenic effects of *A. linicola* are due to the production of a toxin.

## 1.4. THE DISEASE

### 1.4.1. Symptomatology

The disease was first recorded on flax in Canada and described by Groves & Skolko (1944). Later it was found in Denmark by Neergaard (1945). *A. linicola* attacks seedlings and causes damping-off symptoms, with development of a brown moist rot. On older plants (especially plants weakened by drought, manganese deficiency, etc.) the fungus causes spots on the tip or the base of the leaves; in the latter case an elongated spot often appears on the stem above or below the point of attachment of the leaf (Neergaard, 1945). Moore (1946) found the disease on flax seedlings grown at Binsted, Hampshire (UK). Although the infected seedlings appeared to be healthy at first sight, their cotyledons were brown, withered and fell off at a touch.

Closer examination revealed the presence of minute dark spots on the first pair of true leaves. The spots spread irregularly to give necrotic areas 1-2mm across and on some seedlings this pair of infected leaves was discoloured and shrivelled. A pale brown streak was often present on the stem just above the cotyledons and more frequently a canker-like area with reddish brown rim developed on the hypocotyl or at the base of the main root. According to Mercer *et al.* (1991a), *A. linicola* is more pathogenic on seedlings than on mature plants. It may attack the seedlings early in the season as they emerge from their seed coat. Dark-brown lesions are formed on the cotyledons and lower leaves. Brick-red lesions can also appear on the stems and lower leaves. Infected seedlings can be seriously weakened or killed. The upper leaves of linseed crops seem to be free of disease for most of the growing season. Symptoms appear on the upper leaves, sepals and capsule cases late in the season just before harvest (Mercer *et al.*, 1991a).

### 1.4.2. Host range

*A. linicola* has been found on only two species of the genus *Linum*, namely *Linum grandiflorum* and *L. usitatissimum* (Neergaard, 1945). On the latter species it seems that the fungus occurs more frequently on linseed than on fibre flax (Beaudoin, 1989).

### 1.4.3. Epidemiology

There is little information on the epidemiology of *A. linicola* on linseed. The pathogen is carried in the seed coat as resting hyphae (Mercer *et al.*, 1991a). When the infected seed takes up water on sowing, the hyphae are activated and later, depending on the environmental conditions, symptoms appear on the cotyledons and the lower leaves. It seems that the upper parts of the plants grow

free of symptoms for most of the growing season. When the crop matures and the capsules begin to change colour they may become infected, especially if the period between flowering and harvest is wet (Mercer *et al.*, 1991a). It is not fully understood why there are no symptoms on the upper leaves for most of the growing season, or how the fungus is spread from the cotyledons and lower leaves to infect the capsules and seeds. The disease is favoured by wet weather during July and August (Mercer *et al.*, 1991a). In 1987 and 1988, when the rainfall was > 120 mm during these two months, more than 97% of the 986 seed samples tested were infected by *A. linicola*. However, after the hot, dry summers of 1989 and 1990 (approximately 70 mm of rain fell during July and August each year), only 4% of the seed samples were infected (Fitt & Vloutoglou, 1992).

Studies on the epidemiology of *A. macrospora* on cotton plants showed that the pathogen is transferred from the seeds to the developing seedlings by growth of the mycelium, either inside the germinating seed or on the outer surface of the plant (Bashan & Levanony, 1987). Bashan & Hernandez-Saavedra (1992) reported that the cotyledons support the early stages of the disease caused by *A. macrospora* on cotton crops as they appear to be more susceptible than the leaves. The cotyledons therefore provide a reservoir of inoculum to infect the lower leaves as the canopy closes, raising the humidity to a level at which the leaves become more susceptible. *A. macrospora* (Bashan & Hernandez-Saavedra, 1992) and *A. alternata* (Rotem *et al.*, 1988) can produce symptomless or miniature infections on cotton plants. It is not known why these infections do not develop into large, visible lesions although Rotem *et al.*, (1988) suggested that symptom expression is greatly enhanced by sunlight. Young tissues of oilseed rape were also more resistant to *A. brassicae* infection than

older ones (Köhle & Hoffman, 1989). Moisture requirements are more critical than temperature for the establishment of *Alternaria* diseases as most of them can develop over a wide range of temperatures (Strandberg, 1988; Bashan & Hernandez-Saavedra, 1992; Humpherson-Jones, 1992). Leaf wetness and dew are very important for the progress of diseases caused by *A. dauci* on carrots (Hooker, 1944; Netzer & Kenneth, 1969; Langenberg *et al.*, 1977), *A. solani* on potato and tomato plants (Rowell, 1953; Rotem & Reichert, 1964; Barksdale, 1969), *A. macrospora* on cotton ( Ling, 1944; Rane & Patel, 1956) and *A. brassicae* and *A. brassicicola* on brassica crops (Humpherson-Jones, 1992).

### 1.4.4. Control

#### 1.4.4.1. Chemical control

The most effective method for controlling the seed-borne phase of *A. linicola* is by seed treatment with iprodione (Rovral) or prochloraz (Prelude) (Mercer *et al.*, 1985; Mercer & McGimpsey, 1987; Mercer & Hardwick, 1991). However, in the UK iprodione has been replaced by prochloraz since 1986, as there have been indications of an increase in the proportion of iprodione-resistant strains of *A. linicola* (Mercer *et al.*, 1988). Although prochloraz, as a seed treatment, is highly effective against most of the seed-borne pathogens of linseed (including *A. linicola)*, it is considered to have a fungistatic rather than a fungitoxic effect on *A. linicola,* as the fungus can be isolated from seedlings grown from prochloraz-treated seed (Mercer *et al.*, 1989). The effects of fungicide applications on the incidence of *A. linicola* disease and yield of linseed crops differ between different regions in the UK and between different years in the same region. A single spray with iprodione at the green capsule

stage had no effect on the incidence of *A. linicola* on the seed and did not affect yield (Mercer *et al.*, 1992a). Prochloraz applied at weekly intervals had no effect on yield and only a slight effect on *A. linicola* incidence on seed (Mercer *et al.*, 1989). Hardwick & Mercer (1989) reported differences in ripening due to the fungicide sprays and some effect on yield, but no effect on the incidence of *A. linicola*. By contrast, Fitt & Ferguson (1990) reported relatively good control of *A. linicola* disease with fungicide sprays in 1988 and 1989 and associated yield increases.

Iprodione is also used as a seed treatment for controlling *A. brassicicola* infection of *Brassica oleracea* seeds (Maude & Humpherson-Jones, 1980a; Maude *et al.*, 1984) and *A. dauci* on carrot seeds (Strandberg, 1984). Tylkowska & Kryślak (1986) reported that although iprodione as a seed treatment was very effective for controlling low levels (<20%) of *A. radicina* infection on carrot seeds, it was less effective when the seed samples had a higher incidence of infection (>40%). Iprodione applied as a single spray at petal fall not only controlled *A. brassicae* and *A. brassicicola* pod infection but also increased the yield of brassica crops (Cox *et al.*, 1981; Evans & Gladders, 1981).

It is not always possible to develop schemes for forecasting diseases caused by *Alternaria* species because they can develop under various weather conditions and they are also affected by the age of the crop (Rotem, 1994). However, forecasting systems such as EPIDEM (Waggoner & Horsfall, 1969) and FAST (Madden *et al.*, 1978) have been developed to determine periods when environmental conditions are favourable for the development of diseases caused by *A. solani* on potato and tomato, respectively, in order to optimize fungicide spray schedules.

1.4.4.2. Cultural methods

Many *Alternaria* species can survive between crop seasons by a number of mechanisms (seed, volunteers, infected debris, alternate hosts, etc.) to provide inoculum to infect succeeding crops even under conditions that do not appear favourable for survival. This partially explains why crop rotation has been and continues to be important in managing the diseases caused by *Alternaria* species. Seed health tests to exclude heavily infected seed from seedling production have been used to minimize the primary inoculum (Valkonen & Koponen, 1990). Additionally, effective burial of infected debris soon after harvest can reduce the danger that the following crop will be infected by air-borne inoculum. However, seeds are often deposited on the soil during harvest and these should be allowed to germinate before ploughing to reduce the number of infected volunteer plants in succeeding crops (Humpherson-Jones, 1992).

1.4.4.3. Biological control

Recent work (Mercer *et al.*, 1991b; 1992a) investigated control of *A. linicola* with sprays of spore suspensions of *Trichoderma viride* and *Epicoccum nigrum*; with some isolates the level of control was equivalent to that achieved by prochloraz sprays although less than that achieved by iprodione sprays. Biological agents used as seed treatments have been reported to be alternatives to fungicides for control of other *Alternaria* species, although they can control superficial but not internal infections (Vannacci & Harman, 1987). Detailed studies by Tsuneda *et al.* (1976) showed that *Nectria inventa* is a destructive mycoparasite of *A. brassicae*. Some *Trichoderma*, *Gliocladium* and *Penicillium* species reduced seed transmission of *A. brassicicola* in cabbage (Wu & Lu, 1984). Vannacci & Harman (1987) reported that *Periconia* and *Penicillium* species and *Chaetomium globosum* gave control of *A. brassicicola*

on brassica seeds equivalent to that by iprodione. A powdered preparation of *Streptomyces griseoviridis* was also very effective (80 - 90% control) against *A. brassicicola* on artificially inoculated brassica seeds (Tahvonen & Avikainen, 1987).

1.4.4.4. Resistant cultivars

There is a variability in the resistance of linseed cultivars to *A. linicola*. However, this variability is often attributed to differences in cultivar maturity (Mercer & Jeffs, 1988).

## 1.5. OBJECTIVES

As the area sown with linseed in the UK has increased so have the disease problems in the crop. Diseases caused by seed-borne pathogens, like that caused by *A. linicola*, have become very important in the last few years, mainly because these diseases can decrease crop yield, oil quantity and quality. In the case of the disease caused by *A. linicola* on linseed crops there is little information on either the biology of the pathogen or the epidemiology of the disease. Better knowledge of the life cycle of *A. linicola* and of the effects of different environmental factors on conidial germination, infection and sporulation of the pathogen and on spread of the disease in linseed crops would be useful not only in predicting disease development in the crop but also in developing effective disease management strategies, which will minimize the use of fungicides. Therefore, laboratory and controlled environment experiments were designed to :

- Study the effects of environmental factors such as temperature, leaf wetness duration, light regime and their interactions on germination of *A. linicola* conidia. The mode

of penetration of linseed leaf tissues by the pathogen was also studied.

- Test methods for inducing sporulation of *A. linicola in vitro* and to study the effects of temperature, leaf wetness duration, light regime and their interactions on the sporulation of the pathogen on linseed plants.

- Study the effects of inoculum concentration and of the environmental factors such as temperature, leaf wetness duration and light regime on infection and development of symptoms by *A. linicola* on linseed plants.

- Examine the mycoflora present on linseed seeds and to estimate the incidence of *A. linicola* infection on seed samples from different origins. The efficiency by which *A. linicola* is transmitted from infected seeds to the seedlings was also examined.

Field experiments were also done to :

- Study the seasonal and diurnal dispersal of air-borne *A. linicola* conidia above linseed crops by using the Burkard spore sampler. The effects of environmental factors (rainfall, temperature, wind speed) on the dispersal of *A. linicola* conidia were also examined.

- Investigate the inoculum potential of *A. linicola* in a linseed crop by using bait plants.

- Study the *A. linicola* disease gradients and spore dispersal gradients from point or line inoculum sources.

- Examine whether *A. linicola* can survive on infected linseed stem debris, volunteer linseed plants or alternate hosts (weeds) and whether infected debris can initiate an epidemic early in the growing season. The formation by *A. linicola* of structures (chlamydospores, microsclerotia, etc.) which might be involved in a long-term survival of the pathogen on the infected debris was also studied.

- Study the effects of the application of fungicide sprays to the crop on : a) the development of the disease during the growing season, b) the seed-borne phase of *A. linicola* and c) crop yield.

# CHAPTER II. GENERAL MATERIALS AND METHODS

## 2.1. Glasshouse and controlled environment experiments

### 2.1.1. Isolation of *A. linicola* - production of single-spore isolates

*A. linicola* was isolated from naturally infected linseed plants (cv. Antares) during the period 1989-1992. The infected plant tissues (cotyledons, leaves, stems, buds, sepals or seeds) were immersed for 1 min in 200 ml of 1% NaOCl to remove superficial contaminants. The plant tissues were then rinsed in two changes of sterile distilled water and allowed to dry on sterile filter paper (Whatman No 1) before placing them on V-8 agar plates (20 ml per plate). The plates were sealed with parafilm and incubated under diurnal NUV-light [12 h NUV-light (365 nm)/12 h darkness] at 20°C. After 7 days of incubation, the plates were examined under a stereo-microscope (x 40 magnification) and identification of *A. linicola* colonies which emerged from the infected plant tissues was based on the conidial morphology.

Plugs (5 mm in diameter) were cut with a cork borer from the edge of the colonies and transferred singly onto a new V-8 agar plate. The plates were sealed with parafilm and incubated for 7 days under diurnal NUV-light [12h NUV-light (365 nm)/12 h darkness] to induce sporulation. Ten ml of sterile distilled water containing 0.01 ml of 0.01% Tween 80 (polyxyethylene sorbitan monolaurate) as a wetting agent were added to each Petri plate. Conidia were dislodged by gentle rubbing of the agar surface with a sterile bent glass rod. The resulting conidial suspension of each isolate was filtered through two layers of sterile muslin; the concentration of conidia of each isolate was determined with a haemocytometer (4

counts per conidial suspension) and was adjusted to 3 x $10^4$ conidia $ml^{-1}$ by dilution with sterile distilled water. For the production of single-spore isolates, 0.1 ml of each conidial suspension was spread on a water agar (see section 2.1.7) plate by using a sterile bent glass rod. The plates were then examined under a stereo-microscope (x 40 magnification) and *A. linicola* conidia were transferred singly onto V-8 agar plates by using a sterile needle.

### 2.1.2. Long-term storage of isolates

All the isolates were from the Rothamsted Experimental Station collection and the stock cultures were maintained by the following method : McCartney bottles were filled up to 2/3 of their volume with a mixture of loam : compost : sand (1 : 2 : 1). Two ml of distilled water were added to each bottle before autoclaving them for 30 min at 120°C. The bottles with the mixture were autoclaved a second time 24 h later. After cooling, each bottle was inoculated under sterile conditions with 2 plugs (5 mm in diameter) cut with a cork borer from the edge of a culture grown on a V-8 agar plate. The bottles were incubated at room temperature until the mixture of loam, compost and sand was colonized by the fungal hyphae (approximately 4 days after the inoculation) and then they were stored at 4°C until required. To re-isolate the fungus on V-8 agar plates, a small amount of the inoculated mixture of loam, compost and sand was sprinkled onto the plates.

### 2.1.3. Inoculum production

For the production of inoculum for artificial inoculations the method of Shahin & Shepard (1979) was used. Cultures of the isolates were grown initially in 9-cm

diameter Petri plates, each containing 20 ml of V-8 agar medium (see section 2.1.7). After 4 days of incubation of the plates in darkness at 20°C, and before the appearance of 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 individually transferred to the surface of the sporulation medium (S-medium, see section 2.1.7) and 2 ml of sterile distilled water were added to partially cover the blocks. In total one half of the area of the growing colony in the V-8 agar medium was cut into blocks. The plates were sealed with parafilm and incubated under diurnal NUV-light [12 h NUV-light/12 h darkness] at 20°C. The source of NUV-light was two 36W Philips TLD fluorescent "black light" lamps placed 27 cm above the plates. The lamps were 10 cm apart (center to center) and emitted a light spectrum with a peak of 365 nm. After 3 days of incubation, when the agar blocks were covered with conidia, 10 ml of sterile distilled water (containing 0.01 ml of 0.01% Tween 80 as a wetting agent) were added to each plate. Conidia were dislodged by gentle rubbing of the agar surface with a sterile bent glass rod. The resulting conidial suspension was filtered through two layers of sterile muslin. The concentration of conidia was determined with a haemocytometer (4 counts per conidial suspension) and was adjusted to the designated concentration by dilution with sterile distilled water.

**2.1.4. Controlled environment cabinets**

Controlled environment cabinets (length x width x height = 2.5 x 1 x 1.4 m) designed at Rothamsted Experimental Station were used. The temperature set for each cabinet was monitored by a min-max mercury thermometer. The light was provided in each

cabinet by 18 fluorescent lamps placed 70 cm above the plants. The light intensity at plant level (15 cm above the floor of the cabinet), measured with a quantum radiometer photometer light instrument (T & P. J. Crump), varied across the floor of the cabinets in both directions, ranging from 120 to 160 $\mu$ Einsteins $m^{-2}$ $sec^{-1}$ of photosynthetically active radiation (PAR). Humidity was not controlled, but was monitored with a hand-held HMKI 31 Vaisala humidity meter, which had been calibrated with a Vaisala humidity meter calibrator HMKII, employing LiCl and $K_2SO_4$ salt solutions.

### 2.1.5. Plant production

Linseed seed (cv. Antares) was used in the experiments (unless otherwise stated). Seed was obtained from International Seed Producers, Bury St Edmund's, treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem) and stored at 4°C. Seeds were sown 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.]. The pots were placed in controlled environment cabinets (see section 2.1.4) set at 18°C/13°C day/night temperatures (Fig. 2.1). The daylength was 16 h (from 24:00 to 16:00 h) and the light was provided by 18 fluorescent lamps. Because of differences in the light intensity across the cabinets, the plants were periodically moved around in each cabinet. Unless otherwise stated, seedlings were thinned immediately after emergence to give 10 plants per pot.

### 2.1.6. Plant inoculation

Linseed plants were artificially inoculated by spraying them with conidial suspensions

**Figure 2.1.** Linseed seedlings (cv. Antares) growing in the controlled environment cabinet designed at Rothamsted Experimental Station and used in the experiments.

of *A. linicola* prepared by the method described in section 2.1.3. The plants were sprayed until run-off by using a hand-operarated spray gun (Humbrol spray gun, Humbrol, Marfleet, Hull, UK).

### 2.1.7. Culture media

Water agar

Agar (Oxoid No 3)................................ 15 g

Distilled water........................................1 l

Potato dextrose agar (PDA)

Potato dextrose agar (Oxoid)...................39 g

Distilled water........................................1 l

Malt extract agar (MEA)

Malt extract (Oxoid)..............................20 g

Agar (Oxoid No 3)................................20 g

Distilled water........................................1 l

Corn meal agar (CMA)

Coarse cornmeal....................................15 g

Agar (Oxoid No 3).................................15 g

Distilled water........................................1 l

V-8 juice agar (V-8 agar)

V-8 juice........................................165 ml

$CaCO_3$..............................................1 g

Agar (Oxoid No 3)..............................20 g

Distilled water..................................810 ml

0.1 M KOH........................................25 ml

pH 6.5 - 7.0

To prevent bacterial growth the antibiotics streptomycin (100 mg $l^{-1}$) and penicillin (26 mg $l^{-1}$) were added to the V-8 juice agar medium after autoclaving.

S - medium (Shahin & Shepard, 1979)

Sucrose...........................................20 g

$CaCO_3$..........................................30 g

Agar (Oxoid No 3)...........................20 g

Distilled water..................................1 l

pH 7.4

The agar media were sterilized by autoclaving them for 30 min at 120$^0$C at 1.05 kg $cm^{-2}$ and poured into Petri plates (20 ml per plate) under sterile conditions.

**2.1.8. Staining plant tissues**

Prior to examination under the light microscope, plant tissues (cotyledons, leaves and sepals) were stained with cotton blue in lactophenol :

1% w/v cotton blue.............................5 ml

Glacial acetic acid.............................20 ml

Lactophenol.......................................100 ml

## 2.2. Field experiments

### 2.2.1. Induction of *A. linicola* sporulation on infected plant tissues

Since the symptoms caused by *A. linicola* on linseed plants were similar to those caused by other fungi (e.g. *B. cinerea*), the identification of the pathogen was based on conidial morphology. For inducing sporulation of *A. linicola* on plant tissues with symptoms, the plant tissues (cotyledons, leaves, stems, buds or sepals) were placed on two layers of moistened filter paper (Whatman No 1) lining the bottom of Petri dishes (9 cm in diameter) (dew chambers). The dishes were sealed with parafilm and incubated under diurnal NUV-light [12 h NUV-light (365 nm)/12 h darkness] at 20°C until conidia formed (approximately 4-5 days).

### 2.2.2. Spore samplers

For monitoring the daily or hourly concentrations of air-borne *A. linicola* conidia above linseed crops, a Burkard spore sampler was used (Fig. 7.1). This type of sampler is a 7-day recording volumetric spore sampler, which sucks 10 l air $min^{-1}$ through an orifice (width x length = 2 x 14 mm) (Burkard Manufacturing Co. Ltd., Woodcock Hill, Industrial Estate, Rickmansworth, Herts, UK) (Hirst, 1952). The conidia are deposited on a cellophane tape [coated with vaseline wax (see section 2.2.3) to retain the conidia] wrapped round a drum which rotates behind the orifice at approximately 2 mm $h^{-1}$. After 7 days of exposure the tape was removed and cut into 7 sections of 48 mm each (each section corresponding a 24-h period of exposure).

A perspex template was used to divide the tape accurately into daily sections. Each section was mounted on a microscope slide with gelvatol (see section 2.2.3) and covered with a cover glass.

For estimation of the daily concentrations of *A. linicola* conidia, the number of conidia which were deposited on three traverses along each slide were counted under a light microscope (x 250 magnification). The mean daily concentration of the conidia was estimated by using the following equation (McCartney, H.A. & Lacey, M.E., personal communication):

$$N = (Nc \times 972) / f \qquad (2.1)$$

in which Nc = mean number of conidia counted in three traverses along each slide and f = microscope field width ($\mu$m).

For estimation of the hourly concentration of air-borne *A. linicola* conidia, the number of conidia which was deposited on a 2-mm traverse across a slide was counted under the light microscope (x 250 magnification). The diurnal periodicity of *A. linicola* conidia was calculated by using the following equation :

$$N = Nc / V \qquad (2.2)$$

in which Nc = number of conidia counted on one traverse across the slide and V = volume of air sampled by the sampler in 60 min. For a flow rate of 10 l $min^{-1}$ and a microscopic field width of 471 $\mu$m :

$$N = Nc \times 7.1 \text{ conidia } m^{-3} \qquad (2.3)$$

To study the horizontal or vertical dispersal of *A. linicola* conidia in a linseed crop naturally infected by the pathogen, the rotorod spore sampler was used (Fig. 7.6). This type of sampler consisted of a pair of vertical arms (length x width = 5.7 x 0.16 cm), which were rotated at approximately 3500 rpm by a small electric

motor (Fig. 7.6). Conidia were impacted on a cellotape strip mounted at the leading edge of the rotating arms and coated with vaseline wax (see section 2.2.3). At the end of the exposure time, the cellotape strips were removed, mounted on microscope slides with gelvatol (see section 2.2.3) and covered with a cover glass. The numbers of conidia collected were counted under a light microscope (x 250 magnification) and the hourly concentration of conidia was estimated by using the equation :

$$C = (N \times A) / (V \times T) \qquad (2.4)$$

in which N = number of conidia counted, A = total area of strips (2 x width of one strip x length of one strip), V = volume of air sampled by the rotorod (2 arms) and T = time of exposure (min).

### 2.2.3. Coating and slide mounting media

<u>Vaseline wax</u>

Vaseline - petroleum jelly.................150 ml

Paraffin wax...................................18 g

Phenol..........................................10 g

<u>Gelvatol</u>

Gelvatol.........................................35 g

Glycerol........................................50 ml

Phenol............................................2 g

Distilled water..............................100 ml

### 2.2.4. Meteorological data

Daily meteorological data were provided by a meteorological station on the Rothamsted site. Temperatures (minimum and maximum) were measured by standard meteorological thermometers in a Stevenson screen at a height of 1 m above grass. For measuring the minimum and maximum temperatures the types of thermometer used were alcohol in glass and mercury in glass, respectively. Mean daily temperatures were calculated by using the equation :

$$Tmean = (T\,max + Tmin) / 2 \qquad (2.5)$$

Daily rainfall was measured in a "Turf-wall" rain gauge (12.7 cm in diameter). Data on the hourly wind speed were provided by an automatic meteorological station situated approximately 1 km from the experimental site. The hourly wind speed measured by a cup anemometer at a height of 1 m above ground was recorded by a Campbell 21X data logger.

# CHAPTER III. SPORULATION OF *ALTERNARIA LINICOLA*

## 3.1. Introduction

*Alternaria linicola*, like many other *Alternaria* species, is a necrotrophic facultative pathogen. Most of the *Alternaria* species either fail to sporulate in culture or their sporulation capacity declines or is lost after few serial transfers on media. However, production of large numbers of conidia *in vitro* is often necessary for use as inoculum in experimental work such as screening of varieties for resistance to these pathogens, the evaluation of different fungicides *in vitro* and the identification of critical phases in the epidemiology of diseases. For this reason, considerable attention has been directed towards improving the *in vitro* production of conidia of different *Alternaria* species by physical or chemical agents (Rands, 1917; McCallan & Chan, 1943; Agaraki, 1963; Vakalounakis, 1982; Miles & Wilcoxson, 1984; Senior *et al.*, 1987). Among the methods that have been used for inducing sporulation *in vitro*, exposure of the cultures to near ultraviolet (NUV) light or wounding the mycelium are the most common (Rands, 1917; McCallan & Chan, 1943; Charlton, 1953; Lukens, 1960; Ludwig *et al.*, 1962; Barksdale, 1968; Shahin & Shepard, 1979). However, studies of the factors that induce sporulation *in vitro* are often irrelevant to the sporulation patterns of the same fungus *in vivo*, as they ignore a) the influence of the host on reproduction of its parasite and b) the effects of environmental factors.

Of the three *Alternaria* species that are commonly isolated from linseed

plants in the UK, only *A. alternata* sporulates readily in culture. *A. linicola* and *A. infectoria* often (depending on the isolate) fail to sporulate or produce only small numbers of conidia. Moreover, there is no information on the environmental factors that affect the sporulation *in vivo* of *A. linicola,* which is considered to be the most pathogenic *Alternaria* species on linseed in the UK.

## 3.2. Objectives

1. To study the effects of different media, light regime, culture conditions or wounding the mycelium on the sporulation *in vitro* of the three *Alternaria* species isolated from linseed.
2. To examine the effects of continuous or interrupted leaf wetness, light regime and temperature, alone or in combination, on sporulation of *A. linicola* on linseed plants.

## 3.3. Materials and Methods

### 3.3.1. Sporulation *in vitro*

#### 3.3.1.1. Fungal isolates

Sixteen isolates of *A. linicola* (Al 1-Al 16), nine of *A. infectoria* (Ai 1-Ai 9) and nine of *A. alternata* (Aa 1-Aa 9) were used; all were from the Rothamsted Experimental Station collection and were isolated from naturally infected linseed plants (cv. Antares) during the period 1989-91. Stock cultures were maintained by

the method described in section 2.1.2.

**3.3.1.2. Effect of light regime on sporulation**

Cultures of all isolates were grown initially in 9-cm diameter Petri plates, each containing 20 ml of V-8 agar medium (see section 2.1.7). After 4 days in darkness at 20°C, a 4-mm diameter plug was taken from the edge of the actively growing culture and positioned, centrally, mycelium surface down, on a new V-8 agar plate. Four Petri plates per isolate served as replicates. After 5 days of incubation in darkness at 20°C the cultures were exposed to NUV-light, either constant or diurnal [12h NUV-light (365 nm)/12h darkness], for 2 days before being placed in darkness at 10°C for 2 additional days.

The plates were assessed : a) for colony growth (diameter in cm) and sporulation after incubation in darkness at 20°C, and b) for sporulation only, after the exposure to NUV-light and at the end of the second dark period (10°C). Sporulation was assessed under a stereo-microscope (x 20 magnification) and the production of conidia was recorded using an arbitrary scale: (-) : no sporulation, (+) : poor, (++) : moderate and (+++) : abundant sporulation.

**3.3.1.3. Effects of different media, reduction of vegetative growth and wounding of the mycelium on sporulation**

Two isolates of each *Alternaria* species (*A. linicola*, *A. infectoria*, *A. alternata*) were used. The isolates were chosen on the basis of their relative ability to sporulate (sporulating and non- or poorly sporulating isolates) on V-8 agar medium after exposure to diurnal NUV-light for 5 days at 20°C. The isolates in each group

were: a) sporulating isolates: Al 15, Ai 1, Aa 9 and b) non- or poorly sporulating isolates : Al 17, Ai 4, Aa 10.

The media tested were : malt extract agar (MEA), cornmeal agar (CMA), potato dextrose agar (PDA), V-8 juice agar (V-8 agar) and S-medium. The media were prepared by the method described in section 2.1.7.

In order to study the effect of different media on sporulation, plates containing 20 ml of MEA, CMA, PDA or V-8 agar medium were inoculated with 4-mm diameter plugs taken from actively growing cultures of these six isolates. The plates were incubated in darkness for 5 days at 20°C before being exposed to diurnal NUV-light for 5 additional days at 20°C. The effect of reduction of the vegetative growth on sporulation was studied on these media (MEA, CMA, PDA and V-8 agar) by covering one set of the plates with a 9-cm diameter cellophane disc in order to reduce the mycelial growth. These plates were inoculated and incubated in darkness for 5 days, then under NUV-light for 2 days.

The induction of sporulation by wounding the mycelium was tested by using the method described by Shahin & Shepard (1979). All the isolates were grown on the primary media (MEA, CMA, PDA and V-8 agar) in darkness for 5 days at 20°C. After this period and prior to appearance of aerial mycelium, the agar containing the developing colony was cut with a sterile scalpel into small blocks (3 $mm^2$) which were individually transferred to the surface of the sporulation medium (S-medium). In total one half of the area of the growing colony in the primary media was cut into blocks. Sterile distilled water (2 ml) was added to partially cover the mycelial blocks and the plates were sealed with parafilm. One set of plates was incubated in darkness for 3 days at 20°C and

another set was exposed to diurnal NUV-light for 3 days at 20°C. In all the tests there were four replicates. The cultures were assessed for sporulation under a stereo-microscope (x 20 magnification) and the amount of sporulation was recorded using an arbitrary scale : (-) : no sporulation, (+) : poor, (++) : moderate and (+++) : abundant sporulation.

### 3.3.2. Sporulation *in vivo*

#### 3.3.2.1. Isolates and inoculum production

Mixtures of five single-spore isolates of *A. linicola* (Al 12, Al 25, Al 38, Al 39 and Al 46) isolated from naturally infected linseed plants (cv. Antares) during the period 1989-1992, were used in all the experiments. The isolates were from the Rothamsted Experimental Station collection and the stock cultures were maintained by the method described in section 2.1.2. To produce conidia for artificial inoculations the method of Shahin & Shepard (1979), described in section 2.1.3 was used. Ten ml of sterile distilled water containing 0.01 ml of 0.01% Tween 80 (polyoxyethylene sorbitan monolaurate) as a wetting agent were added to each Petri plate. Conidia were dislodged by gentle rubbing of the agar surface with a sterile bent glass rod. The resulting conidial suspension of each isolate was filtered through two layers of sterile muslin; the concentration of conidia of each individual isolate was determined with a haemocytometer (4 counts per conidial suspension) and was adjusted to $3x10^4$ conidia $ml^{-1}$ by dilution with sterile distilled water. The final inoculum was prepared by mixing together 300 ml of the conidial suspension of each individual isolate. The preparation of inoculum took 30 min

and at the end of this period no conidial germination was observed.

### 3.3.2.2. Plant production

All experiments were done on linseed (cv. Antares). Plants were grown in 13 cm diameter plastic pots (15 plants per pot, unless otherwise stated), by the method described in section 2.1.5. The pots were placed in controlled environment cabinets (designed at Rothamsted Experimental Station) set at 18°C/13°C day/night temperatures (Fig. 2.1). The daylength was 16 h (from 24:00 to 16:00 h) and the light was provided in each cabinet by 18 fluorescent lamps placed 70 cm above the plants. The light intensity measured at plant level by the method described in section 2.1.4 was 120-160 $\mu$Einsteins $m^{-2}$ $sec^{-1}$. The relative humidity in the cabinets, measured by the method described in section 2.1.4, ranged from 65 to 75%.

### 3.3.2.3. Plant inoculation

When the plants were at the cotyledon growth stage (10 days old) they were inoculated by the method described in section 2.1.6. Approximately 20 ml of the conidial suspension was sprayed onto the plants in each pot at the beginning of a dark period (at approximately 16:00 h). The inoculated plants were covered for 72h with polyethylene bags sprayed inside with water (100% r.h.) to provide a water-saturated atmosphere favourable for infection.

### 3.3.2.4. Post inoculation treatments

Sporulation tests started approximately 8 days after inoculation. They were done

on attached cotyledons (18 days old) when necrotic lesions had fully developed on them but had produced neither conidiophores nor conidia. The experiments were done at 15$^0$C unless otherwise stated. The temperature inside the polyethylene bags deviated from the temperature set by less than +2$^0$ C. The lights in each cabinet were continuously on from the time the treatments were applied until the end of the experimental period (60 h or 96 h depending on the experiment). During the dark periods the plants were covered with aluminium foil.

In all the experiments the terms "wet light" or "wet darkness" are used when plants exposed to light or darkness were kept in transparent moistened polyethylene bags (100% r.h.). The terms "dry light" or "dry darkness" are used when plants were exposed to light or darkness after removal of the polyethylene bags (65 -75% r.h.) and drying with a hair-drier.

**3.3.2.5. Procedure for counting conidia**

Twenty cotyledons per replicate and treatment were placed in McCartney bottles containing 3 ml of sterile distilled water (0.01 ml of 0.01% Tween 80 was added as a wetting agent) and shaken to detach the conidia produced. By this treatment the conidia which had been used for inoculation were not detached. The concentration of conidia in the resulting conidial suspensions was determined with a haemocytometer (4 counts per conidial suspension); the mean number of conidia produced per cm$^2$ cotyledon area was estimated (mean area of cotyledon c. 1.45 cm$^2$). Numbers of conidia were counted at the end of the incubation time which was 96 h unless otherwise stated.

### 3.3.2.6. Experimental design

All the experiments were in a randomized block design with five blocks, with one pot (15 plants per pot unless otherwise stated) per block for each treatment.

### 3.3.2.7. Effects of temperature, wet period and light regime on sporulation

Two experiments (Experiment I & II) were done to study the effects of temperature, wet period and light regime on sporulation of *A. linicola.* In both experiments the temperatures tested were either constant or alternating. In Experiment I, sporulation of *A. linicola* was studied under the constant temperatures 10°C and 15°C and alternating 15°C/10°C (day/night) temperatures. In Experiment II, the constant temperatures were 15°C and 20°C and the alternating were 20°C/15°C (day/night). In both experiments the plants were incubated for 96 h at each temperature (constant or alternating) under wet or dry light periods (each 12 h) alternating with wet dark periods (each 12 h). Controls were plants kept for 96 h under continuous wet darkness, wet light or dry light at constant temperatures 10, 15 or 20°C (Fig. 3.1). The number of conidia produced under different treatments was counted at the end of the incubation time (96 h) by using the method described in section 3.3.2.5. Additionally, one cotyledon per replicate and treatment was collected every 12 h, stained with cotton blue in lactophenol (see section 2.1.8) and observed under a light microscope at x 400 magnification to assess development of conidiophores or conidia. The production of conidia was also recorded by using an arbitrary scale (0 - 5) : 0 = no sporulation, 1 = very poor, 2 = poor, 3 = moderate, 4 = good, and 5 = abundant sporulation.

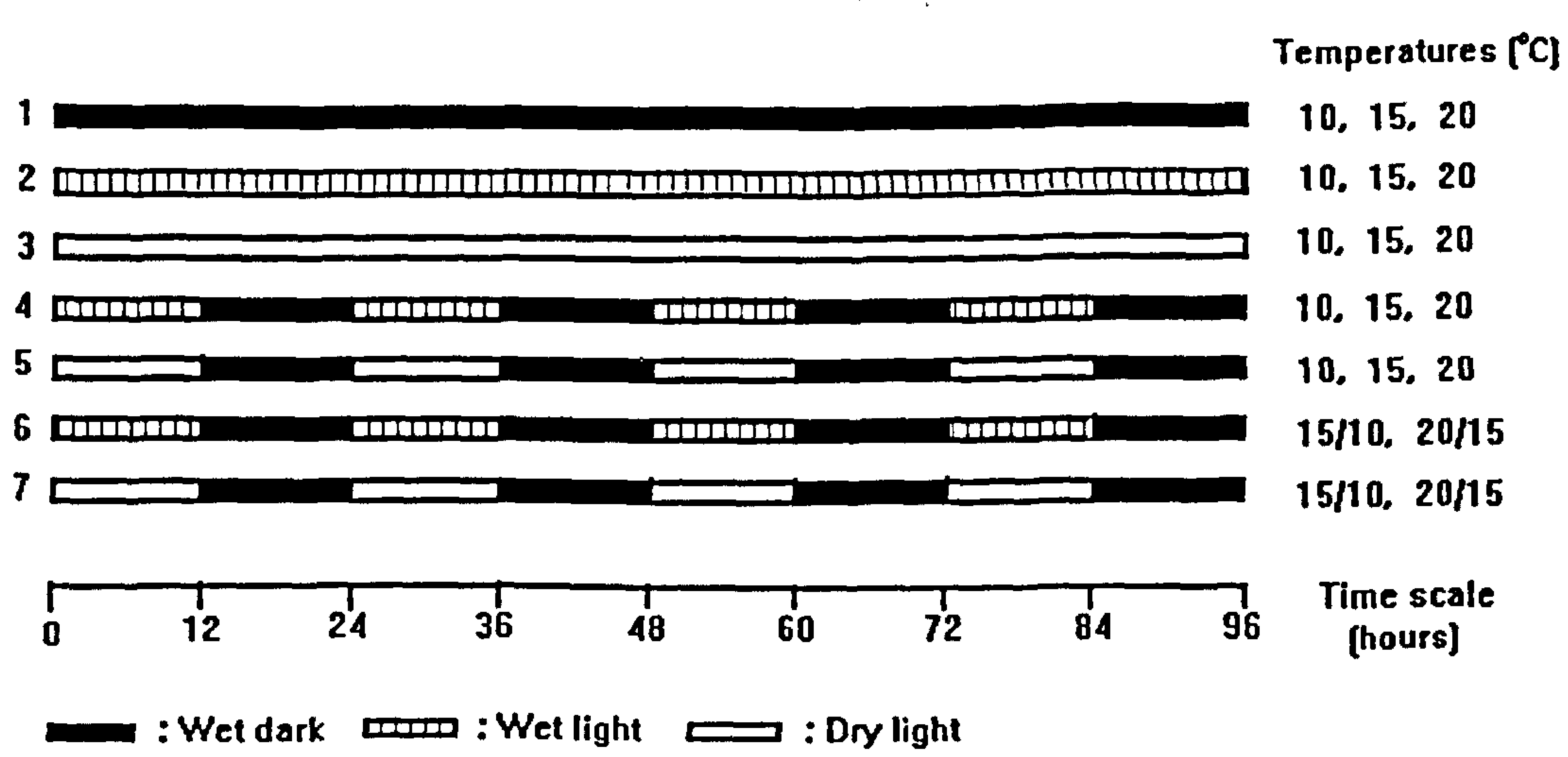



**Figure 3.1.** Treatments used to study the effects of temperature, wet periods and light regime on sporulation *in vivo* of *A. linicola*. Treatments 4 & 5 were tested under constant temperatures : 10°C & 15°C (Experiment I) or 15°C & 20°C (Experiment II). Treatments 6 & 7 were tested under alternating day/night temperatures : 15°C/10°C (Experiment I) or 20°C/15°C (Experiment II). Controls were plants exposed for 96h to continuous wet dark, wet light or dry light periods (treatments 1, 2 and 3, respectively) at constant temperatures 10°C, 15°C or 20°C.

**3.3.2.8. Effects of a) the duration of a wet or dry light period applied before a period of wet darkness, and b) interrupting a continuous wet dark period by several wet or dry light periods on sporulation**

The experiment was done at a constant temperature of 15°C. Plants were exposed either a) to wet or dry light for 12, 24, 48 or 72 h while the rest of the incubation time (total incubation time 96 h) was in wet darkness or b) to wet or dry light periods (each 12 h) alternating with wet dark periods (each 12 h). Controls were plants kept for 96 h under continuous wet light, dry light or wet darkness (Fig. 3.2). The number of conidia produced under different treatments was counted at the end of the incubation time (96 h) by using the method described in section 3.3.2.5. Moreover, one cotyledon per replicate and treatment was collected either at the end of the initial wet or dry light periods or every 12 h for the alternating treatments. It was stained with cotton blue in lactophenol (see section 2.1.8) and observed under a light microscope at x 400 magnification to assess development of conidiophores or conidia. The production of conidia was also recorded, by using the arbitrary scale described in section 3.3.2.7.

**3.3.2.9. Effects of interrupting a continuous wet dark period by wet light, dry light or dry dark periods on sporulation**

The experiment was done at a constant temperature of 15°C. The plants, after being exposed to an initial wet dark period for 24 h, were subjected to wet light, dry light or dry darkness for 2, 6 or 12 h. After the end of these periods the plants were incubated under wet darkness for long enough to complete a period of 48 h in wet darkness (initial plus final). Controls were plants kept under continuous wet

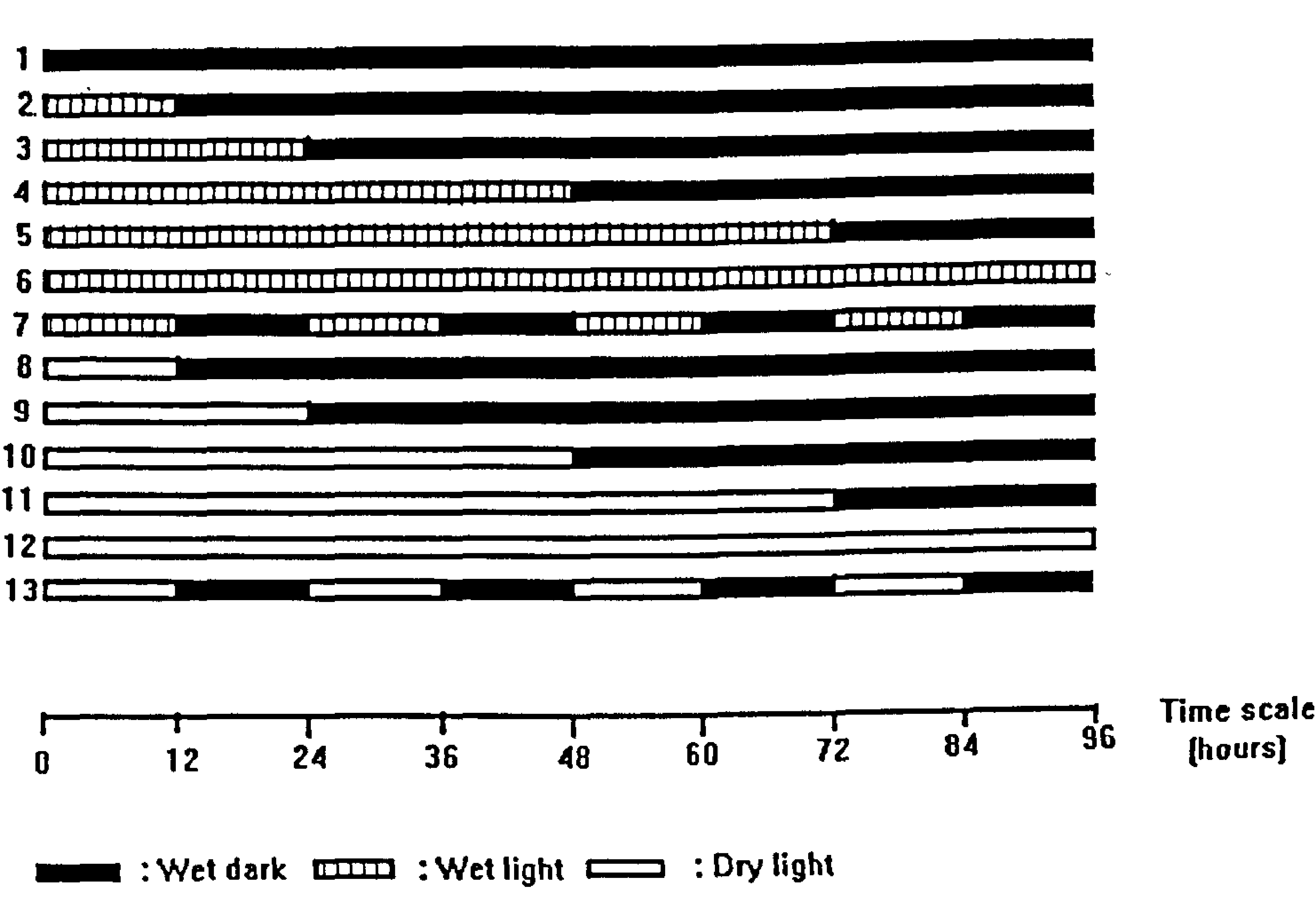



**Figure 3.2.** Treatments used to study the effects of a) the duration of a wet or dry light period applied before a period of wet darkness and b) interrupting a continuous wet dark period by several wet or dry light periods on sporulation *in vivo* of *A. linicola,* at 15°C. Controls were plants exposed for 96h to continuous wet dark (treatment 1), wet light (treatment 6) or dry light (treatment 12) periods.

darkness for 48, 50, 54 or 60 h, and under continuous wet light, dry light or dry darkness for 60 h (Fig. 3.3).

#### 3.3.2.10. Effects of interrupting a) a continuous wet dark period by several dry light or dry dark periods, and b) a continuous wet light period by several dry periods on sporulation

The experiment was done at a constant temperature of 15°C. The plants were incubated for 96 h under a) wet dark periods (each 12 h) alternating with dry light or dry dark periods (each 12 h) or b) under wet light periods (each 12 h) alternating with dry light periods (each 12 h). Controls were plants kept for 96 h under continuous wet darkness, dry darkness, wet light or dry light (Fig. 3.4).

## 3.4. Results

### 3.4.1. Sporulation *in vitro*

#### 3.4.1.1. Effect of light regime on sporulation

The effect of light conditions on conidial production of *A. linicola*, *A. infectoria* and *A. alternata* is shown in Tables 3.1, 3.2 and 3.3. Although there was no significant difference in the colony radial growth between the three *Alternaria* species or between the isolates of each tested, with the only exception of one *A. linicola* isolate (Al 1), the isolates did differ in their ability to sporulate on artificial media, and this process was affected by the light regime.

The formation of conidia by all the *A. linicola* isolates was inhibited under

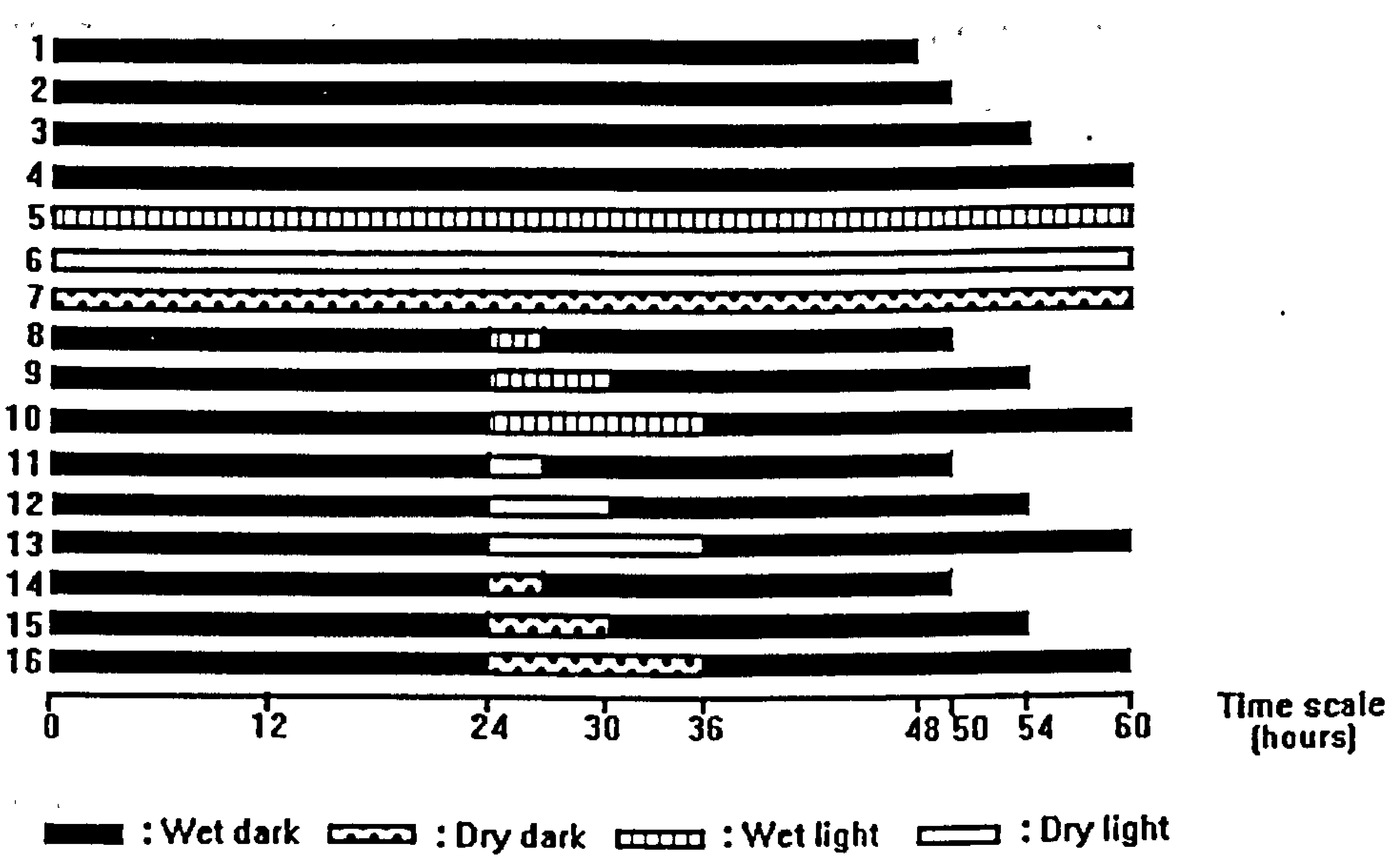



**Figure 3.3.** Treatments used to study the effects of interrupting a continuous wet dark period with wet light, dry light or dry dark periods of different duration (2, 6, or 12h) on sporulation *in vivo* of *A. linicola,* at 15°C. Controls were plants exposed either to wet dark periods for 48, 50, 54 and 60h (treatments 1, 2, 3 and 4, respectively) or to continuous wet light, dry light or dry dark periods (each 60h) (treatments 5, 6 and 7, respectively).

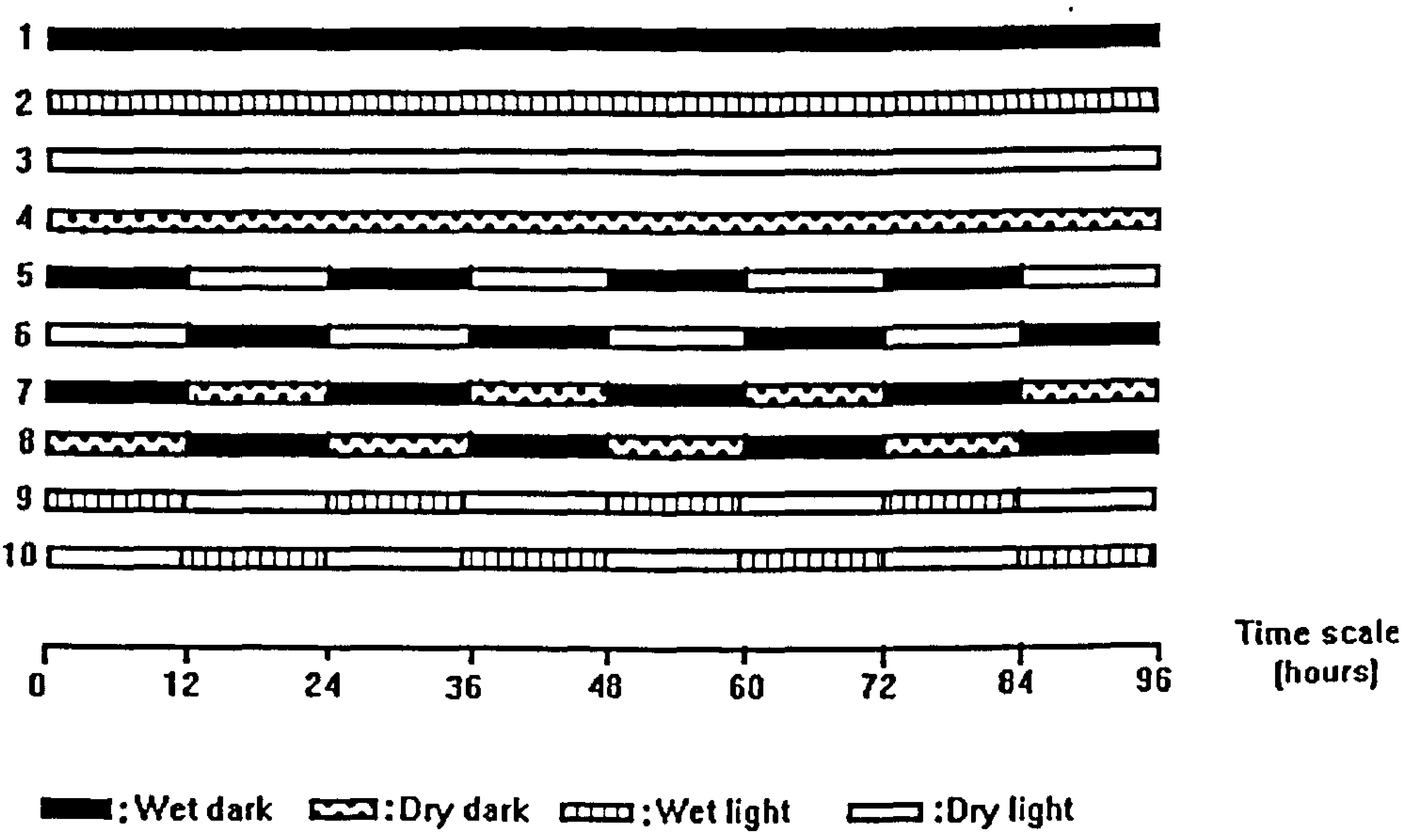



**Figure 3.4.** Treatments used to study the effects of interrupting a) a continuous wet dark period by several dry light periods (each 12h) and b) a continuous wet dark period by several dry periods (each 12h) in darkness or light on sporulation *in vivo* of *A. linicola,* at 15°C. Controls were plants incubated for 96h under continuous wet dark, wet light, dry light or dry dark periods (treatments 1, 2, 3 and 4, respectively).

**Table 3.1.** Effect of light regime on sporulation of *A.linicola* isolates.

| Isolate | Colony diameter[1] (cm) | Sporulation[2] | | | | |
|---|---|---|---|---|---|---|
| | | Light treatment[3] | | | | |
| | | A | A+B | A+C | A+B+D | A+C+D |
| Al 1 | 6.1[4] ± 0.2[5] | - | - | ++ | - | ++ |
| Al 2 | 4.2 ± 0.4 | - | - | ++ | - | ++ |
| Al 3 | 4.2 ± 0.2 | - | - | +++ | - | +++ |
| Al 4 | 4.6 ± 0.2 | - | - | ++ | - | ++ |
| Al 5 | 5.4 ± 0.2 | - | - | ++ | - | ++ |
| Al 6 | 5.2 ± 0.5 | - | - | ++ | - | ++ |
| Al 7 | 4.8 ± 0.3 | - | - | ++ | - | ++ |
| Al 8 | 4.8 ± 0.2 | - | - | ++ | - | ++ |
| Al 9 | 5.0 ± 0.9 | - | - | ++ | - | ++ |
| Al 10 | 3.9 ± 0.7 | - | - | + | - | + |
| Al 11 | 5.6 ± 0.9 | - | - | +++ | - | +++ |
| Al 12 | 5.0 ± 0.5 | - | - | + | - | + |
| Al 13 | 4.7 ± 0.3 | - | - | ++ | - | ++ |
| Al 14 | 5.3 ± 0.3 | - | - | +++ | - | +++ |
| Al 15 | 5.1 ± 0.5 | - | - | +++ | - | +++ |
| Al 16 | 5.4 ± 0.4 | - | - | +++ | - | +++ |

[1] After 5 days exposure to darkness at 20°C.
[2] (-) : no sporulation, (+) : poor, (++) : moderate, (+++) : abundant sporulation.
[3] A : darkness for 5 days at 20°C, B : continuous NUV-light for 2 days at 20°C, C : diurnal NUV-light (12h NUV-light/12h darkness) for 2 days at 20°C, D : darkness for 2 days at 10°C.
[4] Mean of four replicates.
[5] Standard deviation.

**Table 3.2.** Effect of light regime on sporulation of *A.infectoria* isolates.

| Isolate | Colony diameter[1] (cm) | Sporulation[2] Light treatment[3] | | | | |
|---|---|---|---|---|---|---|
| | | A | A+B | A+C | A+B+D | A+C+D |
| Ai 1 | 3.7[4] ± 2.1[5] | - | + | + | + | + |
| Ai 2 | 4.5 ± 0.6 | - | + | + | + | + |
| Ai 3 | 5.2 ± 0.5 | - | + | + | + | + |
| Ai 4 | 5.1 ± 0.4 | - | - | + | - | + |
| Ai 5 | 5.6 ± 0.3 | - | - | + | - | + |
| Ai 6 | 5.9 ± 0.2 | - | - | + | - | + |
| Ai 7 | 5.8 ± 0.2 | - | + | + | + | + |
| Ai 8 | 5.0 ± 0.9 | - | + | + | + | + |
| Ai 9 | 5.8 ± 0.2 | - | + | + | + | + |

[1] After 5 days exposure to darkness at 20°C.
[2] (-) : no sporulation, (+) : poor, (++) : moderate, (+++) : abundant sporulation.
[3] A : darkness for 5 days at 20°C, B : continuous NUV-light for 2 days at 20°C, C : diurnal NUV-light (12h NUV-light/12h darkness) for 2 days at 20°C, D : darkness for 2 days at 10°C.
[4] Mean of four replicates.
[5] Standard deviation.

**Table 3.3.** Effect of light regime on sporulation of *A.alternata* isolates.

| Isolate | Colony diameter[1] (cm) | Sporulation[2] | | | | |
|---|---|---|---|---|---|---|
| | | Light treatment[3] | | | | |
| | | A | A+B | A+C | A+B+D | A+C+D |
| Aa 1 | 6.3[4] ± 0.5[5] | +++ | +++ | +++ | +++ | +++ |
| Aa 2 | 6.0 ± 0.4 | +++ | +++ | +++ | +++ | +++ |
| Aa 3 | 5.8 ± 0.2 | + | +++ | +++ | +++ | +++ |
| Aa 4 | 5.6 ± 0.9 | ++ | ++ | +++ | ++ | +++ |
| Aa 5 | 5.9 ± 0.2 | ++ | ++ | +++ | ++ | +++ |
| Aa 6 | 5.6 ± 0.1 | ++ | ++ | +++ | ++ | +++ |
| Aa 7 | 6.0 ± 0.1 | + | + | ++ | + | ++ |
| Aa 8 | 6.0 ± 0.1 | + | ++ | ++ | ++ | ++ |
| Aa 9 | 5.9 ± 1.1 | +++ | +++ | +++ | +++ | +++ |

[1] After 5 days exposure to darkness at 20°C.
[2] (-) : no sporulation, (+) : poor, (++) : moderate, (+++) : abundant sporulation.
[3] A : darkness for 5 days at 20°C, B : continuous NUV-light for 2 days at 20°C, C : diurnal NUV-light (12h NUV-light/12h darkness) for 2 days at 20°C, D : darkness for 2 days at 10°C.
[4] Mean of four replicates.
[5] Standard deviation.

continuous exposure to NUV-light (Table 3.1). Sporulation occurred only when a dark period followed exposure to diurnal NUV-light. There was, however, variation between the isolates with regard to the amounts of conidia produced.

The effect of irradiation on sporulation of *A. infectoria* differed between the isolates (Table 3.2). All the isolates appeared to be responsive (although their sporulation was poor) to exposure to diurnal NUV-light following the period in darkness. Continuous exposure to NUV-light after the period in darkness induced sporulation in six of the nine *A. infectoria* isolates tested. In darkness, none of the *A. linicola* and *A. infectoria* isolates sporulated. Conidia of *A. alternata* were generally produced most profusely (amounts differed between the isolates) when darkness was followed by constant or diurnal NUV-light but sporulation was still considerable, even when the cultures were kept in darkness for 5 days at 20°C (Table 3.3). The second period of darkness at 10°C which followed the exposure to the NUV-light (constant or diurnal) had no further effect on sporulation of any isolate tested.

**3.4.1.2. Effects of different media, reduction of vegetative growth and wounding of the mycelium on sporulation**

The amount of conidia produced on different media differed between the isolates (Table 3.4). All the isolates tested, with the exception of the non-sporulating isolate of *A. infectoria* (Ai 4), sporulated on CMA with or without cellophane on the surface. More conidia were produced when the cultures were growing on cellophane placed on the top of the media, although the amounts of conidia produced differed between the isolates; the non-sporulating isolates of all the three *Alternaria* species produced

**Table 3.4.** Effects of culture media and inhibition of the mycelial growth on sporulation of *A. linicola, A. infectoria* and *A. alternata* isolates.

| Isolate[1] | Culture Medium | Sporulation[2] under diurnal NUV-light (12h NUV-light/12h darkness) | |
|---|---|---|---|
| | | With cellophane | Without cellophane |
| Al 15 (S) | CMA | +++ | +++ |
| | V-8 | +++ | ++ |
| | MA | ++ | + |
| | PDA | + | - |
| Al 17 (NS) | CMA | +++ | +++ |
| | V-8 | ++ | - |
| | MA | + | - |
| | PDA | + | - |
| Ai 1 (S) | CMA | +++ | + |
| | V-8 | +++ | ++ |
| | MA | ++ | + |
| | PDA | +++ | +++ |
| Ai 4 (NS) | CMA | - | - |
| | V-8 | - | - |
| | MA | - | - |
| | PDA | - | - |
| Aa 9 (S) | CMA | +++ | +++ |
| | V-8 | +++ | +++ |
| | MA | +++ | +++ |
| | PDA | +++ | +++ |
| Aa 10 (NS) | CMA | ++ | ++ |
| | V-8 | + | + |
| | MA | + | + |
| | PDA | +++ | ++ |

[1] (S) : sporulating, (NS) : non- or poorly sporulating on V-8 agar after exposure to diurnal NUV-light.
[2] (-) : no sporulation, (+) : poor, (++) : moderate, (+++) : abundant sporulation.

fewer conidia than the sporulating ones, even when the mycelial growth was reduced.

The influence of wounding the mycelium and exposing it to NUV-light upon sporulation is shown in Table 3.5. All the isolates tested (sporulating and non- or poorly sporulating) produced considerable amounts of conidia on the agar blocks under NUV-light at 20°C even after 24 h. Within 72 h large masses of conidia had developed over the entire surface of the agar blocks although no aerial mycelium was present. Conidiophores with conidia arose abundantly at the edges of the agar blocks where the mycelium was cut (Fig. 3.5). Mycelia proliferated from the agar blocks into the S-medium and produced conidiophores and conidia which covered the entire surface of the S-medium. The composition of the primary media had no effect on the ability of the isolates to sporulate on S-medium. No sporulation occurred (with the exception of the sporulating isolate of *A. alternata*) when the plates with the S-medium were incubated in darkness at 18°C.

### 3.4.2. Sporulation *in vivo*

#### 3.4.2.1. Effects of temperature, wet period and light regime on sporulation

Experiment I. At constant temperatures, 10°C and 15°C, sporulation of *A. linicola* was greater when a continuous wet dark period (total 48 h) was interrupted by four periods (each 12 h) of wet light (960 and 3810 conidia $cm^{-2}$ cotyledon at 10°C and 15°C, respectively) (Fig. 3.6). Conidiophores and conidia were observed after the first wet dark period (24 h) at 15°C and after the second wet dark period (48 h) at 10°C (Fig. 3.7A). However, the number of conidia produced after four periods of dry light was approximately the same as under continuous wet darkness and less

**Table 3.5.** Effect of wounding the mycelium on sporulation of *A. linicola, A. infectoria* and *A. alternata* isolates.

| Isolate[1] | Culture Medium | Sporulation[2] on S-medium | |
|---|---|---|---|
| | | under diurnal NUV-light (12h light/12h darkness) | in darkness |
| Al 15 (S) | CMA | +++ | - |
| | V-8 | +++ | - |
| | MA | +++ | - |
| | PDA | ++ | - |
| Al 17 (NS) | CMA | +++ | - |
| | V-8 | +++ | - |
| | MA | +++ | - |
| | PDA | ++ | - |
| Ai 1 (S) | CMA | +++ | - |
| | V-8 | +++ | - |
| | MA | +++ | - |
| | PDA | +++ | - |
| Ai 4 (NS) | CMA | + | - |
| | V-8 | ++ | - |
| | MA | + | - |
| | PDA | + | - |
| Aa 9 (S) | CMA | +++ | +++ |
| | V-8 | +++ | +++ |
| | MA | +++ | +++ |
| | PDA | +++ | +++ |
| Aa 10 (NS) | CMA | +++ | - |
| | V-8 | +++ | - |
| | MA | +++ | - |
| | PDA | +++ | - |

[1] (S) : sporulating, (NS) : non- or poorly sporulating on V-8 agar after exposure to diurnal NUV-light.
[2] (-) : no sporulation, (+) : poor, (++) : moderate, (+++) : abundant sporulation.

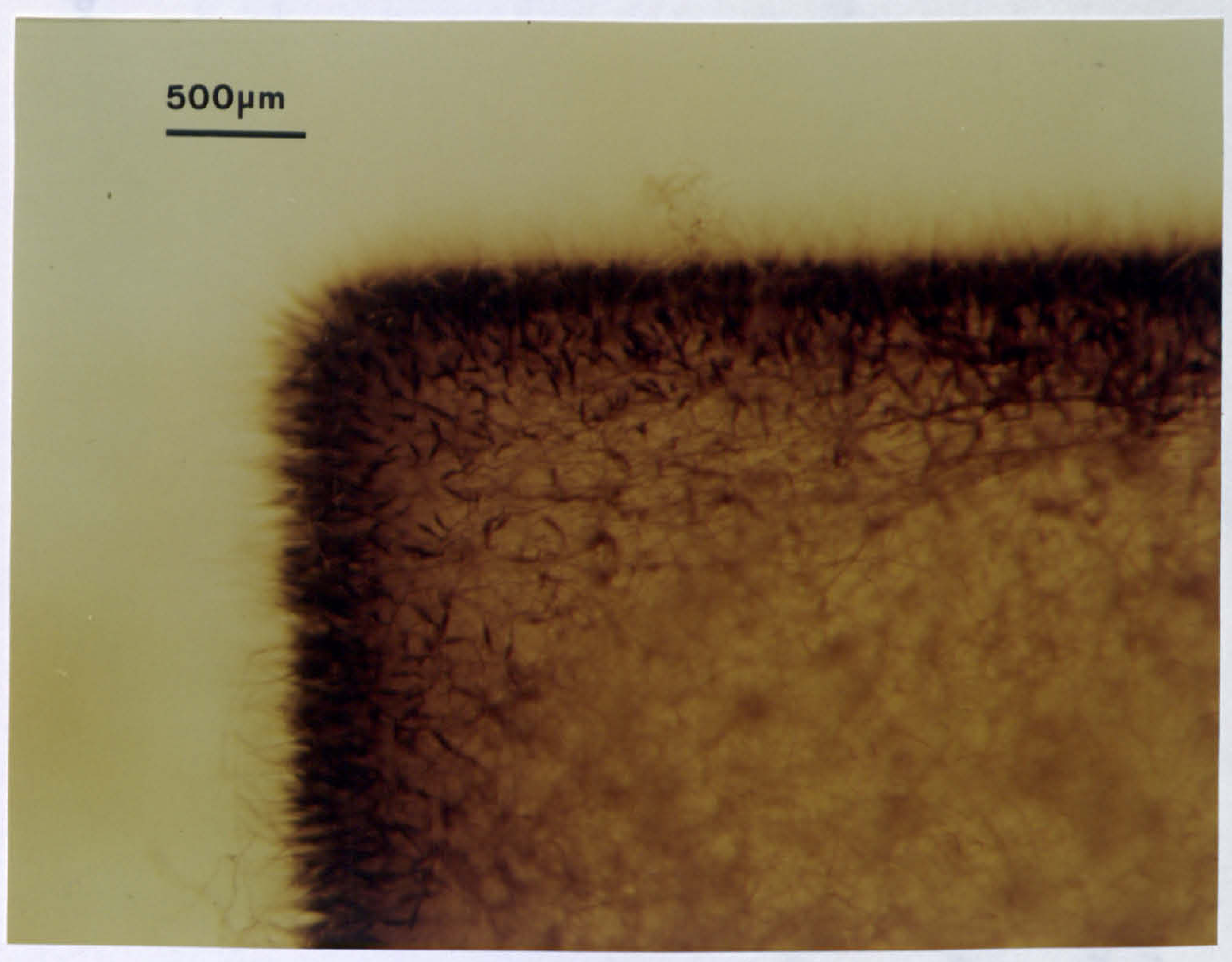



**Figure 3.5.** Sporulation of *A. linicola* on V-8 agar blocks placed on S-medium (Shahin & Shepard, 1979) and incubated for 72h under diurnal NUV-light (12h NUV-light/12h darkness) at 20°C. The intensity of the dark colour reflects abundant sporulation.

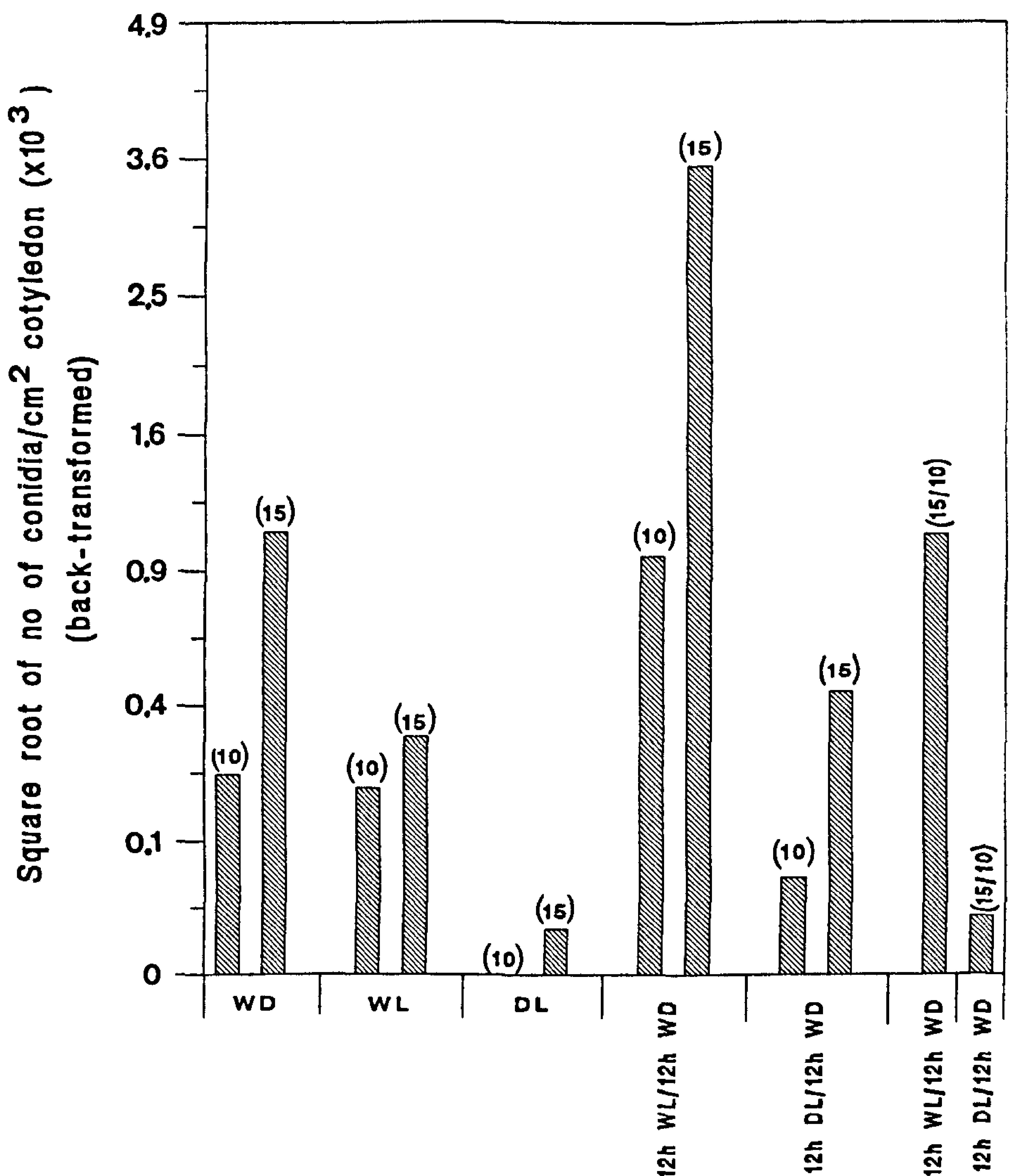



**Figure 3.6.** The effects of temperature, wet periods and light regime on sporulation of *A. linicola* on attached cotyledons of linseed plants (cv. Antares). The temperatures tested (numbers in parentheses) were 10°C or 15°C and they were applied either as constant or as alternating (15°C/10°C) day/night temperatures. The total period of incubation was 96h. WD = wet dark, WL = wet light and DL = dry light period. SED (40 d.f.) = 1.35

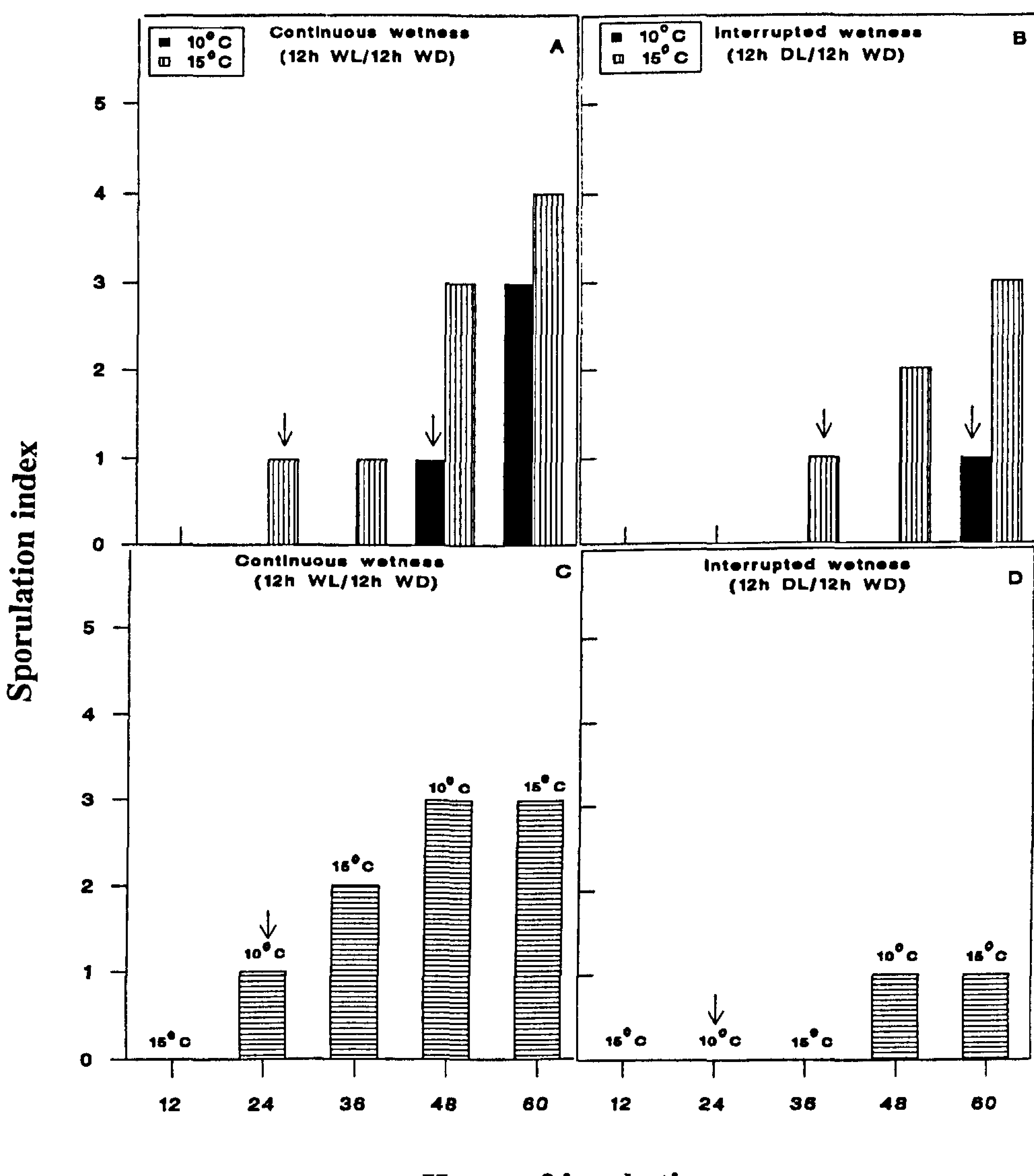



**Figure 3.7.** Sporulation of *A. linicola* with time of incubation on attached cotyledons (18 days old) of linseed plants (cv. Antares) exposed to continuous (A & C) or interrupted (B & D) wet periods at 10°C or 15°C. The temperatures were applied either as constant (A & B) or alternating 15°C/10°C day/night temperatures (C & D). Sporulation index : 0 = no sporulation, 1 = very poor, 2 = poor, 3 = moderate, 4 = good, and 5 = abundant sporulation. WD = wet dark, WL = wet light and DL = dry light period. ↓ : time at which conidiophores were first observed.

than after four periods of wet light (Fig 3.6). Conidiophores and conidia were observed after the second (36 h) and after the third (60 h) dry light period at 15°C and 10°C, respectively (Fig. 3.7B). More conidia were produced under continuous wet darkness than under continuous wet or dry light (Fig. 3.6). Under these conditions a slightly greater number of conidia was produced at 15°C than at 10°C. Under continuous dry light no conidia were formed at 10°C and only 12 conidia $cm^{-2}$ cotyledon were observed at 15°C.

Under alternating temperatures (15°C/10°C) more conidia were produced when a continuous wet dark period (total 48 h) was interrupted by four periods (each 12 h) of wet light than when it was interrupted by four periods (each 12h) of dry light (1060 and 19 conidia $cm^{-2}$ cotyledon, respectively) (Fig. 3.6). The time at which conidiophores and conidia were formed was also affected by the alternating temperatures. When the continuous wet dark period was interrupted by four periods of wet light, formation of conidiophores and conidia was observed after the first wet dark period (24 h) (Fig. 3.7C). However, when the continuous wet dark period was interrupted by four periods of dry light, conidiophores were observed after the first wet dark period (24 h) but the conidia were not observed until after the second wet dark period (48 h) (Fig. 3.7D).

Experiment II. At constant temperatures, 15°C and 20°C, the greatest number of conidia was produced only when a continuous wet dark period (total 48 h) was interrupted by four periods (each 12 h) of wet light (Fig. 3.8). Under these conditions, more conidia were formed at 20°C than at 15°C (11610 and 3810 conidia $cm^{-2}$ cotyledon, respectively). At both temperatures, the formation of conidiophores and conidia was observed after the first wet dark period (24 h) (Fig. 3.9A). The

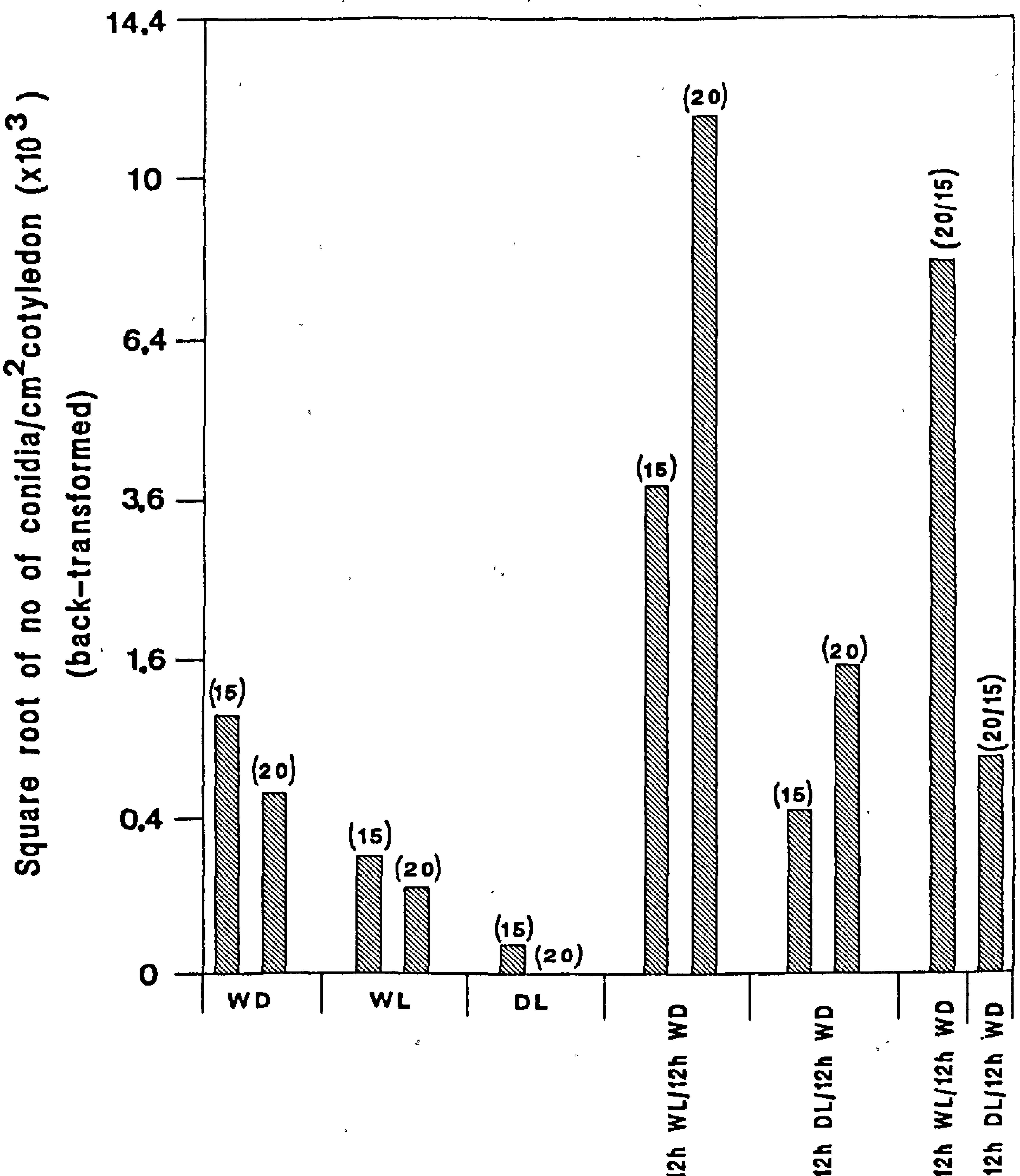



**Figure 3.8.** The effects of temperature, wet periods and light regime on sporulation of *A. linicola* on attached cotyledons of linseed plants (cv. Antares). The temperatures tested (numbers in parentheses) were 15°C or 20°C and they were applied either as constant or as alternating (20°C/15°C) day/night temperatures. The total period of incubation was 96h. WD = wet dark, WL = wet light and DL = dry light period. SED (40 d.f.) = 6.51

number of conidia produced on plants exposed to four periods of dry light (each 12 h) was smaller than on those exposed to four periods of wet light (Fig. 3.8). Conidiophore and conidial formation was observed after the first wet dark period (24 h) at 20°C but not until after the second dry light period (36 h) at 15°C (Fig. 3.9B). Under either continuous wet darkness or continuous wet light more conidia were produced on plants exposed to 15°C than on those exposed to 20°C (Fig. 3.8). No conidia were formed under continuous dry light at 20°C and only 15 conidia $cm^{-2}$ cotyledon were produced at 15°C.

Under alternating temperatures (20°C/15°C), more conidia were produced when a continuous wet dark period (48 h) was interrupted by four periods (each 12 h) of wet light than by four periods (each 12 h) of dry light (8000 and 760 conidia $cm^{-2}$ cotyledon, respectively) (Fig. 3.8). In both treatments conidiophore and conidial formation was observed after the first wet dark period (24 h) (Fig. 3.9C & D).

**3.4.2.2. Effects of the duration of a wet or dry light period applied before a period of wet darkness on sporulation**

Under continuous wetness (96 h) the number of conidia produced was dependent on the length of the light period applied before the period of darkness (Fig. 3.10). When the light period was 12 or 24 h, the number of conidia produced per $cm^2$ cotyledon was small and similar to the number of conidia produced under continuous darkness (c. 780 conidia $cm^{-2}$ cotyledon). When the length of the initial wet light period increased from 24 to 48 or 72 h, the number of conidia produced increased from 780 to 2360 or 2630 conidia $cm^{-2}$ cotyledon, respectively. When a total period of 48 h wet light was divided into four fractions, each followed by a 12-h period

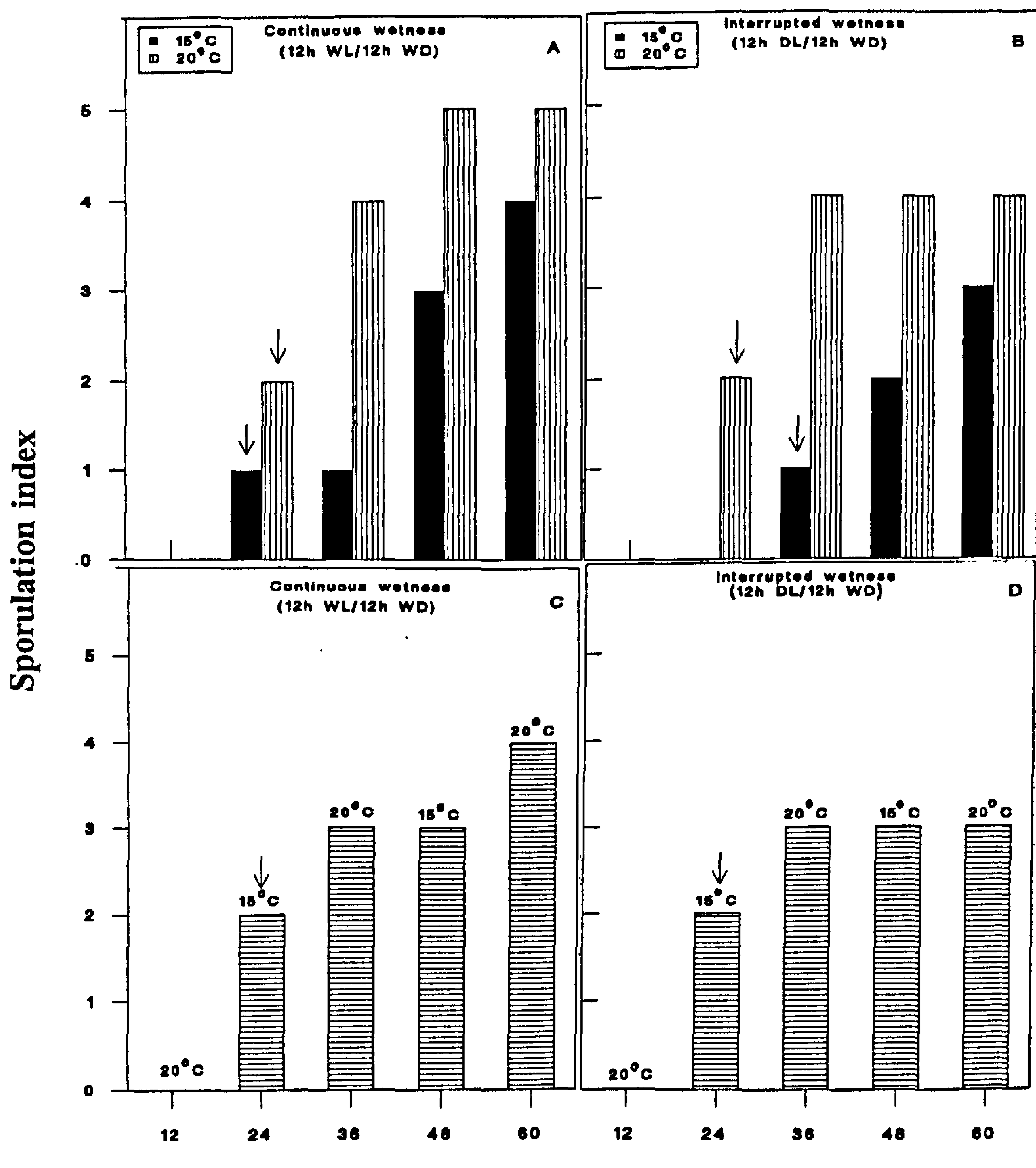



**Figure 3.9.** Sporulation of *A. linicola* with time of incubation on attached cotyledons (18 days old) of linseed plants (cv. Antares) exposed to continuous (A & C) or interrupted (B & D) wet periods at 15°C or 20°C. The temperatures were applied either as constant (A & B) or alternating 20°C/15°C day/night temperatures (C & D). Sporulation index : 0 = no sporulation, 1 = very poor, 2 = poor, 3 = moderate, 4 = good, and 5 = abundant sporulation. WD = wet dark, WL = wet light and DL = dry light period. ↓ : time at which conidiophores were first observed.

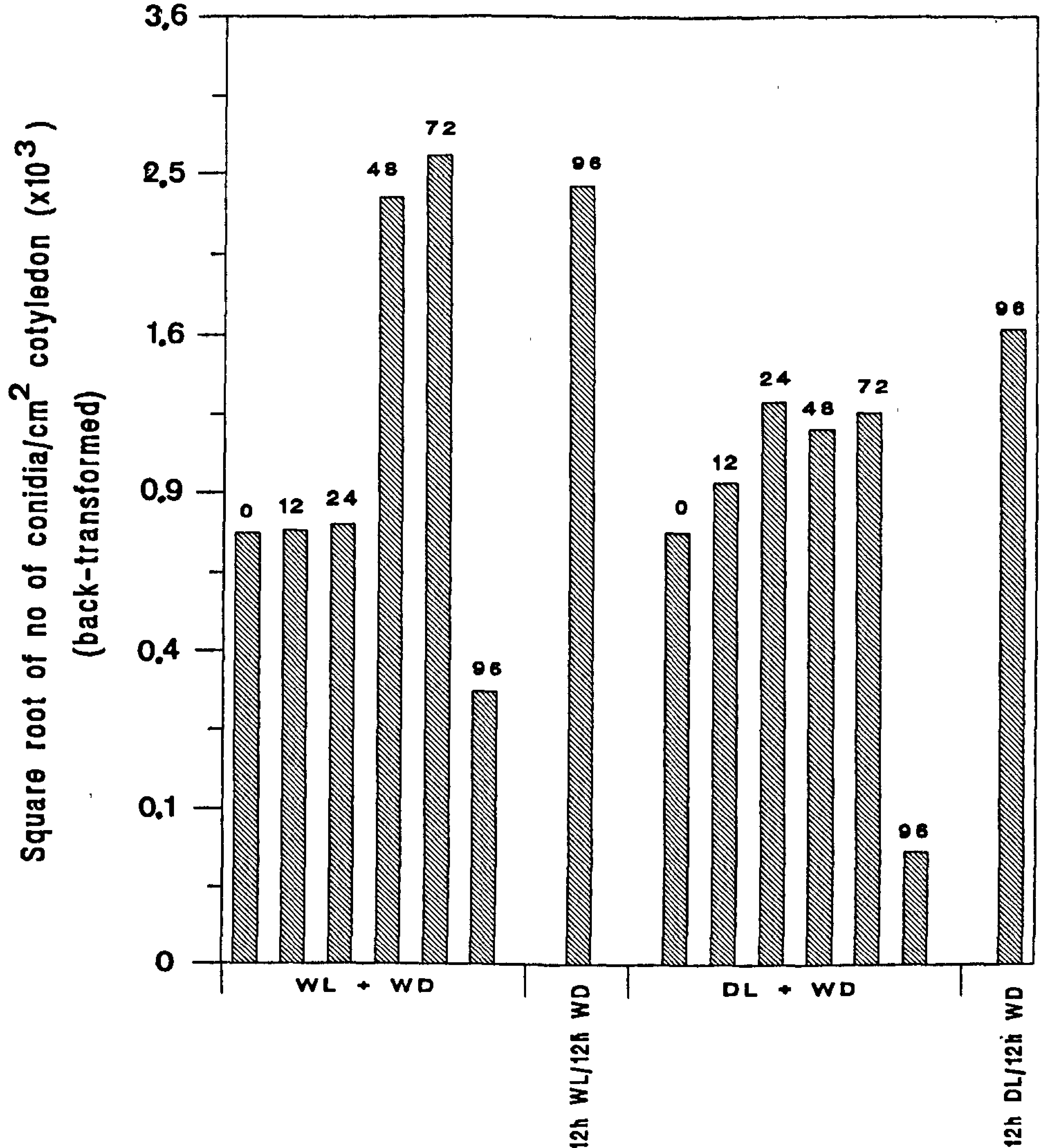



**Figure 3.10.** The effects a) of the duration of a wet or dry light period applied before a period of wet darkness and b) of interrupting a continuous wet dark period by several wet or dry light periods on sporulation of *A. linicola*. The sporulation test was done on attached cotyledons (18 days old) of linseed plants (cv. Antares), at 15°C. Numbers above bars are the hours during which the plants were exposed to wet (WL) or dry (DL) light while the rest of the incubation time (total 96h) was in wet darkness (WD). SED (47 d.f.) = 1.43

of wet darkness (alternating treatment), the number of conidia formed was similar to that produced when this period of wet light (48 h) was applied continuously before a period of darkness (2420 and 2360 conidia $cm^{-2}$ cotyledon, respectively) (Fig. 3.10).

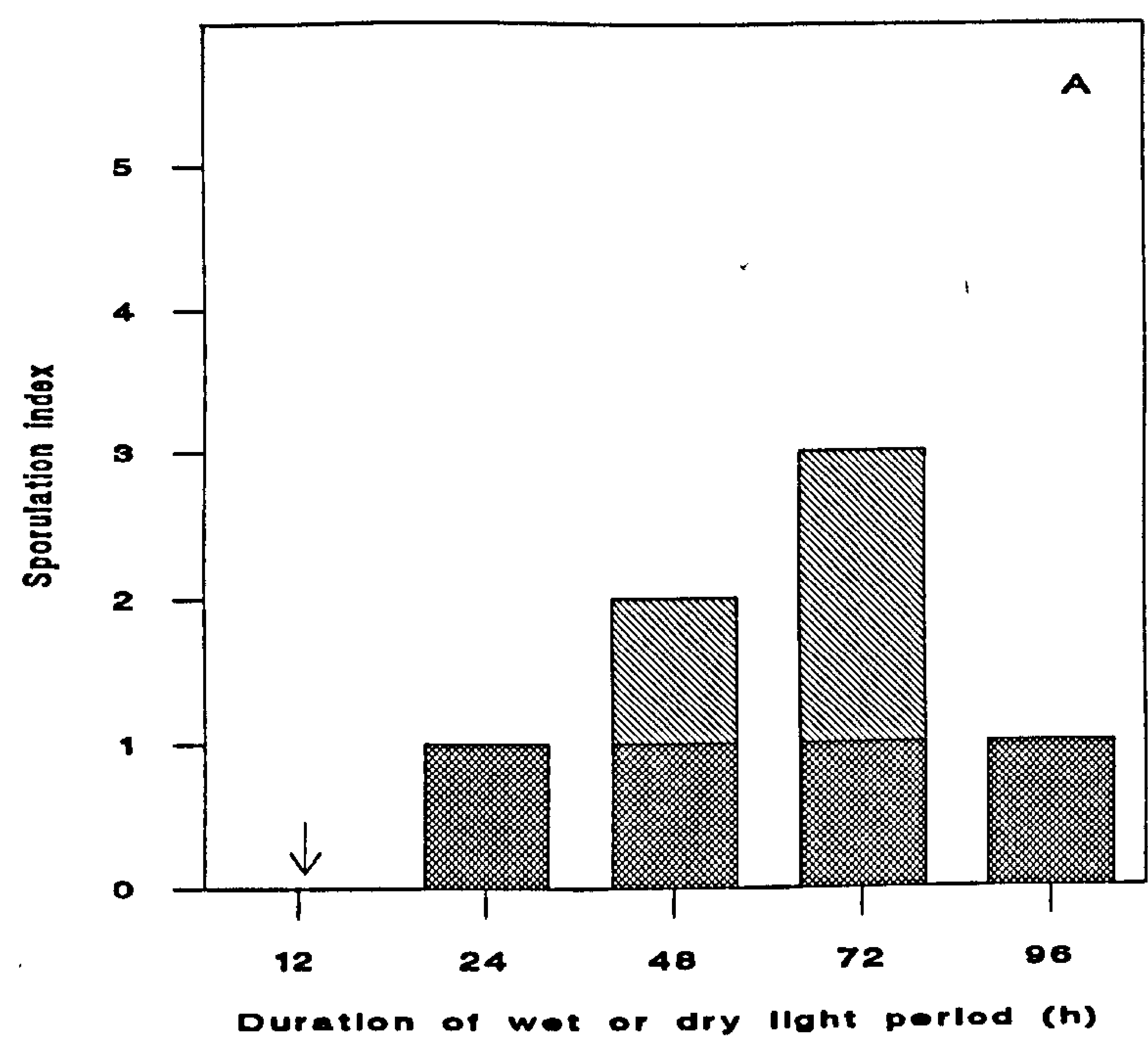
The number of conidia produced on plants exposed to a 24-h dry light period applied before a period of wet darkness was greater than on those exposed to a 12-h dry light period (1270 and 940 conidia $cm^{-2}$ cotyledon, respectively). Further increases (up to 72 h) in the dry light period did not increase the number of conidia produced. Sporulation was slightly increased (1620 conidia $cm^{-2}$ cotyledon) when the 48-h period of dry light was divided into four fractions, each followed by a 12-h period of wet darkness (alternating treatment) (Fig. 3.10).

The number of conidia produced on plants exposed for 96 h to a continuous light period (wet or dry) was significantly lower than on plants exposed to darkness as well for part of this period (Fig. 3.10).

Under all treatments, conidiophore and conidial formation was observed after 12 and 24 h, respectively (Fig. 3.11).

### 3.4.2.3. Effects of interrupting a continuous wet dark period by wet light, dry light or dry dark periods on sporulation

The greatest number of conidia was produced when a 48-h period of wet darkness was interrupted by a 2, 6 or 12-h period of wet or dry light (Fig. 3.12). Under wet light interruptions the number of conidia produced was the same irrespective of the length of the wet light period. The number of conidia produced on plants exposed to short interruptions by dry light (2 or 6 h) was similar to that produced on plants



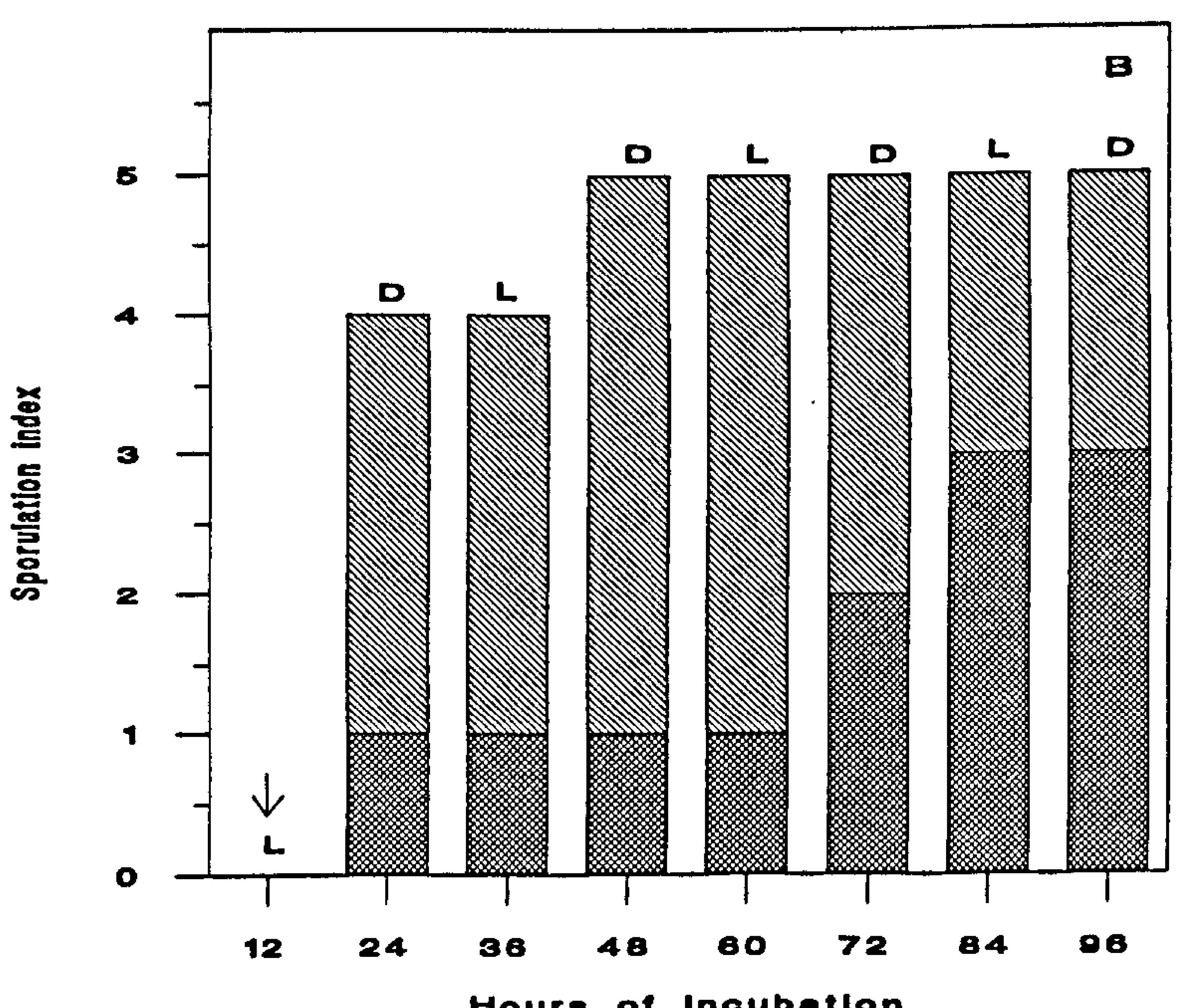




**Figure 3.11.** Sporulation of *A. linicola* with time of incubation either under a continuous wet (▧) or dry (▩) light period applied before a period of darkness (A) or under several wet dark periods (each 12h) interrupted by wet (▧) or dry (▩) light periods(B). The tests were done on attached cotyledons of linseed plants (cv. Antares), at 15°C. D = dark period, L = light period. Sporulation index : 0 = no sporulation, 1 = very poor, 2 = poor, 3 = moderate, 4 = good and 5 = abundant sporulation. ↓: time at which conidiophores were observed.

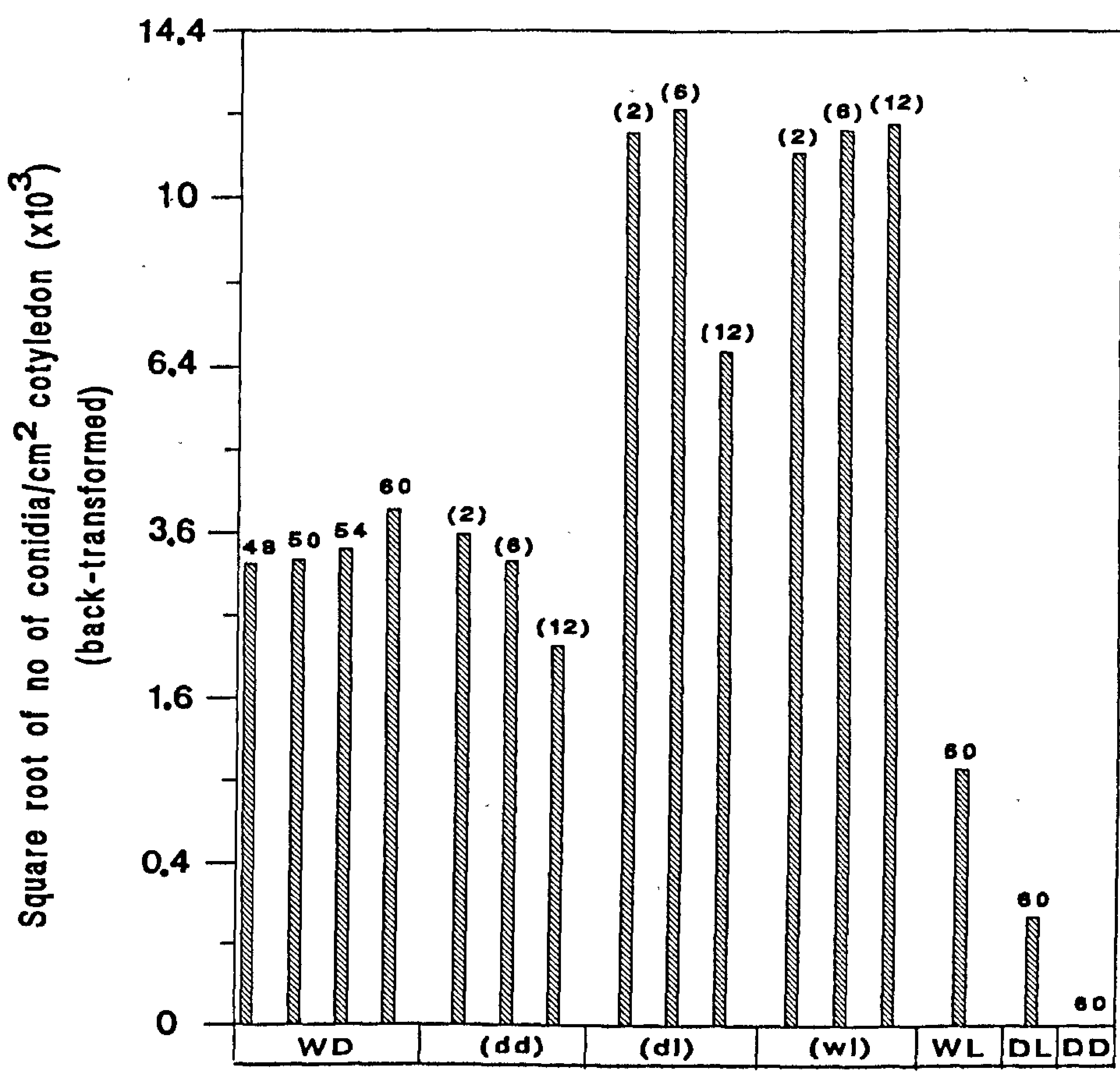



**Figure 3.12.** Effects of interrupting a continuous wet dark (WD) period after 24h with a wet light (wl), dry light (dl) or dry dark (dd) period on sporulation of *A. linicola* on attached cotyledons (18 days old) of linseed plants (cv. Antares) at 15°C. Numbers in parentheses are the hours of interruption. WD, WL, DL and DD : continuous periods of wet darkness, wet light, dry light and dry darkness, respectively. Numbers not in parentheses are the hours of incubation in WD, WL, DL or DD. SED (72 d.f.) = 4.69

exposed to wet light interruptions. However, longer exposure (12 h) to dry light decreased the final number of conidia produced (Fig. 3.12). When the wet dark period (48 h) was interrupted by 2, 6 or 12 h of dry darkness the number of conidia produced was similar to that produced under continuous wet darkness (Fig. 3.12).

Exposure of plants to various continuous wet dark periods (48, 50, 54 or 60 h) did not increase the number of conidia produced (3180, 3230, 3390 and 3970 conidia $cm^{-2}$ cotyledon, respectively). Few conidia were produced on plants incubated under continuous (60 h) wet or dry light (990 and 180 conidia $cm^{-2}$ cotyledon, respectively) and no sporulation occurred on plants exposed to continuous (60 h) dry darkness (Fig. 3.12).

**3.4.2.4. Effects of interrupting a) a continuous wet dark period by several dry light periods, and b) a continuous wet dark or wet light period by several dry periods in darkness or light on sporulation**

The greatest numbers of conidia were produced when the plants were exposed either to a continuous wet dark period (96 h) or to a wet dark period interrupted by four (each 12 h) dry light periods with the wet dark period applied first (1100 and 1470 conidia $cm^{-2}$ cotyledon, respectively) (Fig. 3.13). However, the number of conidia produced was smaller when the continuous wet dark period was interrupted by four dry light periods with the dry light period applied first (506 conidia $cm^{-2}$ cotyledon). Interruption of the wet dark period by four periods (each 12 h) of dry darkness decreased the number of conidia produced, especially when the wet dark period was applied in the beginning (Fig. 3.13). Small numbers of conidia were also produced when a wet light period was interrupted by four dry light periods (each

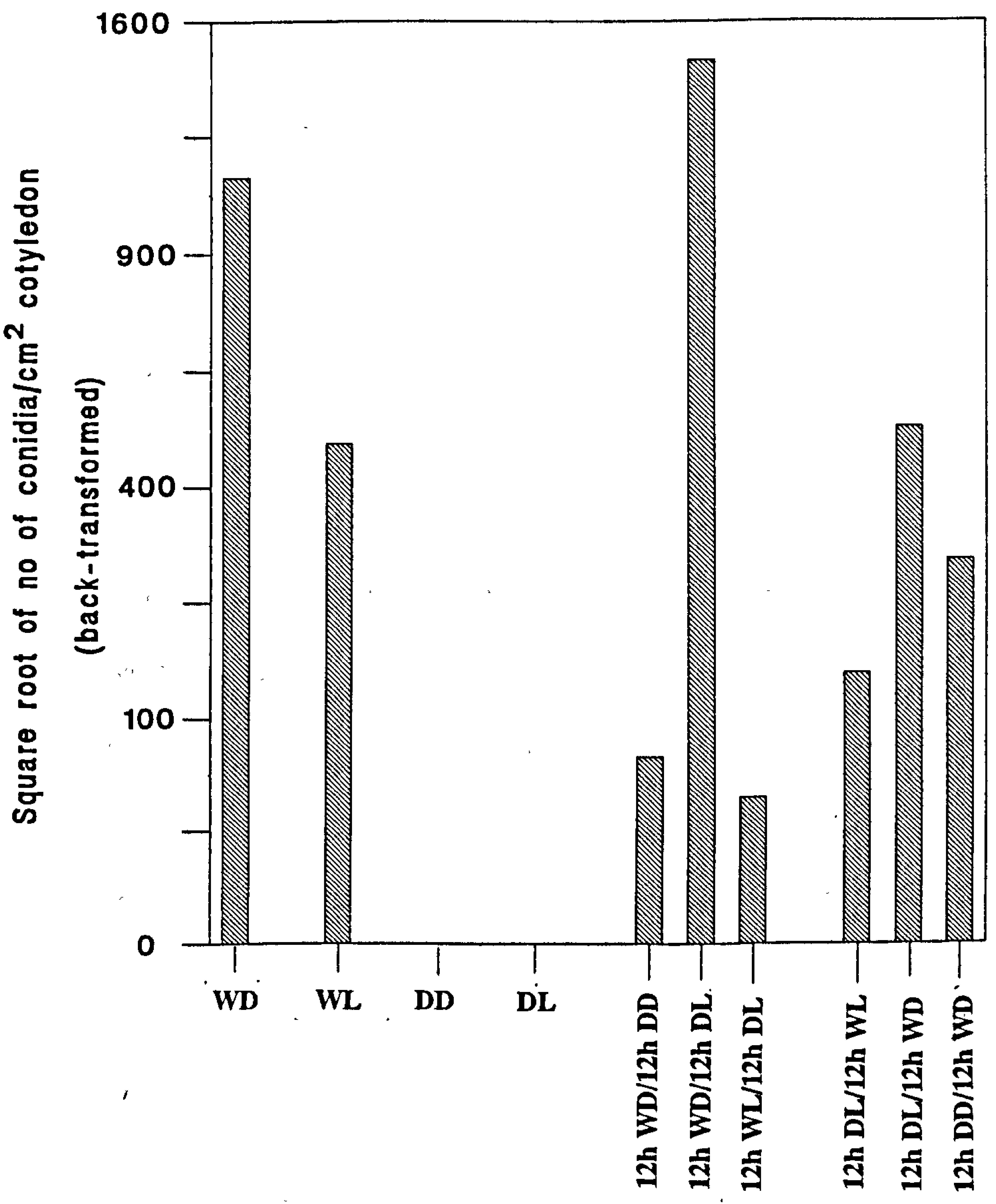



**Figure 3.13.** Effects of interrupting a) a continuous wet dark (WD) period by several dry light (DL) periods and b) a continuous wet dark (WD) or wet light (WL) period by several dry periods in darkness (DD) or light (DL) on sporulation of *A. linicola*. Tests were done on attached cotyledons (18 days old) of linseed plants (cv. Antares), at 15°C. The total period of incubation was 96 h. SED (28 d.f.) = 1.04

12 h), especially when the wet light period was applied first.

The number of conidia produced under continuous wet light was less than under continuous wet darkness (480 and 1100 conidia $cm^{-2}$ cotyledon, respectively). There was no sporulation on plants exposed for 96 h to continuous dry darkness or dry light (Fig. 3.13).

## 3.5. Discussion

The results of the present study showed that the sporulation *in vitro* of *A. linicola* and *A. infectoria* follow the sporulation pattern of "diurnal sporulators" (Leach, 1967). For diurnal sporulators, which include other *Alternaria* species, sporulation is affected by light in two ways: (i) light stimulates conidiophore formation and (ii) light inhibits conidial production. The conidial formation *in vitro* of *A. linicola* and *A. infectoria* was stimulated by exposure to diurnal NUV-light (12h NUV-light/12h dark) at 20°C. Similarly, Lukens (1960) reported that conidiophores of *A. solani* require a 12-h dark period to produce conidia in culture. *A. chrysanthemi* also produces abundant conidia only under diurnal NUV-light (12h NUV-light/12h dark) (Leach, 1962). *A. tomato* failed to sporulate *in vitro* under continuous exposure to NUV-light (Leach, 1967), although these results contrast with those reported by Aragaki (1961) for the same species.

Long exposure either to NUV-light or to darkness seems to inhibit sporulation *in vitro* of *A. linicola* and *A. infectoria* and this has been reported by other workers for *A. chrysanthemi*, *A. porri*, *A. dauci* and *A. chichorii* (Leach, 1962; Fahim, 1966; Zimmer & McKeen, 1968; Vakalounakis & Christias, 1986). Trione & Leach (1965)

first reported the presence in the NUV-irradiated mycelium of a substance called P310, having maximum absorption at 310 nm, which is not present in the dark-grown mycelium of *A. pisi*, *A. chrysanthemi* and *A. dauci*. Synthesis of P310 coincides with the start of photoinhibition and is continued in the dark (Leach, 1965; Trione *et al.*, 1966). Whether this substance is present in *A. linicola* or *A. infectoria* mycelium is not known. *A. alternata* does not seem to follow the same pattern in sporulation as *A. linicola* and *A. infectoria*. All the isolates tested sporulated in darkness as well as under NUV-light (constant or diurnal); it seems that *A. alternata* does not have any specific light requirements for sporulation *in vitro* and this might be one reason why this *Alternaria* species is so widespread. Exposure to NUV-light seems to be a more important factor for inducing sporulation of *A. linicola* and *A. infectoria* than the type of medium used, or the reduction of mycelial growth. Leach (1962) also found that the composition of the media he tested had little effect on the sporulation of irradiated colonies of *A. chrysanthemi*, *A. dauci* and *A. tenuis*. In the present studies, even when the simple water agar medium (S-medium) proposed by Shahin & Shepard (1979) was used, sporulation *in vitro* of *A. linicola* and *A. infectoria* isolates was promoted only after exposure to diurnal NUV-light. However, these results contrast with those of Shahin & Shepard (1979) for *A. solani*, *A. alternata* and *A. dauci*, as these species produced abundant conidia *in vitro* only in darkness at 18°C.

Moreover, physical factors such as wounding the mycelium and high relative humidity (2 ml water was added to the surface of the S-medium) may have enhanced the sporulation *in vitro* of *A. linicola* and *A. infectoria* since more conidia were produced at sites where the mycelium was cut. These factors were amongst those

suggested to be essential for sporulation of *A. solani* (Waggoner & Horsfall, 1969; Rands, 1917).

These studies did not examine the effects of the interaction between light and temperature on sporulation *in vitro* of *A. linicola*, *A. infectoria* and *A. alternata* as all the experiments were done at a constant temperature of 20°C. However, this interaction may affect the sporulation of these *Alternaria* species, as reported for *A. solani*, *A. brassicae*, *A. dauci* and *A. tomato* (Douglas & Pavek, 1971; Senior *et al.*, 1987; Leach, 1967; Zimmer & McKeen, 1969; Aragaki, 1961).

Of all the methods tested, the one described by Shahin and Shepard (1979) appears to be the best for inducing profuse sporulation of *A. linicola*, *A. infectoria* and *A. alternata* in culture. This method, which combines wounding of the mycelium, high relative humidity and exposure to diurnal NUV-light, is simple, consistent and produces in 6 days sufficient numbers of conidia for use in artificial inoculations under completely aseptic conditions.

Unlike some fungi, which require induction by light *in vitro* but which sporulate abundantly without induction *in vivo* (Houston & Oswald, 1946; Hyre, 1972; Zimmer & McKeen, 1969), sporulation of *A. linicola in vivo* was also increased after induction by light. Under controlled environment conditions, *A. linicola* produced the greatest number of conidia when a continuous wet dark period was interrupted by short (each 12 h) periods of wet or dry light. Bashi & Rotem (1976) reported that although *A. porri* f.sp. *solani* sporulated *in vitro* without induction, sporulation *in vivo* was stimulated by light. Similarly, sporulation of *A. macrospora* on cotton was increased when the plants were exposed to a light (wet or dry) period between two wet dark periods (Rotem *et al.*, 1989). Covering a glasshouse with a UV-absorbing vinyl

film inhibited sporulation of *A. porri, A. dauci, A. solani* and *A. brassicae* on their respective hosts (Sasaki *et al.*, 1985) and reduced by 50% the early blight on tomato plants (Vakalounakis, 1991). However, long exposures (96 h) to a continuous light period (wet or dry) suppressed or even inhibited sporulation *in vivo* of *A. linicola.* Although it is not known why the number of conidia produced decreases with long exposure to light according to Zimmer & McKeen (1969), it is possible that the photo-activation of the majority of receptors in the hyphae occurs quickly or with increased exposure formation of an inhibitor takes place. The results of the present study also showed that although the greatest number of conidia was produced after induction by light, some conidia were formed on plants incubated under continuous wet darkness. It is possible that as the inoculated plants were exposed to light before the application of the sporulation treatments, *A. linicola* "memorized" this induction and produced conidia even under continuous darkness. Bashi & Rotem (1976) reported the same phenomenon for the sporulation *in vivo* of *A. porri* f.sp. *solani.* According to their studies, dry conidiophores of *A. porri* f. sp. *solani* "memorized" induction by light and produced conidia after being stored in darkness for almost 3 weeks.

Under controlled environment conditions sporulation *in vivo* of *A. linicola* was favoured by wetness. Few or no conidia were produced on plants exposed to continuous dry periods (in darkness or light). Although short interruptions (2 or 6 h) of a continuous wet dark period by dry light did not affect sporulation, longer interruptions (12 h) significantly reduced the number of conidia produced. It is suggested that the duration of the dry period has a major influence on *A. linicola* sporulation as the equivalent treatment with wet light interruption did not suppress sporulation. The results of this study also showed that under continuous wetness

most of the conidia were formed within the first 24 or 48 h and that prolongation of the wet period did not increase the number of conidia produced. Similarly, sporulation of *A. brassicae* and *A. brassicicola* was not affected by interrupting a continuous wet period by 2 h of dryness but longer dry interruptions (3-4 h) decreased the number of conidia produced (Humpherson-Jones & Phelps, 1989). According to Bashi & Rotem (1975), *A. porri* f. sp. *solani* on potatoes produced up to seven times more conidia under interrupted than under continuous wet periods in darkness. Sporulation of *A. macrospora* on cotton was also favoured more by interrupted than by continuous wet periods (Rotem *et al.*, 1989). However, *A. alternata* produced more conidia under continuous wetness, but tolerated and sporulated well under an interrupted wetness regime (Rotem, 1994).

Under controlled environment conditions wetness may also interact with light in determining the sporulation of *A. linicola* on linseed. More conidia were produced when several (each 12 h) wet dark periods were interrupted by wet than by dry light periods (each 12 h). Moreover, the time during the conidial formation at which the wet or dry light period is applied can affect the number of conidia produced. Although a short interruption (2 or 6 h) of a continuous wet dark period with dry or wet light induced sporulation of *A. linicola,* longer interruption by dry light (12 h) significantly reduced the number of conidia produced. It is not known why sporulation of *A. linicola* is suppressed by long exposures to dry light or if exposure to wet light for longer than 12 h can also decrease sporulation. Wet light applied for 24 h before a continuous wet dark period (96 h) did not increase sporulation but longer light periods (48 or 72 h) increased the number of conidia produced. It seems that under continuous wetness the number of *A. linicola* conidia produced increases as the ratio

light : darkness increases. However, when the light period was accompanied by dryness it suppressed sporulation of *A. linicola* irrespective of the length of the light period. Bashi & Rotem (1975) also reported the stimulatory effect of light applied during the first 48 h of a continuous wet period on sporulation of *A. porri* f.sp. *solani*. Moreover, there was no induction of sporulation of *A. linicola* when the dry periods in light were replaced with dry periods in darkness. Unlike *A. solani* on potatoes (Bashi & Rotem, 1976), dryness alone cannot substitute for induction by light on sporulation of *A. linicola*.

The results of the present study also showed that *A. linicola* can sporulate over a range of temperatures and that the effects of wetness and light on sporulation are temperature-dependent. Although only three temperatures were tested (10, 15 and 20°C), the results showed that under continuous wet dark, wet light or dry light conditions the optimum temperature for sporulation of *A. linicola* is c. 15°C. There is no information on the minimum or maximum temperatures for sporulation of *A. linicola*, as in this study temperatures lower than 10°C or higher than 20°C were not tested. However, when the continuous wet dark period was interrupted by several short (each 12 h) periods of wet or dry light the number of conidia produced increased by increasing temperature from 10 to 20°C. Moreover, fewer conidia were produced under interrupted than under continuous wetness for the same temperatures tested, due to the suppressive effect of dryness on sporulation. The minimum, optimum and maximum temperatures for sporulation of other *Alternaria* species vary depending on the species. In various systems, the minimum temperature varied from 5°C to 15°C, the optimum from 10°C to 30°C and the maximum from 24°C to more than 40°C (Rotem, 1994). Rotem *et al.*,

(1989) demonstrated that under interrupted wetness sporulation of *A. macrospora* on cotton leaves increased with increasing temperature from 15°C to 25°C. According to Strandberg (1977), sporulation of *A. dauci* on carrot petioles incubated under continuous wetness and a 12-h daylength also increased with increasing temperature from 7°C to 19°C.

However, under natural conditions, temperature fluctuates during a 24-h period. The results of this study showed that alternating day/night temperatures (15°C/10°C or 20°C/15°C) decreased the number of conidia produced compared with the constant temperatures 15°C and 20°C, respectively, possibly because of the low temperatures (10°C or 15°C) applied during the night. Temperatures during the night were also very important for the sporulation of *A. dauci* on carrots; although low night temperatures did not inhibit sporulation, they decreased the rate of sporulation (Strandberg, 1977). Sporulation of *A. macrospora* was also affected by the temperature during the wet night and more conidia were produced at 25°C than at 15°C over four nights with 6 h of dew (Rotem *et al.*, 1989). The results of the present study on sporulation of *A. linicola* are referring to chlorotic cotyledons. However, the effects of temperature on sporulation also depend on the stage of the disease development as was demonstrated by Rotem *et al.* (1989) for *A. macrospora* on cotton. According to those studies the optimum temperature for production of *A. macrospora* conidia ranges from 30°C while the leaves are green to 25°C-30°C when they are chlorotic and to 20°C-30°C when they become necrotic.

Under controlled environment conditions the time at which conidiophores and conidia of *A. linicola* were formed depended on the environmental conditions.

At 15°C and 20°C sporulation was observed after the first wet night (24 h) irrespective of the wet or dry conditions during the previous day. At lower temperatures (10°C) sporulation of *A. linicola* required at least two wet nights (48 h). Effects of the interaction between wetness duration and temperature on sporulation have also been reported for other *Alternaria* species. Sporulation of *A. brassicae* and *A. brassicicola* on brassicas requires a 12-h wet period at 14°C, but a longer period (30 h) at 8°C (Maude *et al.*, 1986). Similarly, *A. tomato* sporulated on tomato plants within 9-12 h at 14°C-26°C, but it needed 36-48 h of wetness for sporulation at 10°C (Paulus & Pound, 1955). Strandberg (1977) reported that *A. dauci* produced conidia within 48 h at temperatures ranging from 10°C to 19°C under an alternating 12 h day/night cycle. A wet period of 48 h was necessary for abundant sporulation of *A. solani* on potatoes (Bashi & Rotem, 1975) and of *A. macrospora* on cotton (Rotem *et al.*, 1989).

These results also suggest that under controlled environment conditions, dry day time conditions and low night temperatures both delay the formation of conidia and decrease the rate of sporulation of *A. linicola* on linseed. Leaf moisture or high relative humidity have a big influence on conidial production by *A. linicola.* Although conidia were formed on dry days and wet nights, the greatest number of conidia was produced under wet days and wet nights. The results of studies on the relationship between weather and concentrations of *A. linicola* conidia in the air above linseed crops (see section 7.4.1) showed that the greatest numbers of conidia were observed on dry days following rainy weather. Similarly, concentrations of *A. brassicae* (Louvet & Billotte, 1964) and *A. brassicicola* (Humpherson-Jones & Maude, 1982) conidia in the air within infected oilseed rape crops were higher during warm, dry periods following rain.

The ability to sporulate under interrupted wet periods and over a wide range of temperatures makes *A. linicola* a very efficient pathogen. However, incidence and severity of *A. linicola* symptoms on linseed crops in the UK are higher during the period between flowering and harvest (August & September) than earlier in the season. This has been attributed in the past to the increased susceptibility of the plant tissues to *A. linicola* infection. According to the results of this study it is also possible that the short daylength and the higher temperatures in August and early September not only induce sporulation but also increase the rate of sporulation of *A. linicola.*

# CHAPTER IV. CONIDIAL GERMINATION

## 4.1. Introduction

For a fungus to establish a parasitic relationship with its host plant a number of stages in its life cycle must be successfully completed. For many conidial fungi, the most important of these stages is germination of conidia, the process by which a dispersal unit, a conidium, produces an infection unit which takes the form of a germ tube, with or without an appressorium, and a penetration hypha (Zadoks & Schein, 1979). The principal environmental factors affecting germination of conidia are temperature, relative humidity, leaf wetness duration and the intensity and duration of light. The density of the fungal conidia on the leaf surfaces can also affect germination (Gottlieb, 1950; Cochrane, 1958; Rayner, 1961; Weber & Hess, 1976, Hall, 1981).

The effects of temperature and relative humidity on the conidial germination of *Alternaria* species have been studied in detail, either *in vitro* or *in vivo* (Von Ramm, 1962; Dickinson & Bottomley, 1980; Allen *et al.*, 1983; Ansari *et al.*, 1988). There are also several reports on the mode of penetration of different *Alternaria* species (Angell, 1929; Riley, 1949; Walker, 1952; Chupp & Shurf, 1960; Changsri & Weber, 1963; Fahim & El-Shehedi, 1966; Saad & Hagedorn, 1969; Tsuneda & Skoropad, 1977b; Tewari, 1986). However, there is no information on either the effects of environmental factors on conidial germination or the mode of penetration of *A. linicola*.

## 4.2. Objectives

1. To study the effects of temperature, incubation time, leaf wetness duration, light regime and their interactions on germination of *A. linicola* conidia.
2. To investigate the mode of penetration of linseed leaf tissues by *A. linicola.*

## 4.3. Materials and Methods

### 4.3.1. Preparation of inoculum

Four single-spore isolates of *A. linicola* (Al 10, Al 15, Al 23, Al 24), isolated from linseed plants (cv. Antares) naturally infected by *A. linicola* during the period 1989-1991, were used. Stock cultures were maintained by the method described in section 2.1.2 and for production of inoculum for artificial inoculation the method described in section 2.1.3 was used. The concentration of conidia of each individual isolate was determined with a haemocytometer (4 counts per conidial suspension) and was adjusted to $3 \times 10^4$ conidia $ml^{-1}$ (unless otherwise stated) by dilution with sterile distilled water. The inoculum was prepared by mixing together 100 ml of conidial suspension of each of the four isolates. The preparation of the inoculum lasted 30 min and by the end of this period no conidial germination was observed.

### 4.3.2. Plant production

Unless otherwise stated, the experiments were done on linseed plants [cv. Antares, treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem)

free of *A. linicola* infection] grown in pots by the method described in section 2.1.5. The pots were placed in controlled environment cabinets, designed at Rothamsted Experimental Station, set at 18°C/13°C day/night temperatures (Fig. 2.1). The daylength was 16 h (from 24:00 h to 16:00 h ) and light was provided in each cabinet by 18 fluorescent lamps placed 70 cm above the plants. The light intensity measured at plant level by the method described in section 2.1.4 was 120 - 160 $\mu$Einsteins $m^{-2}$ $sec^{-1}$. The relative humidity in the cabinets, measured by the method described in section 2.1.4 ranged from 65 to 75%.

### 4.3.3. Experimental design

The experiments were in a randomized block design with five blocks, with one replicate pot (10 plants per pot unless otherwise stated) for each block and treatment.

### 4.3.4. Effects of temperature and incubation time on germination

4.3.4.1. Temperatures and incubation times tested

The germination of *A. linicola* conidia on agar and detached leaves was tested at 5, 10, 15, 20 and 25°C. The percentage of conidia germinated, the length of the germ tubes and the percentage of conidia forming appressoria were assessed after 2, 4, 6, 8, 10, 12, 24 and 48 h of incubation at each of these temperatures.

4.3.4.2. Germination on agar

For studying the effects of temperature and incubation time on germination of *A. linicola* conidia *in vitro,* water agar medium (see section 2.1.7) was used. Four replicate plates (20 ml per plate) were used for each temperature and

incubation time tested. Before inoculation, all the plates were preconditioned overnight to the desired temperature. For inoculation of the plates, the following method was used : three drops of a conidial suspension containing 2 x $10^4$ conidia $ml^{-1}$ were placed individually with a Pasteur pipette at the edge of each Petri plate; the plate was slightly tilted to allow the drops to roll down on the surface of the medium. The plates were sealed with parafilm, wrapped individually with aluminium foil to exclude light and placed in incubators at the desired temperatures for the incubation times tested. The inoculation procedure lasted 30 min and by the end of this period no conidial germination was observed. At the end of the designated incubation times the agar plates were removed from the incubators and placed at -18°C to stop germination and preserve the conidia for future observation. To facilitate the assessments, 0.1 ml of cotton blue in lactophenol (see section 2.1.8) was spread on the surface of the agar medium immediately after the removal of the plates from the incubators.

4.3.4.3. Germination on detached leaves

Linseed plants (cv. Antares) were grown in pots (10 plants per pot) by the method described in section 2.1.5. The pots were placed in a heated glasshouse (temperature range 15 - 25°C) with 7 h additional light provided by two 400 W high pressure sodium plant irradiators with integral control (Thermoforce Ltd., Camplex Plantcare Division, Tetbury, Glos., UK). One month after sowing, when the plants had 20 true leaves (GS 6, Fig. 1.2), the third and the fourth leaves of each plant were detached and placed on sterile glass slides (two leaves per slide) with their adaxial surfaces uppermost. Each slide was supported by two plastic rods over two layers of moistened filter paper (Whatman No 1) lining the

bottom of 9-cm plastic Petri dishes. Four dishes, with two leaves each, were used as replicates for each temperature and incubation time tested. Prior to inoculation, all the dishes were preconditioned overnight to the desired temperatures. The leaves were inoculated with a conidial suspension of 2 x $10^4$ conidia $ml^{-1}$ by the method described in section 2.1.6. Approximately 0.3 ml of the suspension was applied to each leaf. Lids were replaced, the dishes were sealed with parafilm, wrapped individually with aluminium foil to exclude light and placed in incubators regulated at the desired temperatures for the incubation times tested. The inoculation lasted 30 min and by the end of this period no conidial germination was observed. At the end of the designated incubation times, the plates with the leaves were removed from the incubators. The leaves were stained with cotton blue in lactophenol (see section 2.1.8), covered with cover slips, heated slightly over a flame and kept for further observation.

4.3.4.4. Assessments

All the assessments were done with a light microscope at x 250 magnification. A conidium was considered to have germinated if the length of the germ tube exceeded the width of the conidium (14 - 19 $\mu$m). The percentage of conidial germination on water agar was determined by observing 50 conidia per plate (total of 200 conidia per temperature and incubation time). For the percent conidial germination on detached leaves, 25 conidia per leaf (total of 200 conidia per temperature and incubation time) were assessed. Germ tube length was measured by using a calibrated eye-piece micrometer (1 division = 4.7 $\mu$m). The germ tubes of 15 conidia per plate or leaf (total of 60 conidia per temperature and incubation time) were measured on water agar and detached leaves. If the

conidium had more than one germ tube only the longest was measured. The percentage of germinated conidia forming appressoria was also assessed.

4.3.4.5. Statistical analyses

The relationship between the incubation time and the germ tube length at each temperature was described by the linear model :

$$y = a + b x \quad (4.1)$$

in which y is the length of the germ tube ($\mu$m) after an incubation time x, a is the intercept on the y-axis and b is the slope of the line.

### 4.3.5. Effect of leaf wetness on germination

4.3.5.1. Pre- and post-inoculation treatments

Linseed plants (cv. Antares) were grown in controlled environment cabinets by the method described in section 2.1.5. Twenty-four hours before inoculation of the plants, the cabinets were set at 15°C under continuous darkness. The relative humidity in the cabinets, measured by the method described in section 2.1.4, ranged from 60 to 75%. 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 by blowing air (at ambient temperature) gently over them with a hair-drier. It took approximately 5 min for the leaves of plants in five pots (one treatment) to dry and therefore this time was not included in the recorded wetness period. 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 (15°C) by less than +2°C.

4.3.5.2. Continuous leaf wetness

Linseed plants at GS 5 - 6 (the plants had 16 - 18 leaves, Fig. 1.2) were artificially inoculated (sprayed until run-off) with a mixed conidial suspension of four single-spore *A. linicola* isolates prepared by the method described in section 4.3.1. Approximately 20 ml of the conidial suspension was sprayed onto the plants in each pot. After inoculation the plants were covered for 8, 10, 12, 16, 20 or 24 h with polyethylene bags sprayed inside with water. At the end of these periods, the plants were uncovered and the third leaf of each plant, counting from the base of the stem, was detached (total of 50 leaves per treatment). The leaves were placed on glass slides with their adaxial surface uppermost, stained with cotton blue in lactophenol (see section 2.1.8) and assessed for conidial germination by using the method described in section 4.3.4.4. All the conidia on each leaf were included in the assessments. The mode of penetration of the leaf tissues by *A. linicola* was also studied but no statistical analyses were done on the data as there were no replicates.

4.3.5.3. Interrupted leaf wetness

Two experiments (I & II) were done to study the effects of interrupting a continuous leaf wetness period on the conidial germination of *A. linicola.* Interruptions of the wetness period were short (2 h) or long (12 h) dry periods applied at various times during the germination process. In both experiments, linseed plants were grown by the method described in section 4.3.2. When the plants were at GS 4 (the plants had 6 - 8 leaves, Fig. 1.2), they were artificially inoculated (sprayed until run-off) with a mixed conidial suspension of four single-spore *A. linicola* isolates. The inoculum was prepared by the method described in

section 4.3.1. Approximately 10 ml of the conidial suspension was sprayed onto the plants in each pot (10 plants per pot). After inoculation the plants were treated by the method described in section 4.3.5.1.

In Experiment I, the plants were given an initial period of leaf wetness of 0, 2, 4, 6, 8 or 10 h followed by a 2-h dry period and a final period of 10, 8, 6, 4, 2 or 0 h of leaf wetness, respectively, to complete a 12-h incubation time (Table 4.1). There were two different controls : a) plants which were inoculated, dried immediately after inoculation and left uncovered for 12 h (0 h of leaf wetness) and b) plants which were inoculated and covered for 12 h with polyethylene bags sprayed inside with water (12 h leaf wetness). The experiment was done on attached leaves and at the end of each wet or dry period, the third leaf of one plant per pot (counting from the base of the stem) (5 leaves per treatment) was collected. The leaves were prepared for examination by the method described in section 4.3.4.4. All the conidia on each leaf were included in the assessments which were done by the method described in section 4.3.4.4.

In Experiment II, a 36-h wet period was interrupted after 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18 or 24 h by a 12-h dry period (Table 4.2). There were two different controls : a) plants which were inoculated, dried immediately after inoculation and left uncovered for 36 h (0 h of leaf wetness) and b) plants which were inoculated and covered for 36 h with polyethylene bags sprayed inside with water (36 h leaf wetness). The conidial germination was assessed on the attached cotyledons and at the end of each wet or dry period, one cotyledon per pot (5 cotyledons per treatment) was collected. The cotyledons were prepared for examination by the method described in section 4.3.4.4. All the conidia on each

**Table 4.1.** Treatments used to study the effects of interrupting a continuous leaf wetness period by a 2-h dry period on conidial germination of *A. linicola* on cotyledons of linseed plants (cv. Antares).

| Treatment | Wet (h) | Dry (h) | Wet (h) | Total period (h) |
|---|---|---|---|---|
| 1 | 0 | 2 | 10 | 12 |
| 2 | 2 | 2 | 8 | 12 |
| 3 | 4 | 2 | 6 | 12 |
| 4 | 6 | 2 | 4 | 12 |
| 5 | 8 | 2 | 2 | 12 |
| 6 | 10 | 2 | 0 | 12 |
| Control 1 | Inoculated, dried, left uncovered for 12 h | | | |
| Control 2 | Inoculated and covered with polyethylene bags for 12h | | | |

**Table 4.2.** Treatments used to study the effect of interrupting a continuous leaf wetness period by a 12-h dry period on conidial germination of *A. linicola* on cotyledons of linseed plants (cv. Antares).

| **Treatment** | **Wet (h)** | **Dry (h)** | **Wet (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 and covered with polyethylene bags for 36h | | | |

cotyledon were included in the assessments which were done by the method described in section 4.3.4.4.

### 4.3.6. Effects of light regime on germination

4.3.6.1. Pre- and post-inoculation treatments

Linseed plants (cv. Antares) were grown in controlled environment cabinets (5 plants per pot) by the method described in section 4.3.2. Twenty-four hours before inoculation of the plants, the cabinets were set at 15°C under continuous light. The relative humidity in the cabinets, measured by the method described in section 2.1.4 ranged from 60 to 75%. The light intensity measured at the plant level using the method described in section 2.1.4 ranged from 120 to 160 $\mu$Einsteins $m^{-2}$ $sec^{-1}$. The wet or dry periods were produced by the method described in section 4.3.5.1. During the wet dark periods, the plants were covered with polyethylene bags (sprayed inside with water) and wrapped with aluminium foil to exclude light. The temperature inside the bags or the alumunium foil deviated from the temperature set (15°C) by less than +2°C.

4.3.6.2. Under continuous leaf wetness

When the plants were at GS 4 (the plants had 6 - 8 leaves, Fig. 1.2) they were artificially inoculated (sprayed until run-off) with a mixed conidial suspension of four single-spore *A. linicola* isolates. The inoculum was prepared by the method described in section 4.3.1. Approximately 10 ml of the conidial suspension was sprayed onto the plants in each pot. After inoculation the plants were treated according to the method described in section 4.3.6.1. The plants were given the different light treatments described in Figure 4.1 for 24 h. There were two

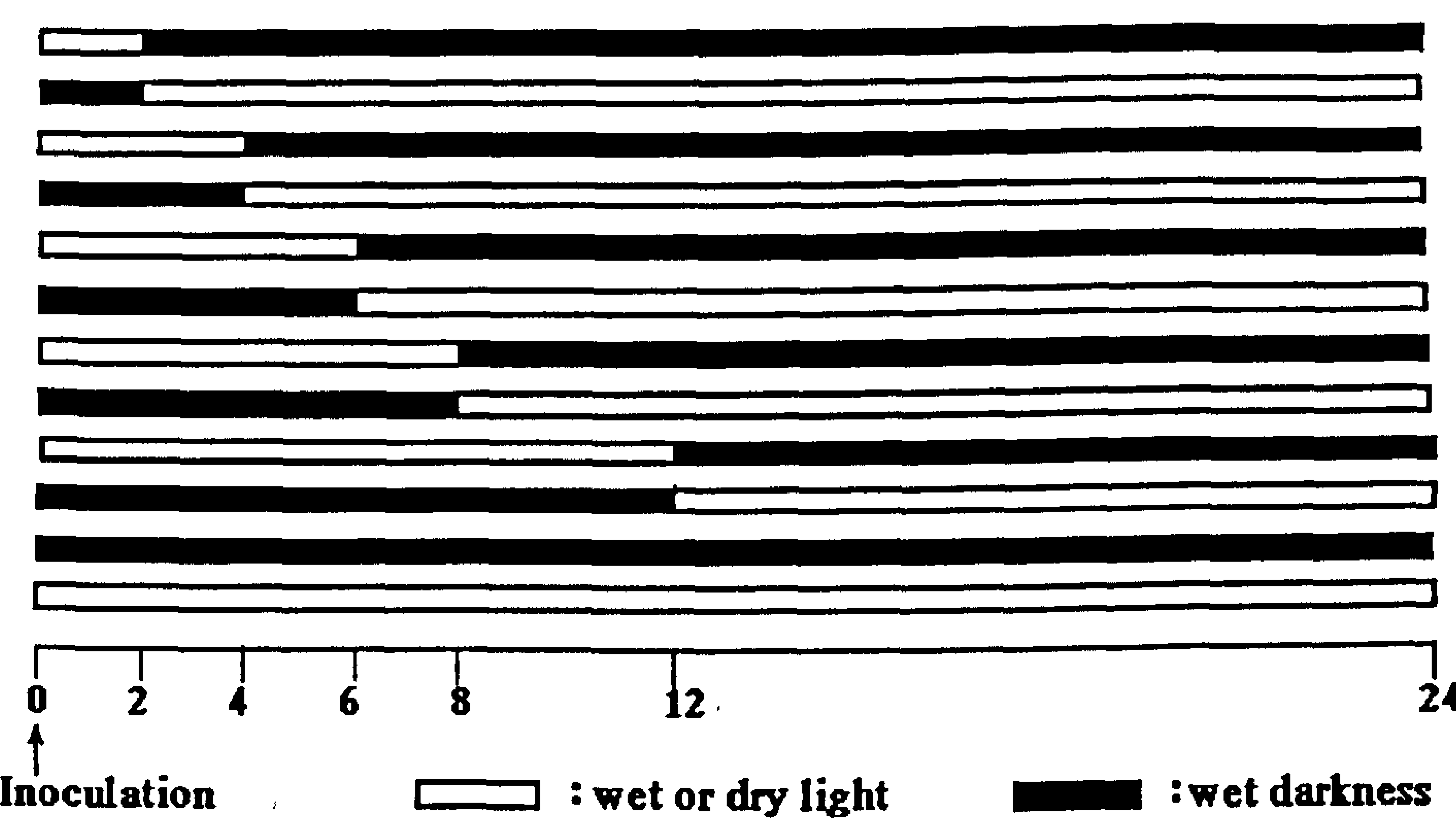



**Figure 4.1.** Treatments used to study the effects of a wet or dry light period of various lengths applied before or after a period of wet darkness on the percentage of germination and germ tube elongation of *A. linicola* conidia on attached linseed leaves (cv. Antares) at 15°C.

different controls : a) plants which were inoculated and exposed to light for 24 h and b) plants which were inoculated and exposed to darknesss for 24 h. At the end of each light or dark period the third leaf of each plant (counting from the base of the stem) was collected (25 leaves per treatment). The leaves were prepared for examination by the method described in section 4.3.4.4. All the conidia on each leaf were included in the assessments which were done according to the method described in section 4.3.4.4.

4.3.6.3. Under interrupted leaf wetness

Plants at GS 4 (the plants had 6 - 8 leaves, Fig. 1.2), were artificially inoculated (sprayed until run-off) with a mixed conidial suspension of four single-spore *A. linicola* isolates. The inoculum was prepared according to the method described in section 4.3.1. Approximately 10 ml of the conidial suspension was sprayed onto the plants in each pot. After inoculation the plants were treated by the method described in section 4.3.6.1. The plants were given the dry light and wet dark treatments described in Figure 4.1 for 24 h. There were two different controls : a) plants which were inoculated, dried immediately after inoculation and left uncovered for 24 h (24 h of dry light) and b) plants which were inoculated and covered for 24 h with polyethylene bags (sprayed inside with water) and aluminium foil (24 h of wet darkness). At the end of each period (wet light, dry light or wet darkness), the third leaf of each plant (counting from the base of the stem) was collected (25 leaves per treatment). The leaves were prepared for examination according to the method described in section 4.3.4.4. All the conidia on each leaf were included in the assessments which were done by the method described in section 4.3.4.4.

## 4.4. Results

### 4.4.1. Effects of temperature and incubation time on conidial germination on agar and detached leaves

*A. linicola* conidia germinated over a wide range of temperatures (5 - 25°C) on both agar and detached leaves (Fig. 4.2), mainly by producing germ tubes and occasionally by producing secondary conidia (Fig. 4.3). All the cells of a conidium could germinate (Fig. 4.3). In general, the percentage of conidia which germinated increased with increasing temperature and increasing length of incubation time. The percentage germination of conidia was greater on agar than on leaves, at least during the first 12 h of incubation. At 5°C germination was slower on agar and detached leaves and 40% and 21% of the conidia had failed to germinate, respectively, even after 48 h of incubation. All the conidia germinated at temperatures higher than 5°C, although there were some differences in the rate of germination (Fig. 4.3).

The effects of temperature and incubation time on germ tube elongation on agar and detached leaves were similar to those on the percentage of germination of conidia (Fig. 4.4). Linear regressions fitted all sets of data quite well. On average, linear regressions of y (length of germ tube) on x (incubation time) at each temperature accounted for 98% and 97% of the variance on water agar and detached leaves, respectively (Table 4.3). Although the germ tube length increased with increasing temperature and incubation time on both surfaces, the rate of elongation was slower on detached leaves than on agar. At 5°C germ tubes grew very slowly, reaching lengths of less than 100 $\mu$m after 48h. Elongation was rapid at temperatures above 5°C and after 12 h of incubation

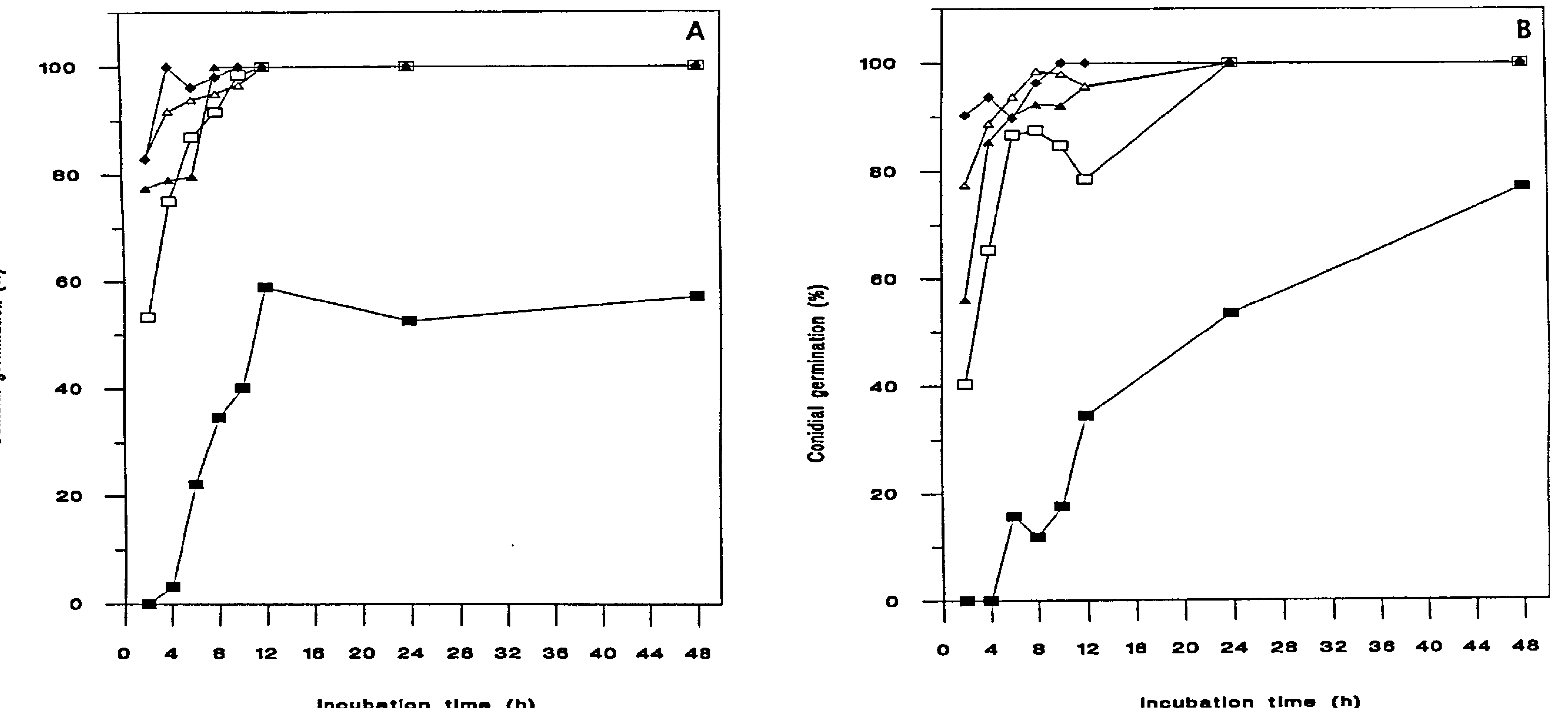



**Figure 4.2.** Effects of temperature and incubation time on the rate of germination of *A. linicola* conidia on water agar (A) and detached linseed leaves (cv. Antares) (B); ■ : 5°C; □ : 10°C; ▲ : 15°C; △ : 20°C; ◆ : 25°C. SED (120 d.f.) = 2.74 (water agar); SED (120 d.f.) = 2.94 (detached leaves).

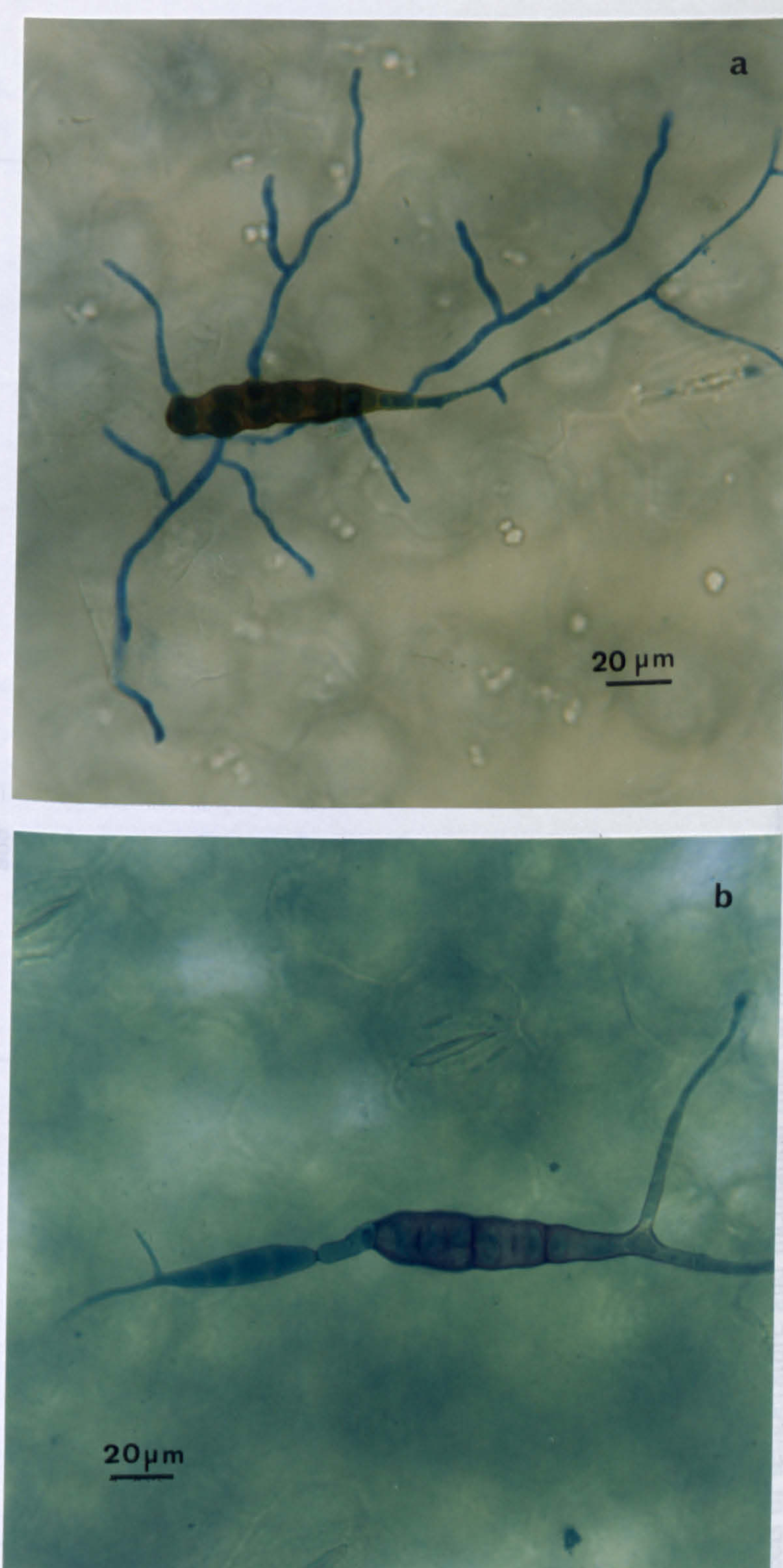



**Figure 4.3**. *A. linicola* conidia germinating on linseed leaves producing either several germ tubes (a) or a secondary conidium (b), 24 h after inoculation and stained with cotton blue in lactophenol.

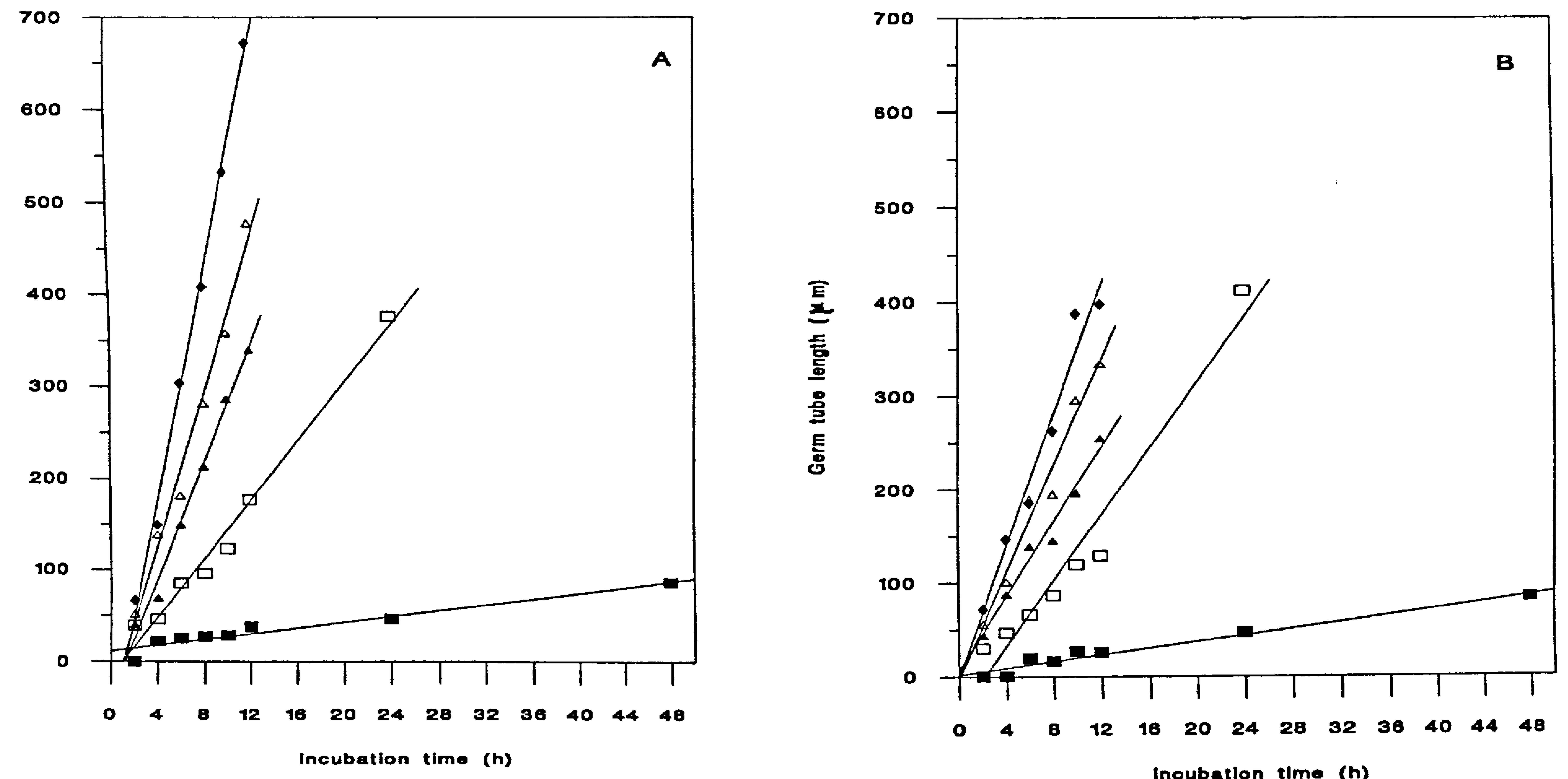



**Figure 4.4.** Effects of temperature and incubation time on the rate of germ tube elongation of *A. linicola* conidia on water agar (A) and detached linseed leaves (cv. Antares) (B); ■ : 5°C; □ : 10°C; ▲ : 15°C; △ : 20°C; ◆ : 25°C. The percentages of variance accounted for ($r^2$) are given in Table 4.3.

**Table 4.3.** Parameters estimated for the linear regressions ($y^* = a + b x^\dagger$) used to describe the elongation of germ tubes of *A. linicola* conidia with time at 5, 10, 15, 20 or 25°C on water agar or detached leaves.

| Temperature (°C) | Water agar | | | Detached leaves | | |
|---|---|---|---|---|---|---|
| | $a^{\dagger\dagger}$ | $b^{\ddagger}$ | $r^{2**}$ | $a^{\dagger\dagger}$ | $b^{\ddagger}$ | $r^{2**}$ |
| 5 | 11.6 | 1.6 | 92.7 | 1.8 | 1.8 | 96.0 |
| 10 | -15.0 | 15.9 | 98.4 | -38.6 | 17.6 | 95.6 |
| 15 | -38.1 | 31.6 | 99.1 | 8.1 | 19.8 | 97.2 |
| 20 | -40.5 | 41.3 | 98.6 | -2.9 | 28.3 | 97.1 |
| 25 | -72.7 | 81.2 | 99.6 | -1.2 | 34.7 | 96.8 |

* y : germ tube length.
† x : incubation time.
†† a : constant equal to the value of y at x=1.
‡ b : slope of the linear regression.
** $r^2$ : % variance accounted for.

the germ tubes were too long to be measured (Fig. 4.4).

Appressoria were formed as a terminal swelling of the germ tubes of the germinated conidia on detached leaves (Fig. 4.5); no formation of appressoria was observed on water agar. Conidia that germinated at 5°C had not formed appressoria at any of the incubation times tested (Fig. 4.6). Formation of appressoria was observed less than 4 h after inoculation at 15, 20 and 25°C. At 10°C only 11% of the conidia which had germinated had formed appressoria after 10 h of incubation. However, this percentage increased rapidly during the next few hours and 24 h after inoculation all the germinated conidia had formed appressoria (Fig. 4.6).

### 4.4.2. Effects of leaf wetness on germination

#### 4.4.2.1. Continuous leaf wetness

4.5.2.1.1. Germination and germ tube elongation on attached leaves

After 8 h of incubation under continuous leaf wetness, 73% of the conidia had germinated (Fig. 4.7). By increasing the length of the leaf wetness period from 8 to 24 h the percentage of conidia which germinated did not increase significantly. However, the length of the germ tubes increased with increasing leaf wetness duration reaching 290 $\mu$m after 24 h (Fig. 4.8). Approximately 30% of the germinated conidia had formed appressoria after 8 h of incubation under continuous leaf wetness. Although there were differences between subsequent samples in the percentage of germinated conidia which formed appressoria, this percentage did not increase significantly with further incubation (Fig. 4.8).

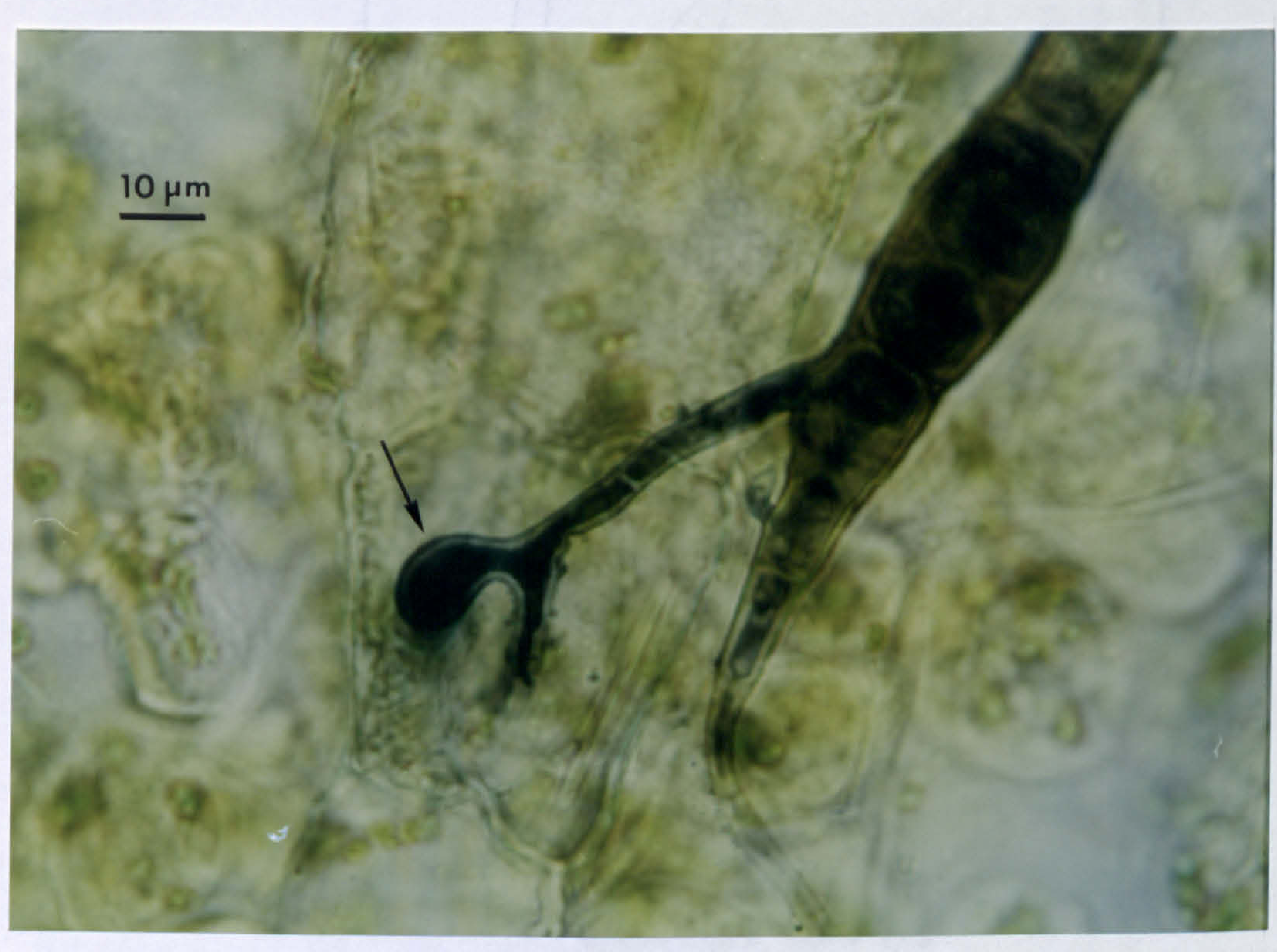



**Figure 4.5.** Formation of an appressorium (arrow) by a germinating *A. linicola* conidium on a linseed leaf, 4 h after inoculation at 25°C.

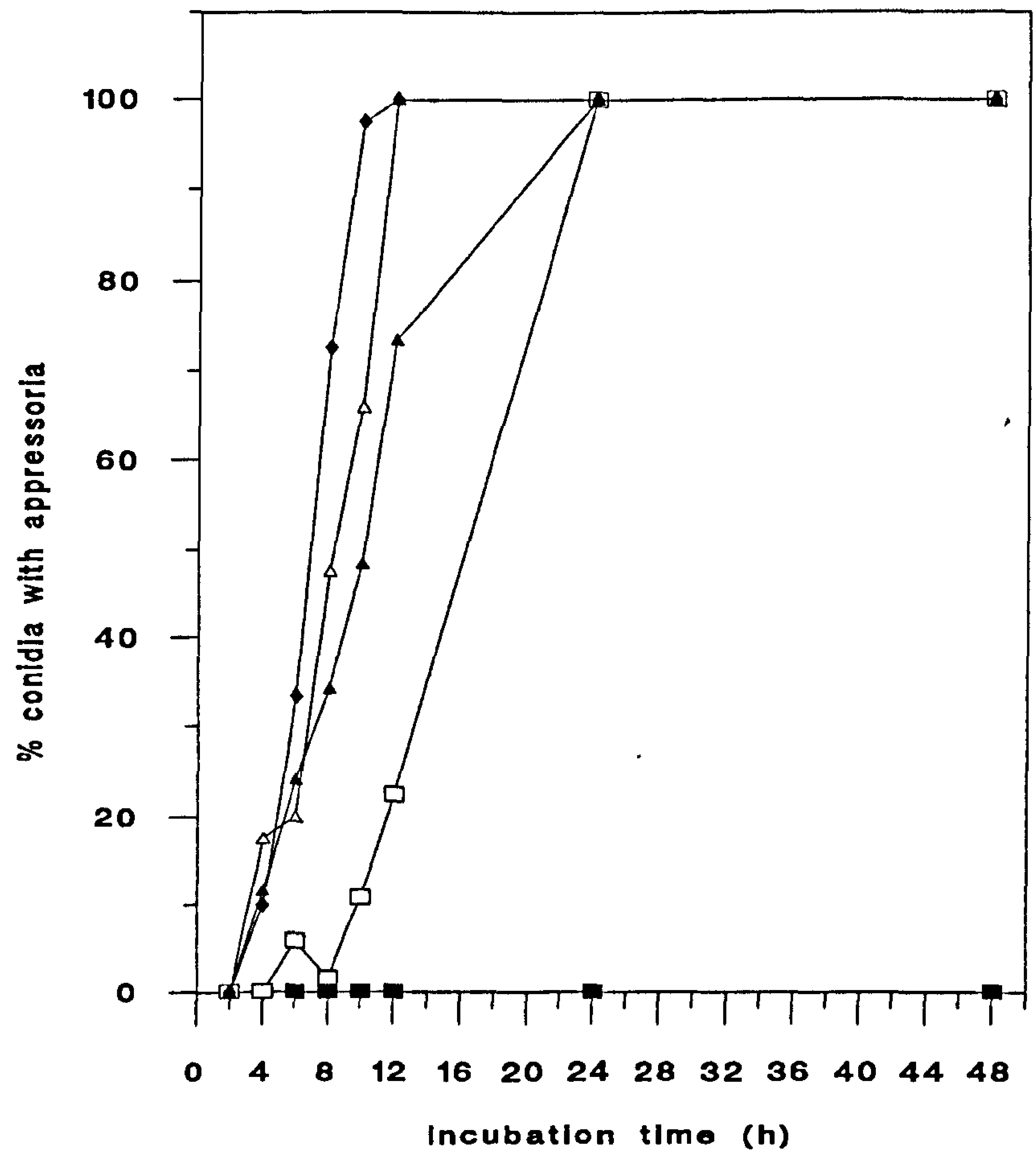



**Figure 4.6.** Effects of temperature and incubation time on the percentage of *A. linicola* conidia forming appressoria on detached linseed leaves (cv. Antares). ■ : 5°C; □: 10°C; ▲: 15°C; △ : 20°C; ◆ : 25°C. SED (120 d.f.) = 5.81

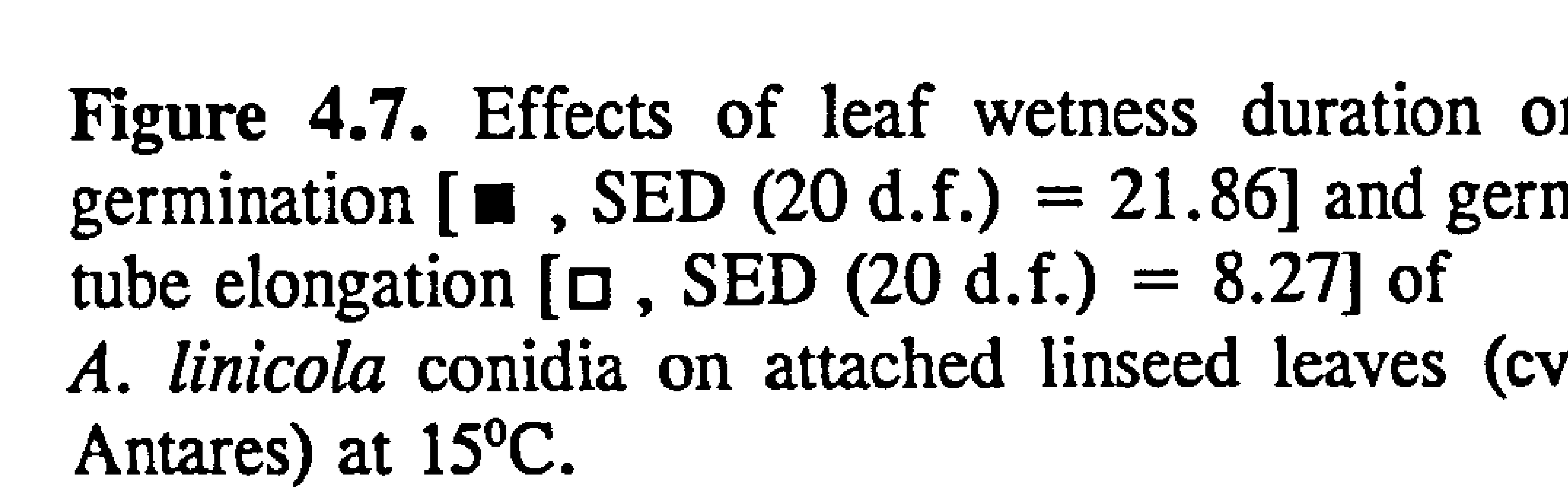


Figure 4.7. Effects of leaf wetness duration on germination [■ , SED (20 d.f.) = 21.86] and germ tube elongation [□ , SED (20 d.f.) = 8.27] of *A. linicola* conidia on attached linseed leaves (cv. Antares) at 15°C.

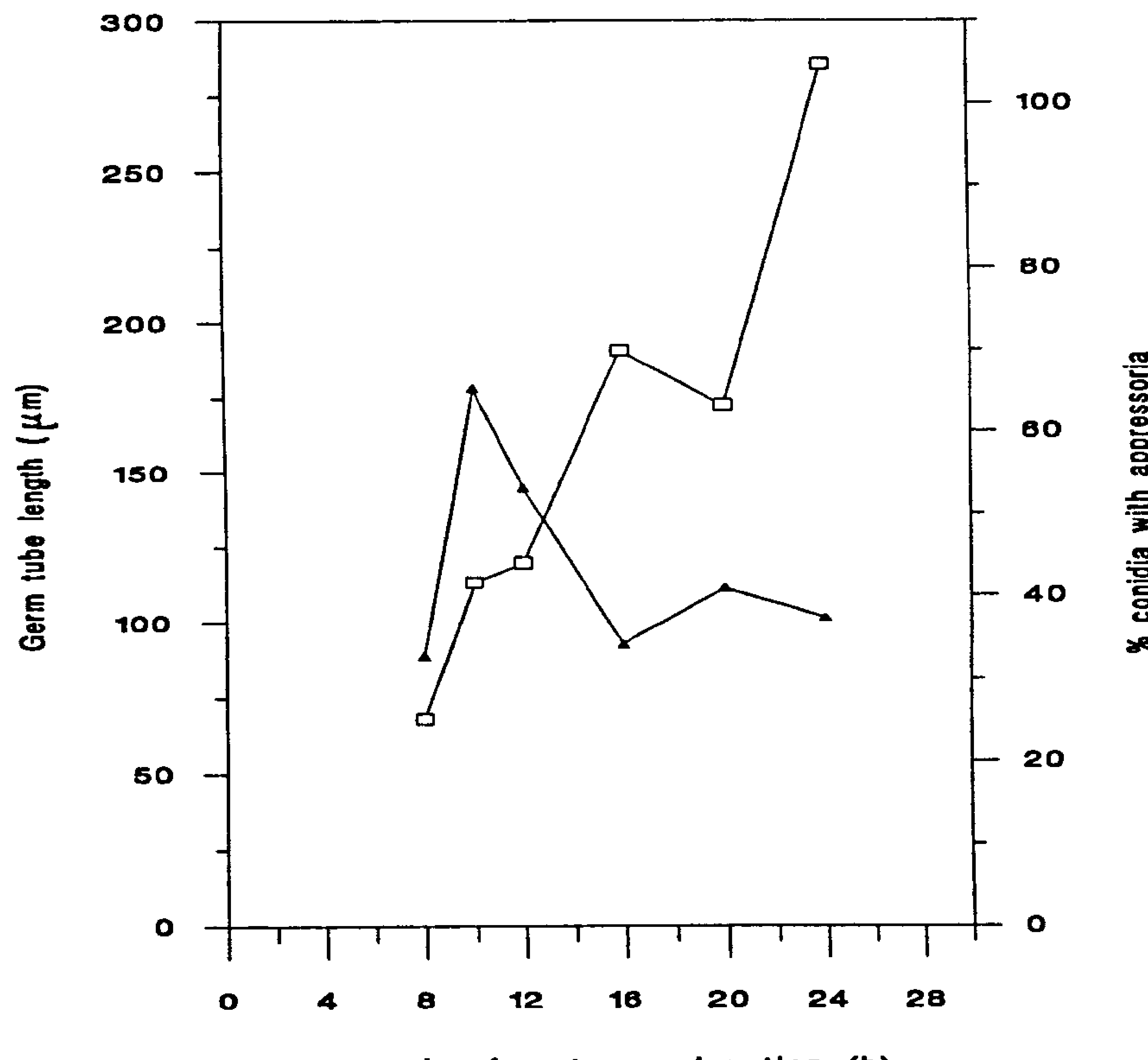



Figure 4.8. Effects of leaf wetness duration on germ tube length [□ , SED (20 d.f.) = 8.27] and on the percentage of *A. linicola* conidia forming appressoria [ ▲ , SED (20 d.f.) = 13.64] on attached linseed leaves (cv. Antares) at 15°C.

4.4.2.1.2. Penetration of leaf tissues

Penetration of the leaf tissues by *A. linicola* conidia was observed 12 h after inoculation and occurred either with (Fig. 4.9a) or without appressoria (Fig. 4.9b). The appressoria adhered to the epidermis and produced infection pegs (Fig. 4.9a) that penetrated into the epidermal cells, forming vesicles (Fig. 4.9c). Hyphae of *A. linicola* penetrated the leaf tissue, either directly through epidermal cells or indirectly through stomata (Fig. 4.9). After 24 h of incubation under continuous leaf wetness, 10% and 2% of the germinated conidia had penetrated the leaf tissue directly or indirectly, respectively (Fig. 4.10). Stomatal penetration appeared to occur by chance; hyphae of *A. linicola* sometimes grew over stomata without penetrating (Fig. 4.11). After the fungus entered the leaf tissue, hyphal growth was intercellular. Necrosis of epidermal cells around some *A. linicola* conidia was also observed after 24 h of incubation (Fig. 4.12). The walls of the dead epidermal cells became swollen, refracted the light more intensely and readily stained with cotton blue in lactophenol (Fig. 4.12).

**4.4.2.2. Interrupted leaf wetness**

The effects of interrupting a period of continuous leaf wetness by a short (2 h) or long (12 h) dry period during the germination process on the percentage of conidial germination and the germ tube elongation depended not only on the time at which the interruption occurred, but also on the duration of the dry period.

A 2-h dry period applied immediately after inoculation (0 h) did not affect germination and all the conidia (100%) germinated after an additional period of 12 h of leaf wetness (Fig. 4.13A). However, a dry period applied 2 h after

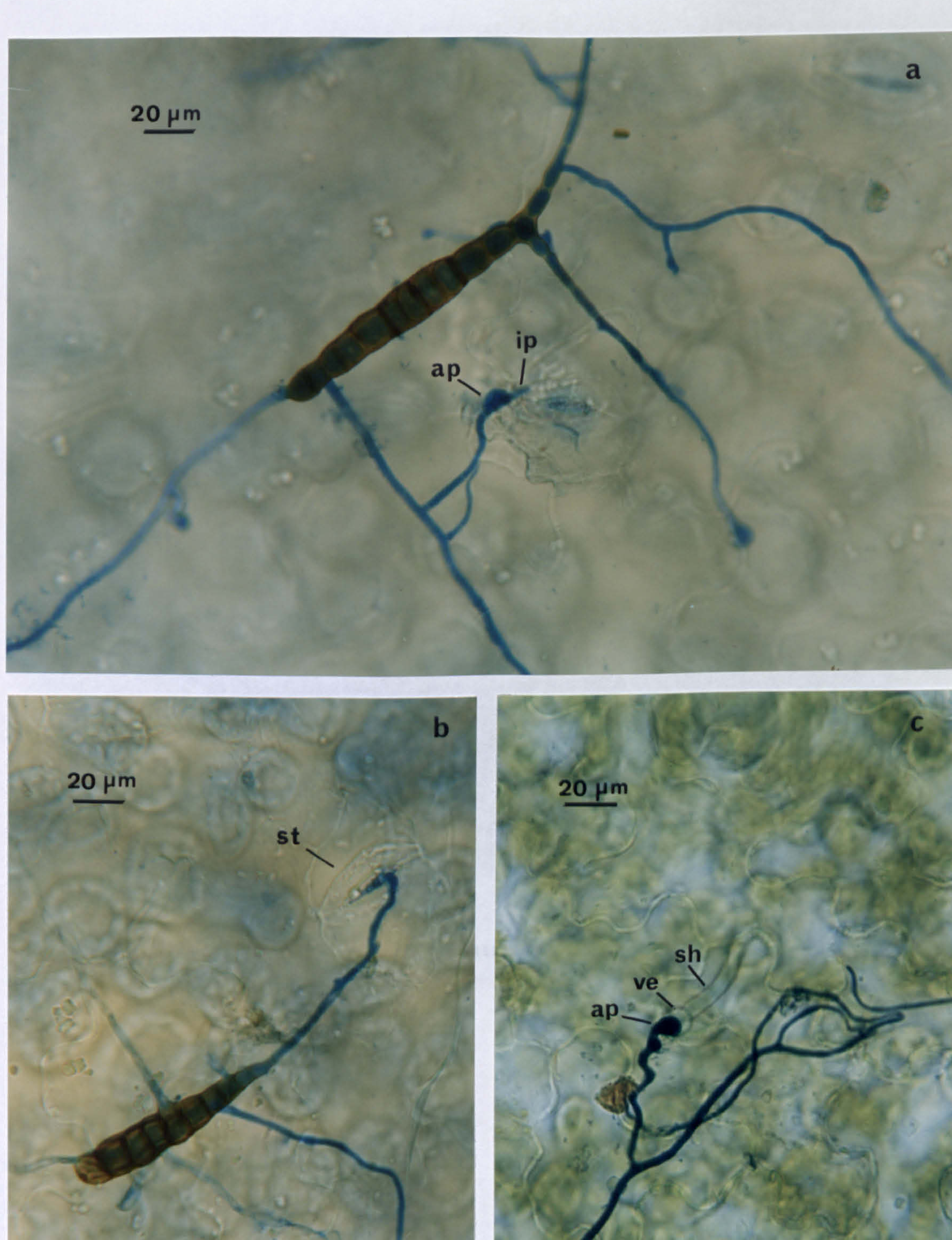



**Figure 4.9.** Penetration of linseed leaves by *A. linicola* conidia, 12 h after inoculation; (a) : direct penetration of epidermal cell with the formation of an appressorium (ap) and infection peg (ip); (b) : indirect penetration through a stoma (st) without the formation of an appressorium; (c) : direct penetration with formation of an appressorium (ap), vesicle (ve) and subcuticular hypha (sh). Preparations were stained with cotton blue in lactophenol.

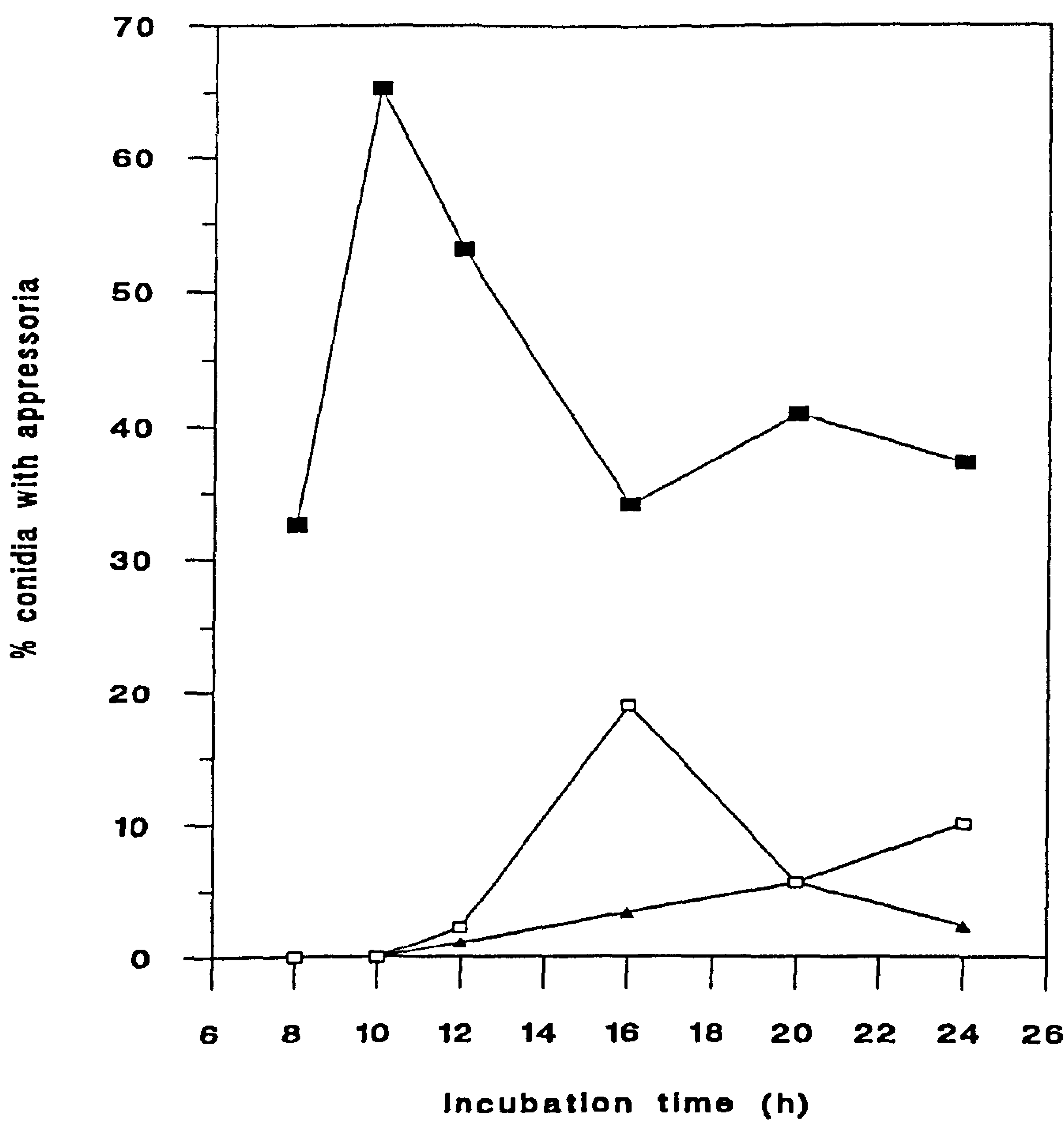



**Figure 4.10**. Percentage of *A. linicola* conidia with appressoria (■) penetrating the linseed leaf tissue directly ( □ ) or indirectly through stomata ( ▲ ).

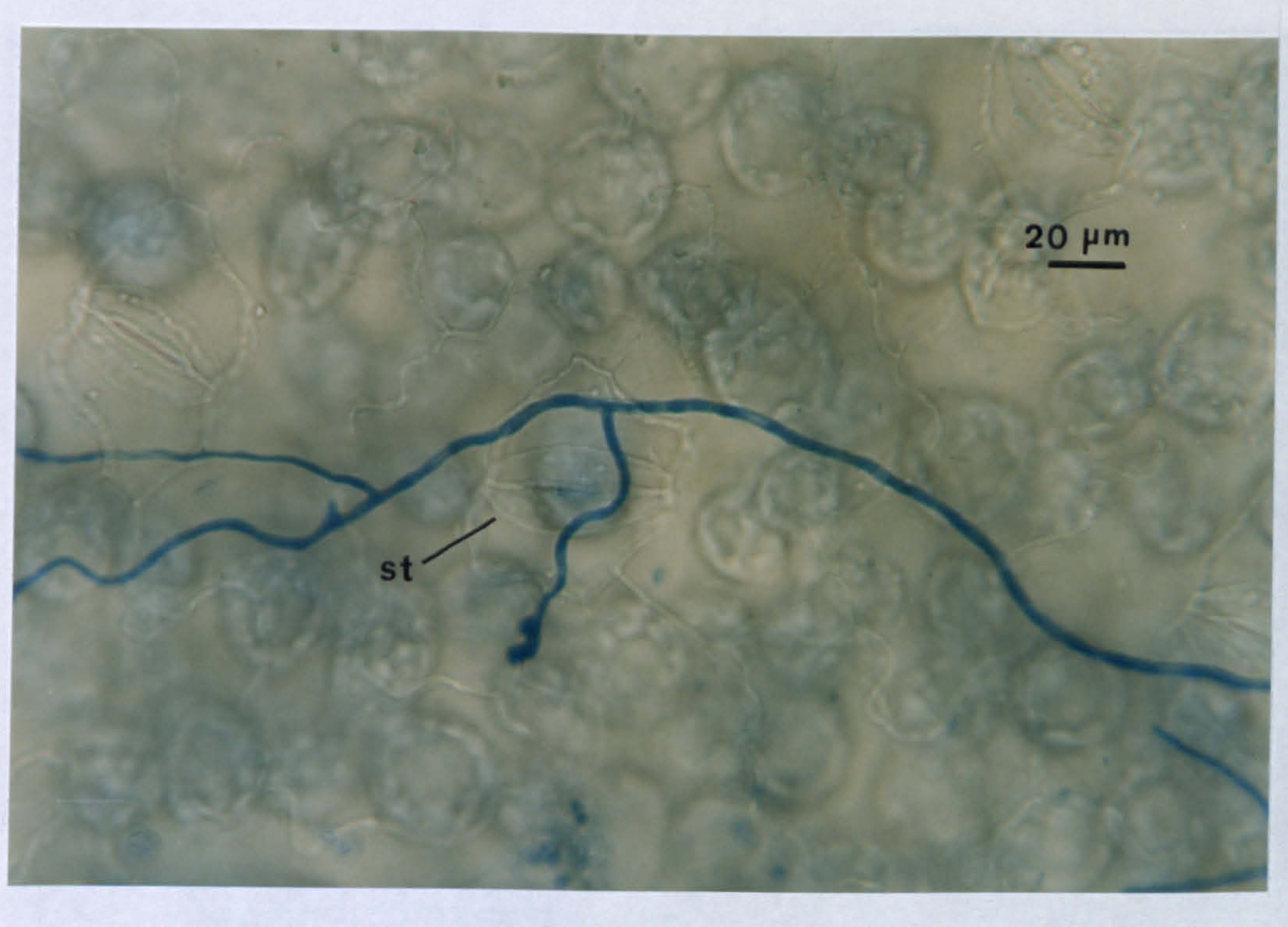


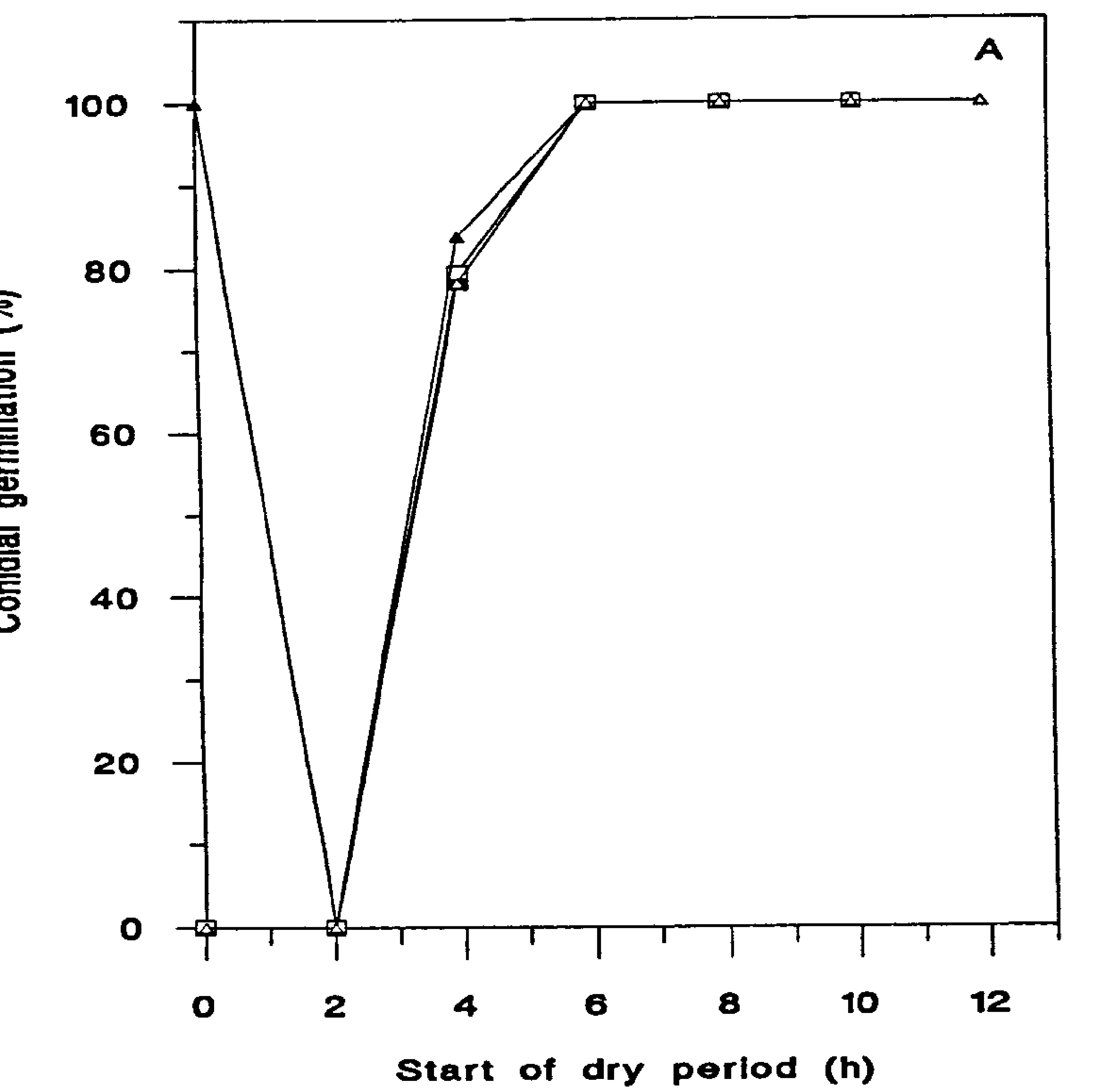

**Figure 4.11**. *A. linicola* hypha (stained with cotton blue in lactophenol) growing over a stoma (st) without penetrating it, 24 h after inoculation at 20°C.

**Figure 4.12.** Necrosis of epidermal cells of linseed leaves (arrows) around conidia of *A. linicola*. Preparation stained with cotton blue in lactophenol.

inoculation inhibited the germination of the conidia, which did not recover even when an additional period of leaf wetness was applied (Fig. 4.13A). Similarly, a 2-h dry period applied 4 h after inoculation, when 78% of the conidia had already germinated, stopped the germination process and only an additional 6% of conidia germinated when an additional period of leaf wetness (8 h) was applied subsequently (Fig. 4.13A). However, all the conidia (100%) germinated within 12 h under continuous leaf wetness. No conidial germination was observed when the plants were incubated under a 12-h continuous dry period (control 2).

Short (2 h) interruptions of continuous wetness periods by dry periods affected germ tube elongation in the same way that they affected conidial germination (Fig. 4.13B). However, there was a slight increase in the lengths of the germ tubes when an additional period of leaf wetness was applied after the dry periods, although no further elongation of the germ tubes occurred during the dry periods (Fig. 4.13B).

Longer (12 h) interruptions of the continuous leaf wetness periods by dry periods either suppressed or stopped the germination process depending on the time at which they were applied during the germination process. Dry periods applied after 1 or 2 h of incubation suppressed germination and only 19 and 21% of the conidia germinated during the second period of leaf wetness that followed the 12-h dry period (Fig. 4.14A). When a 12-h dry period interrupted the 24-h period of leaf wetness after 3, 4, 6, 7, 8, 10, 12, 18 or 24 h of incubation, it prevented the germination of any more conidia, irrespective of the length of the subsequent period of leaf wetness (Fig. 4.14A). All the conidia germinated after a 36-h period of continuous leaf wetness (control 2) but no germination of conidia was observed after



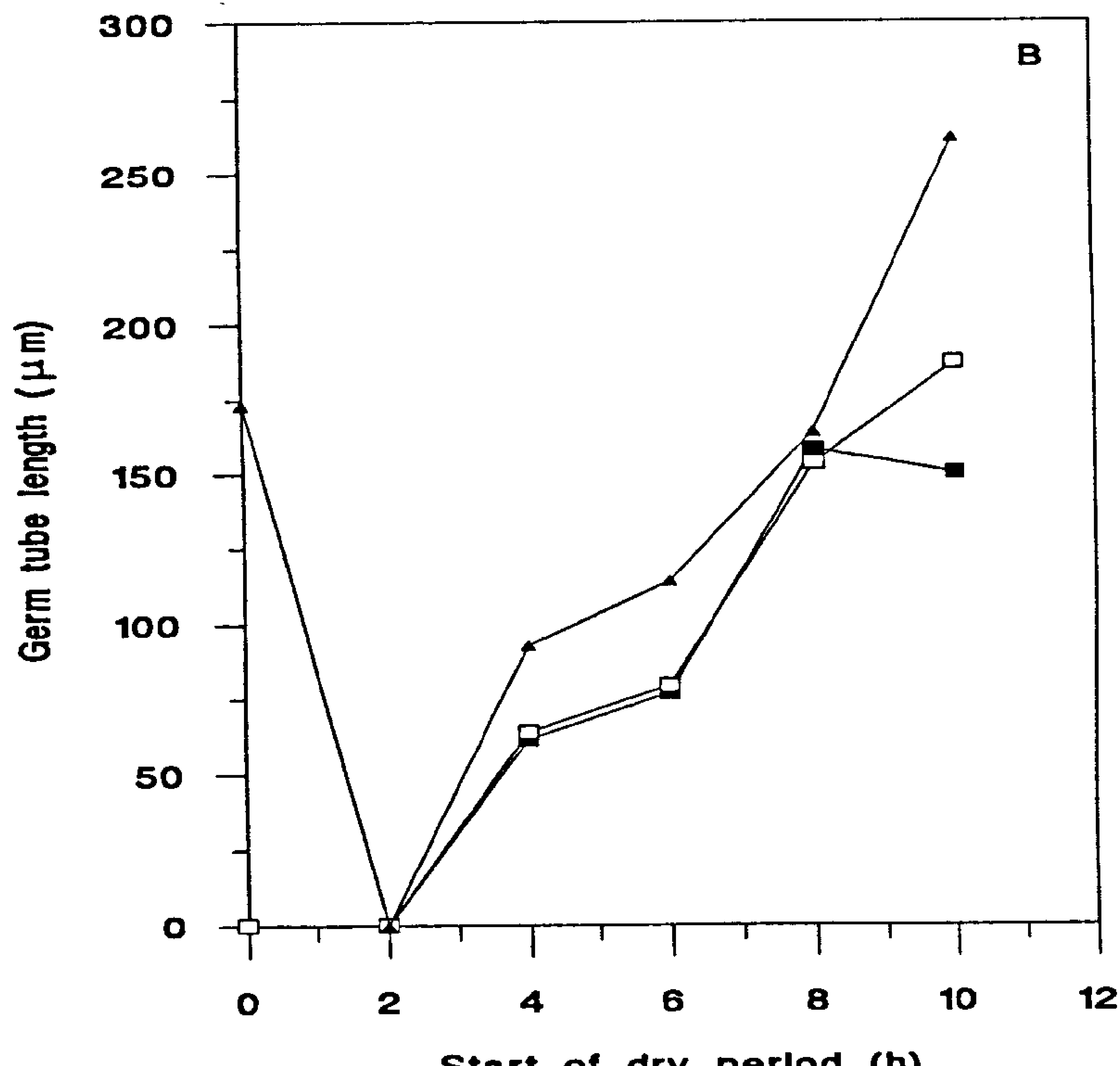




**Figure 4.13.** The effects of interrupting a 12-h continuous period of leaf wetness by a 2-h dry period in darkness at different times during the germination process on the percentage of germination (A) and germ tube length (B) of *A. linicola* conidia on attached leaves at 15°C; ■ : initial wet period; □ : 2 h dry period; ▲ : second wet period. The total incubation period was 12 h. As there was no variation between replicates (except in one case), no statistical analysis was possible for the percentage of germination. SED (20 d.f.) = 10.5 (germ tube elongation).

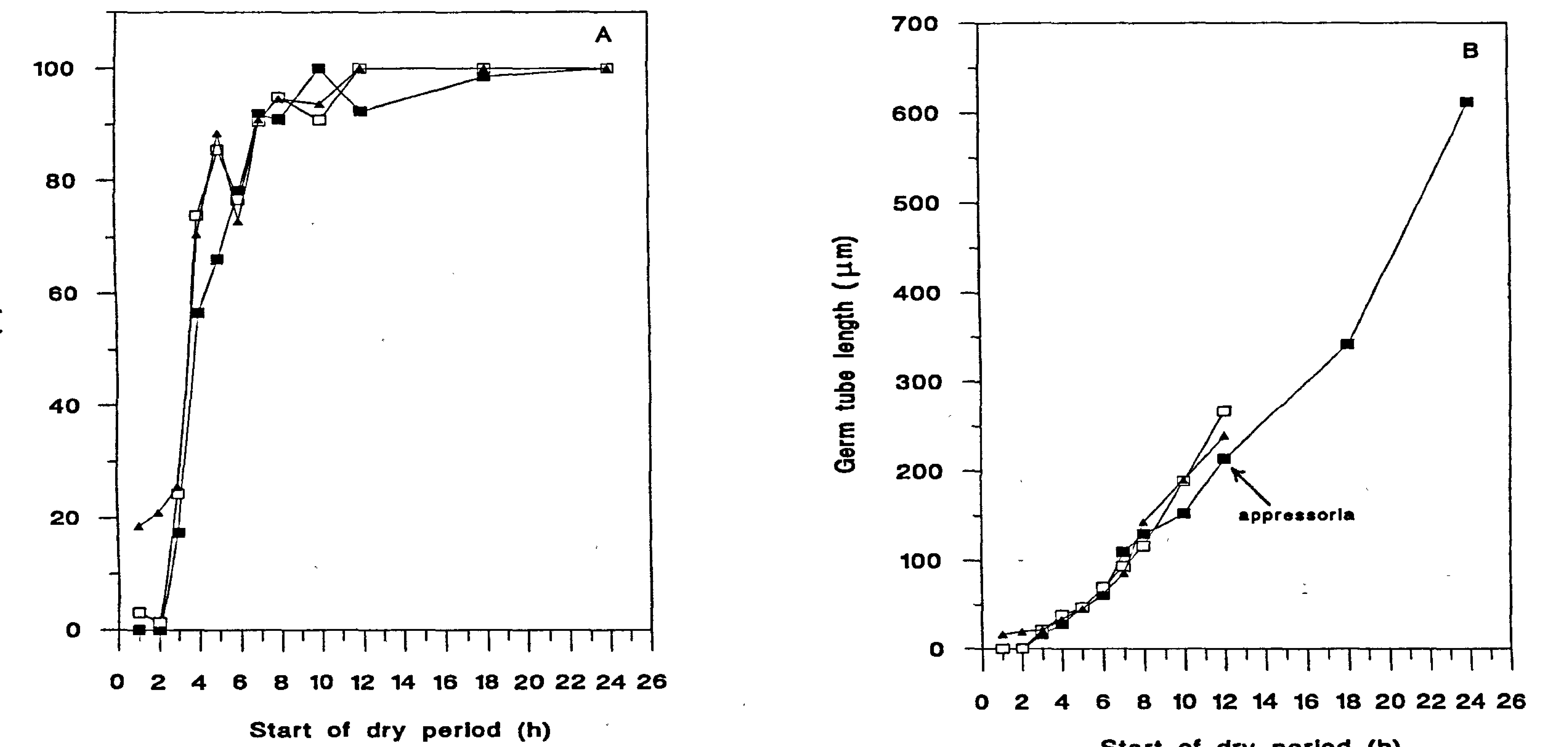



**Figure 4.14.** The effects of interrupting a 36-h continuous period of leaf wetness by a 12-h dry period in darkness at different times during the germination process on the percentage of germination (A) and germ tube length (B) of *A. linicola* conidia on attached leaves at 15°C; ■ : initial wet period; □ : 12 h dry period; ▲ : second wet period. The total incubation period was 12 h. SED (72 d.f.) = 6.56 (percentage of germination); SED (1031 d.f.) = 13.54 (germ tube elongation).

a 36-h dry period (control 1). The 12-h dry period also stopped the elongation of germ tubes when it was applied at any time during the period between 1 h and 8 h after inoculation (Fig. 4.14B). However, a 12-h dry period applied 10, 12, 18 or 24 h after inoculation did not affect the elongation of germ tubes which continued to grow to lengths of more than 700 $\mu$m after 18 h of incubation (not shown on the graph). The germ tubes of conidia incubated for 36 h under continuous wet darkness were too long to be measured. The first appressoria were observed after 12 h of incubation in wet darkness (Fig. 4.14B).

**4.4.3. Effects of light regime on germination**

4.4.3.1. Under continuous leaf wetness

Light applied for 2, 4, 6, 8 or 12 h immediately after inoculation inhibited the germination of *A. linicola* conidia (Fig. 4.15). However, the conidia did not lose their viability and germinated (100% germination) during the dark period that followed the light period (Fig. 4.15). However, germ tube elongation was affected by the initial period in light. Light periods of 2 h or 4 h did not affect the elongation of the germ tubes which reached approximately 500 $\mu$m after 24 h of incubation (Fig. 4.16). However, the length of the germ tubes decreased with increasing exposure to light and reached only 125 $\mu$m and 61 $\mu$m when the conidia were exposed to initial light periods of 12 h or 24 h, respectively (Fig. 4.16).

Light applied after a dark period of various lengths when germination had started affected conidial germination, depending on the time during the germination process at which the light period was applied (Fig. 4.15). Wet light applied after 2 or 4 h of incubation in wet darkness did not stop conidial

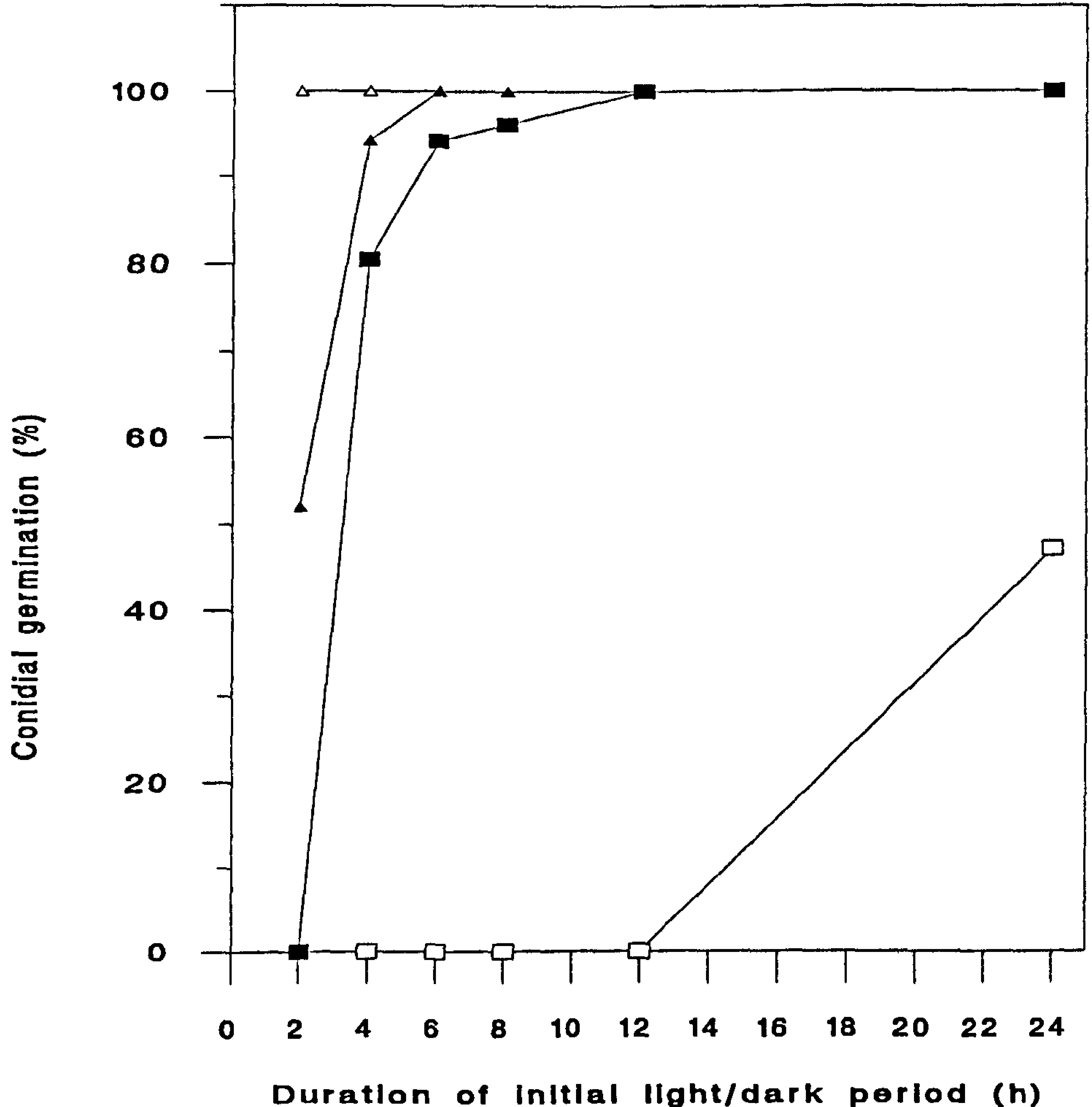



**Figure 4.15.** The effects of a wet light period of various lengths applied before ( □ , △ ) or after ( ■ , ▲ ) a wet dark period on the percentage germination of *A. linicola* conidia on attached leaves at 15°C; □ : initial assessment, after light period (analysis not possible); △ : final assessment (24 h), after light + dark periods, SED (4 d.f.) = 2.60; ■ : initial assessment, after dark period, SED (8 d.f.) = 3.77; ▲ : final assessment (24 h), after dark + light periods, SED (8 d.f.) = 2.30

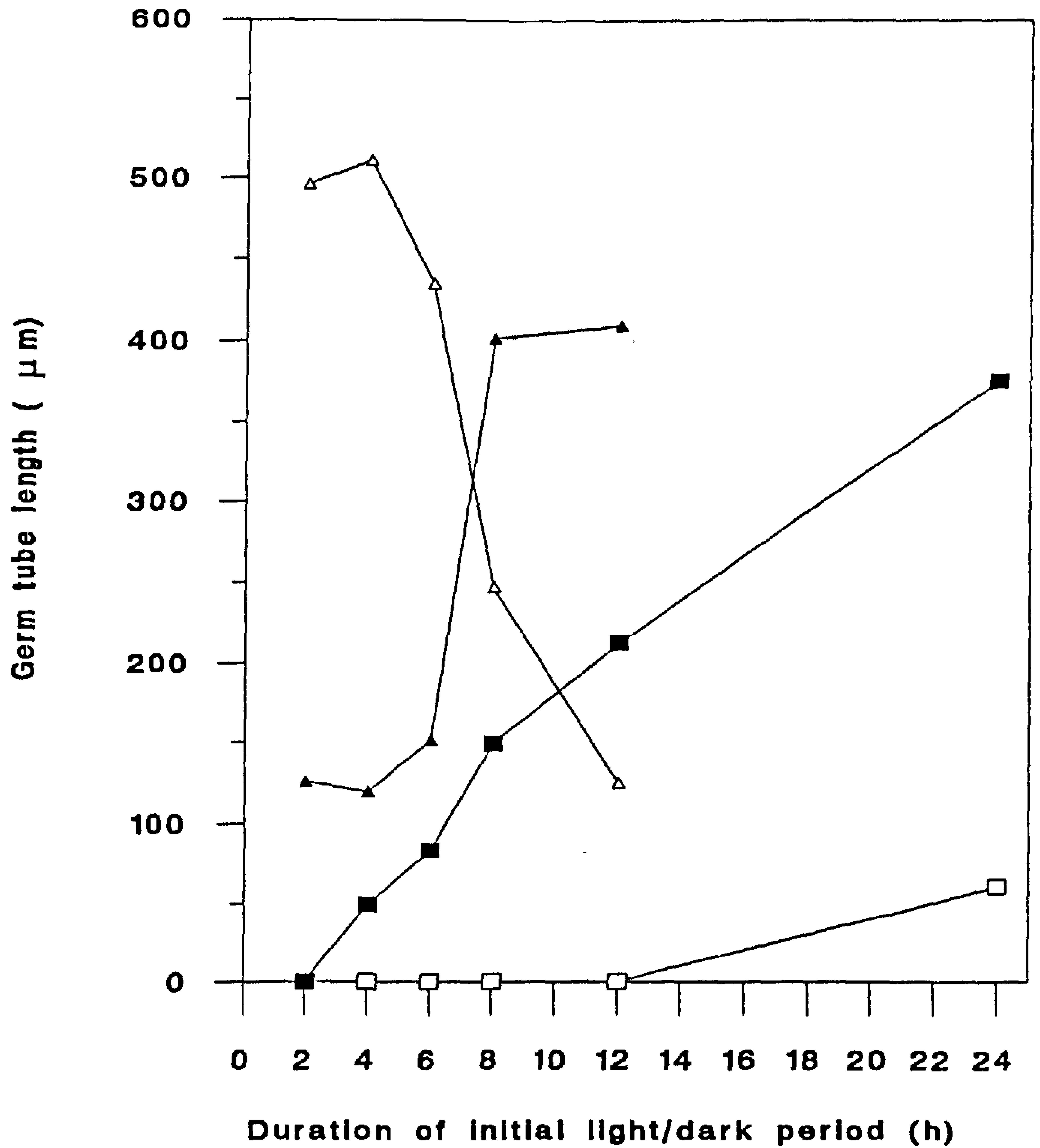



**Figure 4.16.** The effects of a wet light period of various lengths applied before ( □ , △ ) or after ( ■ , ▲ ) a wet dark period on the germ tube elongation of *A. linicola* conidia on attached leaves at 15°C; □ : initial assessment, after light period (analysis not possible); △ : final assessment (24 h), after light + dark periods, SED (24 d.f.) = 12.77; ■ : initial assessment, after dark period, SED (16 d.f.) = 6.69; ▲: final assessment (24 h), after dark + light periods, SED (16 d.f.) = 8.57

germination, but the percentage of conidia which germinated at the end of the incubation time (24 h) was smaller (52% and 94%, respectively) than that of the conidia incubated for 24 h under continuous darkness (100%) (Fig. 4.15). However, light period applied 6, 8 or 12 h after inoculation, when most of the conidia had already germinated (> 90%) did not affect germination (Fig. 4.15).

The length of the germ tubes also increased with increasing exposure to darkness and reached 376 μ after 24 h of incubation (Fig. 4.16). Light periods of 22, 20 or 18 h applied 2, 4 or 6 h after inoculation, respectively, decreased the rate of germ tube elongation, so that the lengths of the germ tubes were smaller (126, 120 and 152 μm, respectively) than those of conidia exposed to continuous darkness for 24 h (376 μm) (Fig. 4.16). However, light periods of 16 or 12 h applied 8 or 12 h after inoculation, respectively, did not affect the elongation of the germ tubes; the germ tubes continued to grow to lengths of approximately 400 μm and 410 μm, respectively, 24 h after inoculation (Fig. 4.16).

4.4.3.2. Under interrupted wetness

Dry light applied immediately after inoculation inhibited the germination of *A. linicola* conidia irrespective of the duration of the light period. The conidia did not recover even when they were exposed to a 22-h wet dark period following the dry light period (Fig. 4.17). Similarly, dry light periods applied after an initial wet dark period of various lengths stopped the germination of the remainder of the conidia (Fig. 4.17). The percentage germination of the conidia exposed to a wet dark period immediately after inoculation increased with increasing duration of the wet dark period and was 90% and 97% after 8 and 12 h, respectively (Fig. 4.17). Germination started between 2 and 4 h after inoculation and all the conidia had germinated (100%) within 24h

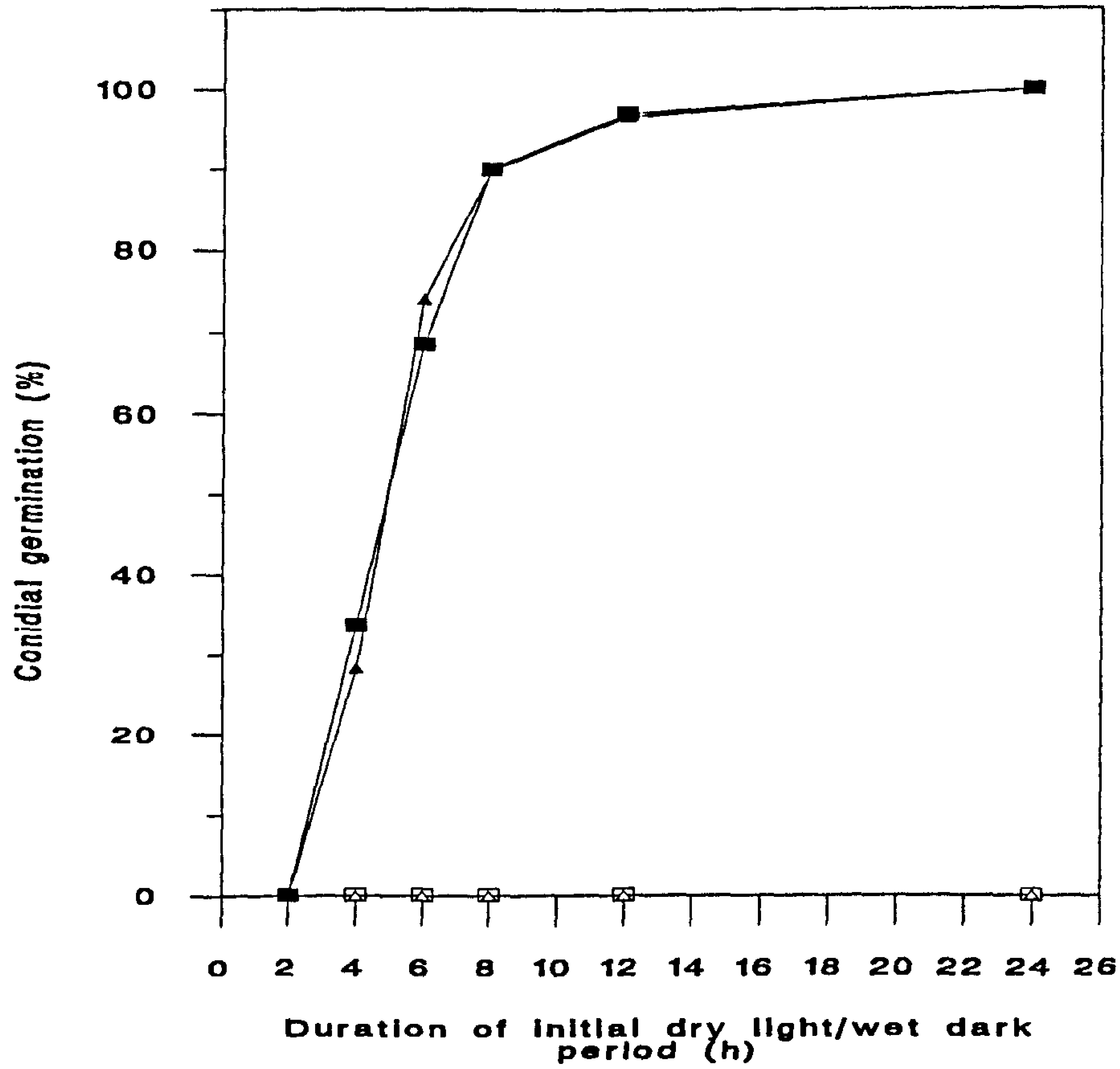



**Figure 4.17.** The effects of a dry light period of various lengths applied before (□, △) or after (■, ▲) a wet dark period on the percentage germination of *A. linicola* conidia on attached leaves at 15°C; □ : initial assessment, after light period (analysis not possible); △ : final assessment (24 h), after light + dark periods (analysis not possible); ■: initial assessment, after dark period, SED (12 d.f.) = 3.19; ▲: final assessment (24 h), after dark + light period, SED (12. d.f.) = 3.06

during their exposure to wet darkness (Fig. 4.17). However, no more conidia germinated during the period of dry light that followed the period of wet darkness (Fig. 4.17). The dry light period applied after an initial wet dark period also stopped further elongation of the germ tubes, irrespective of its duration or the time at which it was applied during the germination process (Fig. 4.18).

**4.5. Discussion**

These results suggest that temperature is one of the most important environmental factors which influences germination of *A. linicola* conidia since it affects not only the percentage of conidia which germinate but also the time required for germination and the rate of germ tube elongation.

The results showed that germination and germ tube elongation of *A. linicola* conidia can occur over a wide range of temperatures (5 - 25°C) in darkness and high relative humidity (100%). Germination of *A. linicola* conidia seems to be a very rapid process, especially at temperatures between 10°C and 25°C. At these temperatures, germination started within 2 h after inoculation on both water agar and detached leaves and was completed after 12 h and 24 h on water agar and detached leaves, respectively. At 5°C, the onset of the germination of *A. linicola* conidia was delayed and the rate of germination was slower than that at higher temperatures (10, 15, 20 or 25°C). At 5°C conidia of *A. linicola* started germinating 4 h (on water agar) and 6 h (on detached leaves) after inoculation and only 60% and 75% of the conidia had germinated after 48 h of incubation on water agar and detached leaves, respectively. The length of the germ tubes also increased with

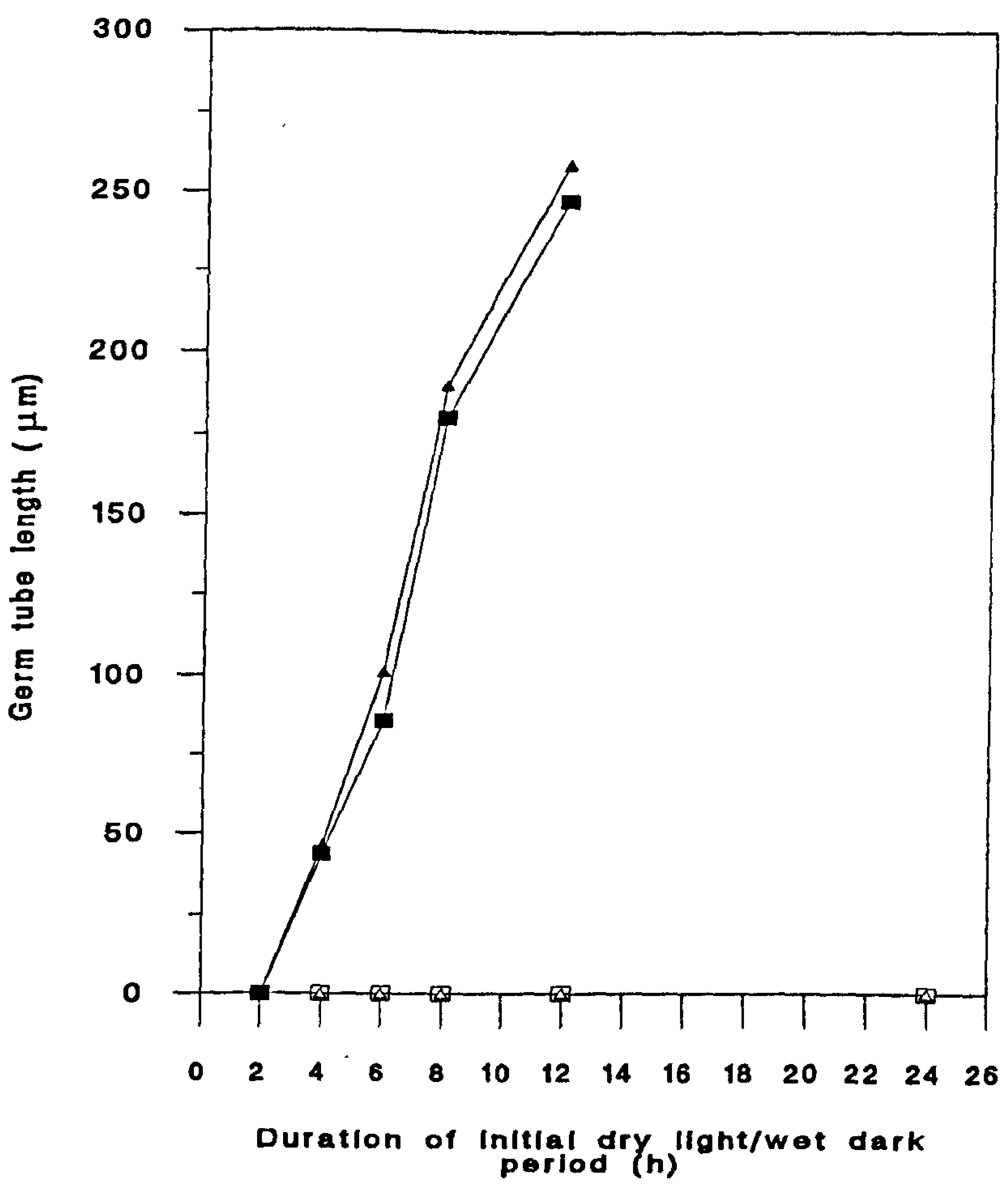



**Figure 4.18.** The effects of a dry light period of various lengths applied before (□ , △ ) or after ( ■ , ▲ ) a wet dark period on the germ tube elongation of *A. linicola* conidia on attached leaves at 15°C; □ : initial assessment, after light period (analysis not possible); △ : final assessment (24 h), after light + dark periods (analysis not possible); ■ : initial assessment, after dark period, SED (12 d.f.) = 5.08; ▲ : final assessment (24 h), after dark + light period, SED (12. d.f.) = 3.19

increasing temperature, but the rate of elongation was much slower at 5°C than that at higher temperatures (10, 15, 20 or 25°C).

These results suggest that early in the growing season (April, May), when the minimum temperatures in the SE of England are frequently around 5°C, germination of *A. linicola* conidia and subsequent infection of linseed by *A. linicola* may be very slow processes. Therefore, the slow germination of *A. linicola* conidia during that period may account for the delay in the appearance of symptoms on linseed plants, despite the presence of inoculum in the field (see section 7.4.2). Furthermore, in these experiments, the germination of *A. linicola* conidia was studied under constant temperatures. Under field conditions, conidia are exposed to fluctuating temperatures which may increase the time required for germination and decrease the rate of germination or germ tube elongation (Waggoner & Parlange, 1974).

Conidia of other *Alternaria* species can also germinate over a wide range of temperatures. For the majority of *Alternaria* species the minimum, optimum, and maximum temperatures for germination are approximately 3°C, 25°C and 35°C, respectively (Weimer, 1924; Angell, 1929; Riley, 1949; Von Ramm & Lucas, 1962; Norse, 1973; Humpherson-Jones *et al.*, 1983; Malathrakis, 1983; Strandberg, 1988; Evans *et al.*, 1992).

The results of this study showed that the duration of the period of high relative humidity (100%) or leaf wetness affects germination and germ tube elongation of *A. linicola* conidia. At each temperature, the percentage of conidia that germinated and the length of the germ tubes on both water agar and detached leaves increased with increasing exposure to high relative humidity. At temperatures ≥15°C, the minimum periods of high relative humidity or leaf wetness required for the germination

of 50% and 90% of the conidia were 2 h and 6 h, respectively. These results suggest that in the SE of England and during June, July, August or early September, when the mean daily temperatures are higher than 15°C, a few hours of dew during the night might be sufficient to allow germination of *A. linicola* conidia deposited on the leaf surfaces during the previous day.

Under optimum temperatures and high relative humidity (100%) or in the presence of water (leaf wetness), conidial germination of many *Alternaria* species starts within 3 h after inoculation (Strandberg, 1987; Waggoner & Horsfall, 1969). Moreover, for some *Alternaria* species the time required for germination at marginal temperatures is influenced by the duration of exposure to high relative humidity or leaf wetness. Conidia of *A. macrospora, A. alternata* and *A. solani* failed to germinate in 24 h at 2°C but they did germinate when they were exposed at this temperature for 48 h (Rotem, unpublished). The germination of conidia of *A. helianthi* was favoured by temperatures between 25°C and 28°C and by the presence of free water on the leaf surfaces (Allen *et al.*, 1983).

However, under field conditions, conidia of *A. linicola* deposited on the plant tissues are exposed to alternating wet and dry periods. The results of this study show that dry periods interrupting a continuous wet period during the germination process may influence the time required for germination, the percentage of conidia which germinate and the growth of the germ tubes. Moreover, the effects of the dry interruptions on germination of *A. linicola* conidia depended not only on the time at which these interruptions occurred, but also on the duration of the dry period.

The effects of the dry interruptions on conidial germination and germ tube elongation at the early stages of the germination process (between 0 h and 2 h after

inoculation), when no visible germ tubes were present, depended on the duration of the dry period. Conidia of *A. linicola* could withstand short (2 h) dry interruptions occurring at any time during this period without losing their viability. All these conidia subsequently started germinating by producing germ tubes which elongated during the wet period that followed the dry period. However, a long (12 h) dry interruption occurring at any time during the period between 1 h and 2 h after inoculation, stopped conidial germination and germ tube elongation. Moreover, only 20% of the conidia retained their viability and started germinating by producing very short germ tubes when rewetted.

Generally, *A. linicola* conidia seemed to be very sensitive to drying during the period between 2 h and 6 h after inoculation. Short (2 h) or long (12 h) dry interruptions occurring at any time during this period stopped conidial germination and no more conidia germinated after rewetting. Dry periods (short or long) occurring at any time between 2 h and 8 h after inoculation stopped the growth of the germ tubes and no further elongation occurred during the wet period that followed the dry period.

Short (2 h) or long (12 h) dry interruptions occurring at any time after 10h of incubation in wet darkness did not stop the elongation of the germ tubes, which continued to grow even during the dry period. It is possible that the growth of germ tubes which had reached a certain length (approximately 200 $\mu$m after 10 h incubation in wet darkness) depended on factors which were not influenced by the presence of water on the leaf surfaces.

Unfortunately there is little information on the effects of dry interruptions on the germination of conidia of other *Alternaria* species. However, Bashi & Rotem

(1974) reported that conidia of *A. porri* f. sp. *solani* could withstand two dry periods (22 h each) interrupting a continuous wet period every 2 h and continued to germinate after rewetting.

The results of this study showed that light (wet or dry) may influence the time required for germination, the rate of germination as well as the germ tube elongation of *A. linicola* conidia. Conidia of *A. linicola* are dispersed and subsequently deposited on the leaf surfaces during the day (see section 7.4.1), although the effects of temperature, incubation time and leaf wetness duration on germination of *A. linicola* conidia were studied under continuous darkness. The effects of light on germination of *A. linicola* conidia depended on the time during the germination process at which the light period occurred, the duration of exposure and the presence or not of wetness on the leaf surfaces.

Wet light applied immediately after inoculation delayed rather than stopped conidial germination. Although no germination occurred when the conidia were exposed to light for 2, 4, 6, 8 or 12 h after inoculation, some conidia (47%) did germinate by producing short germ tubes (average length 61 $\mu$m) after 24 h of exposure to light. However, for conidia germinating in darkness, germination was completed within 6 h after inoculation and the average length of the germ tubes was approximately 350 $\mu$m after 24 h. Conidia exposed to light for up to 12 h immediately after inoculation did not lose their viability and, although they did not germinate during the light period, they did so during the dark period that followed the exposure to light. For conidia germinating during the dark period that followed the initial period of light, the length of the germ tubes was negatively correlated with the duration of the initial exposure to light.

Wet light applied after the onset of the conidial germination may also affect the number of conidia which germinate, depending on the time during the germination process at which the light period occurs. Conidia of *A. linicola* seemed to be very sensitive to light and the percentage of germination was decreased by approximately 50% when they were exposed to light after 2 h of incubation in darkness. It is possible that various metabolic processes which occurred during the first 2 h after inoculation were very sensitive to light. As the majority of the conidia had germinated after 4 h in darkness, light applied after this period had no effect on germination.

Wet light applied after the onset of conidial germination influenced the growth of the germ tubes, depending on the time during the germination process at which the light period was applied. Generally, germ tubes of *A. linicola* conidia seemed to be very sensitive to light between 2 h and 6 h after inoculation, when their average length was less than 200 μm. Light applied during that period decreased the length of the germ tubes by approximately 50% compared with the length of the germ tubes of conidia incubated in darkness. However, light applied after this period increased rather than decreased the length of the germ tubes. These results support the suggestion that once the germ tubes of *A. linicola* have reached a length of approximately 200 μm, their further growth is not influenced by the presence of water or light.

The results of this study showed that dry light applied immediately after inoculation stopped conidial germination and germ tube elongation, irrespective of the length of the light period. No more conidia germinated even when they were rewetted and incubated in darkness after their exposure to dry light. Moreover, no more conidia germinated and the growth of the germ tubes stopped when a dry light

period was applied after an initial wet period in darkness, irrespective of the time during the germination process at which the dry light period was applied.

There is little information on the effects of light on the conidial germination of other *Alternaria* species. However, pigmented conidia like those of *Alternaria* species are considered to survive long exposures to ultraviolet radiation better than non-pigmented conidia (Leach, 1971). Germination of *A. solani* conidia *in vitro* was inhibited when the conidia were irradiated with simulated sunlight (300 - 500 nm) (Stevenson & Pennypacker, 1988). Low light intensity (3500 lux) did not inhibit conidial germination of *A. helianthi,* but increased the number of germ tubes produced per conidium (Allen *et al.*, 1983). However, conidial germination of *A. oleracea* was stimulated by exposure to light (Hawker, 1950).

The results of this study showed that the time required for germination, the rate of germination and the rate of germ tube elongation of *A. linicola* conidia depended also on the substrate on which germination was studied. More time was required for germination and the rate of germination and germ tube elongation was less rapid on leaves than on water agar. Similarly, the growth rate of the germ tubes of *A. alternata* f. sp. *tabaci* was two or three times lower on tobacco leaves than on agar (Norse, 1973). These results indicate the danger of interpreting the behaviour of *A. linicola* conidia in the field on the basis of *in vitro* studies. It is possible that the delay in germination on leaves was associated with the presence of either spores of other antagonistic or competitive fungi or various compounds which might have affected germination, although neither of these possibilities was investigated.

At a constant temperature of 15°C, germination of *A. linicola* conidia was

slower on attached leaves than that on detached leaves. Moreover, the number of conidia which germinated on attached leaves after 18 h of leaf wetness did not increase when leaf wetness duration increased to 24 h. There are two possible explanations for these differences in germination : a) as the detached leaves were more senescent than the attached leaves (Tukey, 1971), they supplied sufficient nutrients to *A. linicola* conidia for a rapid germination or b) the germination on detached leaves was studied under high relative humidity (100%), whereas the germination on attached leaves was studied in the presence of water drops (leaf wetness). The low oxygen supply due to the presence of water drops on the leaves might have delayed the germination process (Lilly & Barnett, 1951).

Germination of *Alternaria* conidia may also be affected by host factors. Conidial germination of *A. alternata* on tomato fruit was stimulated, even at marginal temperatures, by nutrients which leached from the fruit and accumulated on their surface (Pearson & Hall, 1975). Sunflower pollen stimulated germination of *A. helianthi* conidia (Allen *et al.*, 1983). Sometimes the nature of the host may affect conidial germination, although it has been reported that *Alternaria* conidia germinate equally well on host (resistant or susceptible) and on non-host plants (McRoberts & Lennard, 1991). *A. triticina* conidia failed to germinate on leaves of a resistant cultivar of wheat (*Triticum sphaerococcum*), but they germinated well on leaves of the susceptible cultivar Lemarojo Amber (Kumar *et al.*, 1974). Germination of conidia of *Alternaria* species may also be inhibited by self-inhibitors. These inhibitors, which are produced in small quantities by the conidia, may prevent conidia from germinating, especially when the conidia are clumped together (Rotem, 1981).

Such inhibitors have been reported to be produced by *A. porri* f.sp. *solani* (Rotem, 1963) and *A. brassicicola* (Mukadam, 1982) conidia. Self-inhibitors might have also been produced by *A. linicola* conidia, since the disease severity on the cotyledons was the same when inoculum concentrations of 5 x $10^4$ and 1 x $10^5$ conidia $ml^{-1}$ were used for artificial inoculations (see section 5.4.1).

The results of this study also showed that *A. linicola* conidia germinated mainly by producing germ tubes which penetrated the leaf tissues directly through the epidermal cells. However, formation of secondary conidia was also observed, although it was not common. During the first 12 h after inoculation, *A. linicola* grew on the leaf surface without penetrating it. At and beyond 12 h the fungus started penetrating the leaf tissue directly through epidermal cells. Indirect penetration through stomata was also observed but it was less common. Stomatal penetration necessitates growth of hyphae over the leaf surface until a stoma is reached. If the fungus is sensitive to stomatal stimuli then hyphae should orientate towards the stomata. This was not observed and stomatal penetration by *A. linicola* appeared to occur by chance. Conidia of most *Alternaria* species germinate by producing germ tubes (Young, 1926; Von Ramm, 1962; Fahim & El-Shehedi, 1966; Saad & Hagedorn, 1969; Norse, 1973; Allen *et al.*, 1983; Ansari *et al.*, 1988). Exceptions seem to be *A. brassicae* (Tsuneda & Skoropad, 1977a) and *A. alternata* (Skidmore, 1976) conidia which germinate by producing either germ tubes or secondary conidia.

The mode of penetration (direct or indirect) by other *Alternaria* depends on the species. However, there are contradictory reports on the mode of penetration by the same *Alternaria* species. Angell (1929) reported stomatal penetration of onion leaves by *A. porri*. Walker (1952) reported penetration by the same fungus through

both stomata and wounds in the epidermis. Chupp & Sherf (1960) and Fahim & El-Shehedi (1966) observed that *A. porri* penetrates through stomata and directly through the unwounded epidermis. *A. brassicae* penetrates through stomata and *A. raphani* either through stomata or directly (Changsri & Weber, 1963). Allen *et al.* (1983) observed that the most common route by which *A. helianthi* entered sunflower leaves was directly through the cuticle and epidermis, and that penetration through stomata was less common. Direct penetration was also reported for *A. longipes* on tobacco leaves (Riley, 1949), *A. solani* on potato and tomato leaves (Rands, 1917) and *A. cucumerina* on curcubits (Jackson, 1958). *A. dauci* penetrated carrot leaves only through stomata (Strandberg, 1983). Tsuneda & Skoropad (1977b) reported that *A. brassicae* had different modes of penetration of rapeseed leaves depending on the cultivar; penetration of leaves of *Brassica napus* (cv. Midas) occurred only indirectly through stomata but the fungus penetrated leaves of *Brassica campestris* (cv. Torch) through epidermal cells as well as through stomata.

*A. linicola* conidia are dispersed by the wind on dry days (see section 7.4.1). The results of this study suggest that in the SE of England, where the mean daily temperatures in June, July, August and early September exceed 15$^0$C, factors limiting for the germination of *A. linicola* conidia on the linseed surfaces will be water availability and light. Conidia deposited on the leaf surfaces on a dry, sunny day are unlikely to germinate even during the following night, when the conditions will be favourable for germination (darkness, dew). However, if moisture is present during the day (rain), germination and germ tube elongation may initiate and proceed, although at a slow rate due to the presence of light. Generally, it is likely that most of the conidia deposited on the leaf surfaces a few hours before dusk, when there is a decrease

in the light intensity, will germinate during the night and complete the infection process in the dark. However, in this study conidia of *A. linicola* were applied as a spore suspension, whereas in nature and during sunny, dry days conidia are deposited on the leaf surfaces as dry conidia. Therefore, the behaviour of these conidia in the crops might be different from that observed in the present study.

# CHAPTER V. EFFECTS OF INOCULUM DENSITY, TEMPERATURE, LEAF WETNESS AND LIGHT REGIME ON INFECTION AND SYMPTOM DEVELOPMENT

## 5.1. Introduction

There are no detailed studies on the effects of important environmental variables such as temperature, leaf wetness (continuous or interrupted) and light on infection and symptom development of *A. linicola* on linseed plants. Moreover, the symptom development and the severity of the disease may be affected by the amount of inoculum present on the plant tissues, as for other host-pathogen systems (Eyal & Scharen, 1977; Shearer, 1978; Eisensmith *et al.*, 1982; Jeger *et al.*, 1985; Makowski, 1993). Few studies on the pathogenicity of *A. linicola* to linseed plants and on the control of the disease have been done in controlled environments (Fitt & Coskun, 1991; Fitt *et al.*, 1991b; 1991c; Davis & Fitt, 1992). Field observations have suggested that periods of wet weather and relatively high temperature during the interval between flowering and harvest favour the development of the disease in the linseed crop (Mercer *et al.*, 1991a; Fitt *et al.*, 1991b).

Determination of the optimal environmental conditions for infection of linseed plants by *A. linicola* should help not only in understanding the development of epidemics in linseed crops, but also in improving the strategies for management of the disease.

## 5.2. Objectives

To study the effects of inoculum density and of environmental factors such as temperature, leaf wetness (continuous or interrupted) and light regime on the development of symptoms caused by *A. linicola* on linseed plants under controlled environment conditions.

## 5.3. Materials and Methods

### 5.3.1. Preparation of inoculum

Mixtures of single-spore isolates of *A. linicola*, isolated from naturally infected linseed plants (cv. Antares) during the period 1989-1992, were used in all experiments. The isolates were from the Rothamsted Experimental Station collection and the stock cultures were maintained by the method described in section 2.1.2. For the production of inoculum for artificial inoculation the method of Shahin & Shepard (1979) (see section 2.1.3) was used. Ten ml of sterile distilled water containing 0.01 ml of 0.01% Tween 80 as a wetting agent, were added to each Petri plate. The resulting conidial suspension of each isolate was filtered through two layers of sterile muslin. The concentration of conidia of each isolate was determined with a haemocytometer (4 counts per conidial suspension) and was adjusted to $3 \times 10^4$ conidia $ml^{-1}$ (unless otherwise stated) by dilution with sterile distilled water. The final inoculum was prepared by mixing together 300 ml of the conidial suspension of each isolate. The preparation of the inoculum took approximately 30 min and at the end of this period no conidial germination was observed. The viability of conidia (% conidia germinated) used for artificial

inoculation was tested by the following method : three water agar plates (1.5% water agar, 20 ml of medium per plate) were sprayed with the conidial suspension, sealed with parafilm and incubated in darkness at 20°C. After 6 h of incubation the percentage of conidia which had germinated was assessed under a light microscope (x 250 magnification). In all experiments the viability of conidia ranged from 97 to 100%.

### 5.3.2. 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) and free from *A. linicola* infection). Plants were grown in pots by the method described in section 2.1.5. The pots were placed in controlled environment cabinets, designed at Rothamsted Experimental Station, set at 18°C/13°C day/night temperatures (unless otherwise stated) (Fig. 2.1). The daylength was 16 h (from 24:00 h to 16:00 h) and the light was provided in each cabinet by 18 fluorescent lamps placed 70 cm above the plants. The light intensity, measured at plant height by the method described in section 2.1.4 was 120 - 160 $\mu$Einsteins $m^{-2}$ $sec^{-1}$. The relative humidity in the cabinets, measured by the method described in section 2.1.4 ranged from 60 to 75%.

### 5.3.3. Plant inoculation

When the plants were at GS 5-6 (the plants had 16 - 18 leaves, Fig.1.2) (unless otherwise stated) they were artificially inoculated (sprayed until run-off) with the mixed conidial suspension of *A. linicola* isolates. The inoculum for artificial

inoculation was prepared according to the method described in section 5.3.1. Approximately 20 ml of the conidial suspension (unless otherwise stated) was sprayed onto the plants in each pot (10 plants per pot) by the method described in section 2.1.6. The inoculation procedure lasted 30 min and at the end of this period no conidial germination was observed. The plants were inoculated at the beginning of a dark period (approximately 16:00 h).

**5.3.4. Experimental design**

All experiments were in a randomized block design with five blocks, with one pot (10 plants per pot) per block for each treatment.

**5.3.5. Effects of inoculum density on infection and symptom development**

A mixture of three single-spore isolates of *A. linicola* (Al 1, Al 5, Al 15) was used for the artificial inoculations. Linseed plants (cv. Antares, GS 5-6) grown by the method described in section 5.3.2 were artificially inoculated (sprayed until run-off) with conidial suspension containing $1 \times 10^3$, $1 \times 10^4$, $5 \times 10^4$ and $1 \times 10^5$ conidia $ml^{-1}$. prepared by the method described in section 5.3.1. The inoculation of the plants was done according to the method described in section 5.3.3. The 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.

The disease incidence on the cotyledons (% cotyledons with symptoms) was assessed 4, 5, 6, 7, 9, 11, 13, 15 and 18 days after inoculation. All the cotyledons were included in the assessments (20 cotyledons per replicate, 100 cotyledons per

treatment). The disease incidence on leaves (% leaves with symptoms) was assessed 5, 6, 7, 9, 11, 13, 15 and 18 days after inoculation. All the leaves present on each plant at the time of the inoculation (average of 17 leaves) were included in the assessments (170 leaves per replicate, 850 leaves per treatment). Disease incidence on stems and hypocotyledons (% stems or hypocotyledons with lesions or cankers) was assessed 18 days after inoculation (total of 10 stems or hypocotyledons per replicate, 50 stems or hypocotyledons per treatment). Disease severity (% area with symptoms) was assessed only on the cotyledons 6, 7, 9, 11, 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 was calculated as :

$$DI = [(0xA)+(1xB)+(2xC)+(3xD)+(4xE)+(5xF)+(6xG)] / 100 \qquad (5.1)$$

in which A, B, C, D, E, F and G were the mean percentages of area of the cotyledons with symptoms in each of the scale categories (0, 1, 2, 3, 4, 5 and 6, respectively).

Statistical analyses

For analyzing the data on the percentage of stems or hypocotyledons with symptoms (lesions or cankers) at different inoculum concentrations the linear regressions used were :

$$y_1 = a_1 + b_1 \ln(x) \qquad (5.2)$$

$$y_2 = a_2 + b_2 \ln(x) \qquad (5.3)$$

$$y_3 = a_3 + b_3 \ln(x) \qquad (5.4)$$

$$y_4 = a_4 + b_4 \ln(x) \qquad (5.5)$$

in which $y_1$, $y_2$, $y_3$ and $y_4$ are the percentages of stems with lesions, stems with

cankers, hypocotyledons with lesions and hypocotyledons with cankers, respectively, x is the inoculum concentration used, $a_1$, $a_2$, $a_3$ and $a_4$ are the intercepts on the $y_1$-axis, $y_2$-axis, $y_3$-axis and $y_4$-axis, respectively and $b_1$, $b_2$, $b_3$ and $b_4$ are the slopes of the lines.

**5.3.6. Effects of temperature and leaf wetness duration on infection and symptom development**

Two experiments were done to study the effects of temperature and leaf wetness duration on disease development. In both experiments, linseed plants (cv. Antares) were grown in controlled environment cabinets by the method described in section 5.3.2. When the plants were at GS 5-6 (the plants had 16-18 leaves, Fig. 1.2) they were artificially inoculated (sprayed until run-off) with a mixture of four single-spore isolates of *A. linicola* (Al 10, Al 15, Al 23, Al 24) by the method described in section 5.3.3. The conidial suspension prepared by the method described in section 5.3.1 contained 3 x $10^4$ conidia $ml^{-1}$. 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 and dried immediately by blowing air (at ambient temperature) gently over them with a hair-drier. 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.

Experiment I. Two controlled environmental cabinets were set at 15°C and 25°C, respectively, 24 h before inoculation. Only the 3rd, 4th, 5th and 6th leaf of each

plant, counting from the base of the stem, were inoculated by the method described in section 5.3.3. The inoculated plants in each cabinet were exposed to 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48 or 72 h periods of leaf wetness immediately after inoculation. At the end of these periods the plants were dried and transferred to a controlled environment room set at 18°C/13°C day/night temperatures with a 16-h photoperiod until the symptoms developed. The light intensity in the room, measured at plant level by the method described in section 2.1.4 was 120-160 $\mu$Einsteins $m^{-2}$ $sec^{-1}$. 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).

The disease incidence on plants (% plants with symptoms) and on leaves (% leaves with symptoms) was assessed 4 days after inoculation. The disease incidence on leaves 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).

Experiment II. Linseed plants (cv. Antares) were grown in a controlled environment cabinet set at 15°C by the method described in section 5.3.2. All the leaves of each plant (average 17 leaves) were inoculated by the method described in section 5.3.3. The inoculated plants were exposed to 8, 10, 12, 16, 20 or 24 h leaf wetness periods, immediately after inoculation. At the end of these periods the plants were dried and replaced in the cabinet (in darkness) until the symptoms developed. There were three

different controls : a) plants which were inoculated and covered for 24 h with polyethylene bags sprayed inside with water (24 h leaf wetness), b) plants which were inoculated, dried immediately after inoculation and left uncovered for 24 h (0 h leaf wetness) and c) plants which were sprayed with sterile distilled water containing 0.01% Tween 80 and covered for 24 h with polyethylene bags sprayed inside with water (uninoculated plants).

The disease incidence on plants (% plants with symptoms) and on leaves (% leaves with symptoms) and the disease severity on leaves (% leaf area with symptoms) were assessed 4 days after inoculation. The disease incidence on leaves was based on the total number of leaves present on each plant at the time of the inoculation (average 17 leaves per plant, 170 leaves per pot or replicate, 850 leaves per treatment).

### 5.3.7. Effects of interrupted leaf wetness period on infection and symptom development

#### 5.3.7.1. Pre- and post-inoculation treatments

Linseed plants (cv. Antares) were grown in controlled environment cabinets set at 15°C by the method described in section 5.3.2. 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-drier by the method described in section 5.3.6. It took approximately 5 min for the leaves of the plants in five pots (one replicate) to dry and therefore this time was not included in the recorded leaf wetness period. 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 (15°C) by less than +2°C. All plants were kept in darkness after the inoculation until the symptoms developed.

### 5.3.7.2. Interruption by one dry period

Linseed plants (cv. Antares) at GS 4 (the plants had 6-8 leaves, Fig. 1.2) were artificially inoculated (sprayed until run-off) (see section 5.3.3) with a mixed conidial suspension of four single-spore isolates of *A. linicola* (Al 10, Al 24, Al 36, Al 38). The inoculum was prepared by the method described in section 5.3.1. Approximately 10 ml of the conidial suspension was sprayed onto the plants in each pot (10 plants per pot) by the method described in section 2.1.6. 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 36-h leaf wetness period in total (Table 5.1). For the wet or dry periods the plants were treated according to the method described in section 5.3.7.1. 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 all the plants were uncovered and incubated in the cabinets in darkness until the symptoms developed.

**Table 5.1.** 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** | **Wet (h)** | **Dry (h)** | **Wet (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 36h | | | |

### 5.3.7.3. Interruption by several dry periods

Linseed plants (cv. Antares) at GS 4 (the plants had 6-8 leaves, Fig. 1.2) were artificially inoculated (sprayed until run-off) (see section 5.3.3) with a mixed conidial suspension of four single-spore *A. linicola* isolates (Al 10, Al 24, Al 36, Al 38). The inoculum was prepared by the method described in section 5.3.1. Approximately 10 ml of the conidial suspension was sprayed onto the plants in each pot (10 plants per pot) by the method described in section 2.1.6. Plants were exposed either 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 the inoculation by one, two or three dry periods (12 h each), respectively (Table 5.2). For the wet or dry periods, the plants were treated according to the method described in section 5.3.7.1.

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 48h 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 darkness until the symptoms developed.

### 5.3.7.4. Disease assessments

The incidence of the disease on plants (% plants with symptoms) and the disease severity on the cotyledons (% area with symptoms) were assessed 6 days after

**Table 5.2.** Treatments used to study the effects of interrupted or continuous leaf wetness 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[1] W- 12 D - 12 W | 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 |
| 0 W (Control 1) | 0 |
| 48 W (Control 2) | 48 |
| Uninoculated (Control 3) | 48 |

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

inoculation. For the disease severity on the cotyledons, an arbitrary 0 - 6 scale was used : 0 = no symptoms, 1 = 1 - 10%, 2 = 11 - 30%, 3 = 31 - 50%, 4 = 51 - 70%, 5 = 71 - 90% and 6 = 91 - 100%. The disease index (equation 5.1) described in section 5.3.5 was calculated. For this index A, B, C, D, E, F and G were the mean percentages of the area on the cotyledons with symptoms in each of the scale categories (0, 1, 2, 3, 4, 5 and 6, respectively).

### 5.3.8. Effects of light regime on infection and symptom development

Linseed plants (cv. Antares) were grown in a controlled environment cabinet set at 15°C by the method described in section 5.3.2. When the plants were at GS 5-6 (the plants had 16 - 18 leaves, Fig. 1.2) they were artificially inoculated (sprayed until run-off) with a mixed conidial suspension of four single-spore *A. linicola* isolates (Al 10, Al 15, Al 23, Al 24) by the method described in section 2.1.6. The inoculum prepared by the method described in section 5.3.1 contained 3 x $10^4$ conidia $ml^{-1}$. Approximately 10 ml of the conidial suspension was sprayed onto the plants in each pot (10 plants per pot). At the end of the inoculation procedure 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 regime described in Figure 5.1. During the dark periods, the plants 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 darkness) or exposed for 72 h to light. The temperature inside the bags and the aluminium foil deviated from the temperature set (15°C) by less than +2°C.

LIGHT CONDITIONS

: light
: dark

Time scale
(hours)

0 24 48 72

**Figure 5.1.** Treatments used to study the effects of light regime on the development of symptoms on linseed plants (cv. Antares) inoculated with *A. linicola*.

The incidence of the disease was assessed on cotyledons (% cotyledons with symptoms) and on stems (% stems with symptoms) 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 disease index was calculated as:

$$DI = [(0xA)+(0.1xB)+(1xC)+(2xD)+(3xE)+(4xF)+(5xG)] / 100 \qquad (5.6)$$

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

Statistical analyses

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

$$y = a - b\,x \qquad \text{(for the light period)} \qquad (5.7)$$

$$y = c + d\,z \qquad \text{(for the dark period)} \qquad (5.8)$$

in which y is the disease incidence on stems (% stems with symptoms), x, z are the durations of the light or dark period, respectively, a, c are the intercepts on the y-axis in equations 5.7 and 5.8, respectively and b, d are the slopes of the lines for equations 5.7 and 5.8, respectively.

For analyzing the data on the severity of the disease on leaves under light or dark periods of different durations the linear regressions used were :

$$w = f - g\,x \qquad \text{(for the light period)} \qquad (5.9)$$

$$w = k + m\,z \qquad \text{(for the dark period)} \qquad (5.10)$$

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 5.9 and 5.10, respectively and g, m are the slopes of the lines for equations 5.9 and 5.10, respectively.

## 5.4. Results

### 5.4.1. Symptoms of *A. linicola* under controlled environment conditions

Symptoms caused by *A. linicola* on cotyledons and leaves of linseed plants under controlled environment conditions were necrotic areas without a definite margin which had a Warm Buff colour (Ridgway, 1912, Fig. 9.9) (Fig. 5.2). However, these symptoms seem to be different from those observed on linseed plants under field conditions (see section 8.4.4). Under natural conditions the symptoms caused by *A. linicola* either on cotyledons or on leaves of linseed plants were lesions with a definite margin and a dark brown colour (see section 8.4.4) (Fig. 8.5 & Fig. 8.15.). The lesions observed on stems and hypocotyledons of linseed plants under controlled environment conditions (Fig. 5.2) were similar to those caused by *A. linicola* under field conditions (Fig. 9.8 & Fig. 8.16). However, under controlled environment conditions some of these lesions developed into cankers (the epidermis and the cortex of the stem or the hypocotyledon beneath the central area of the lesion were split along the lesion), whereas cankers were not observed either on stems or hypocotyledons under field conditions.

**Figure 5.2.** Symptoms of *A. linicola* on cotyledons (a), leaves and stems (b) of linseed plants (cv. Antares) under controlled environment conditions 8 days after inoculation. Plants were artificially inoculated (sprayed until run-off) with 3 x $10^4$ conidia $ml^{-1}$ and covered for 24 h with polyethylene bags sprayed inside with water.

### 5.4.2. Effects of inoculum density on infection and symptom development

Symptoms were observed first on the cotyledons 4 days after inoculation at all the inoculum densities tested (Fig. 5.3A). The percentage of cotyledons with symptoms increased with increasing inoculum concentration and increasing time. However, the rate of increase in the incidence of the disease on the cotyledons was less rapid at the lowest inoculum density (1 x $10^3$ conidia $ml^{-1}$) than that at the higher inoculum densities. Four days after inoculation, the percentage of cotyledons with symptoms was 10% on plants inoculated with 1 x $10^3$ conidia $ml^{-1}$, whereas the percentage of cotyledons with symptoms was > 70% on plants inoculated with higher inoculum concentrations (1 x $10^4$, 5 x $10^4$ or 1 x $10^5$ conidia $ml^{-1}$) (Fig. 5.3A). When the highest inoculum density (1 x $10^5$ conidia $ml^{-1}$) was used, 100% of the cotyledons had developed symptoms 6 days after inoculation (Fig. 5.3A). With the lowest inoculum concentration (1 x $10^3$ conidia $ml^{-1}$), 65% of the cotyledons had developed symptoms 15 days after inoculation and this percentage did not increase in the next three days (Fig. 5.3A). However, it was not clear whether the disease incidence had reached a maximum (65%) 18 days after inoculation, as no more assessments were done after that date.

The severity of the disease on the cotyledons (% area with symptoms) also increased with increasing inoculum concentration and increasing time (Fig.5.3B). Moreover, the rate of increase in the disease severity was less rapid on plants inoculated with the lowest inoculum density (1 x $10^3$ conidia $ml^{-1}$) than that on plants inoculated with higher inoculum concentrations (Fig. 5.3B). When the inoculum densities 1 x $10^3$ and 1 x $10^4$ conidia $ml^{-1}$ were used, less than 1% of the area of the cotyledons was showing symptoms 4 days after inoculation (Fig. 5.3B). However, this percentage increased with time to 40% and > 80% 18 days after inoculation on plants inoculated

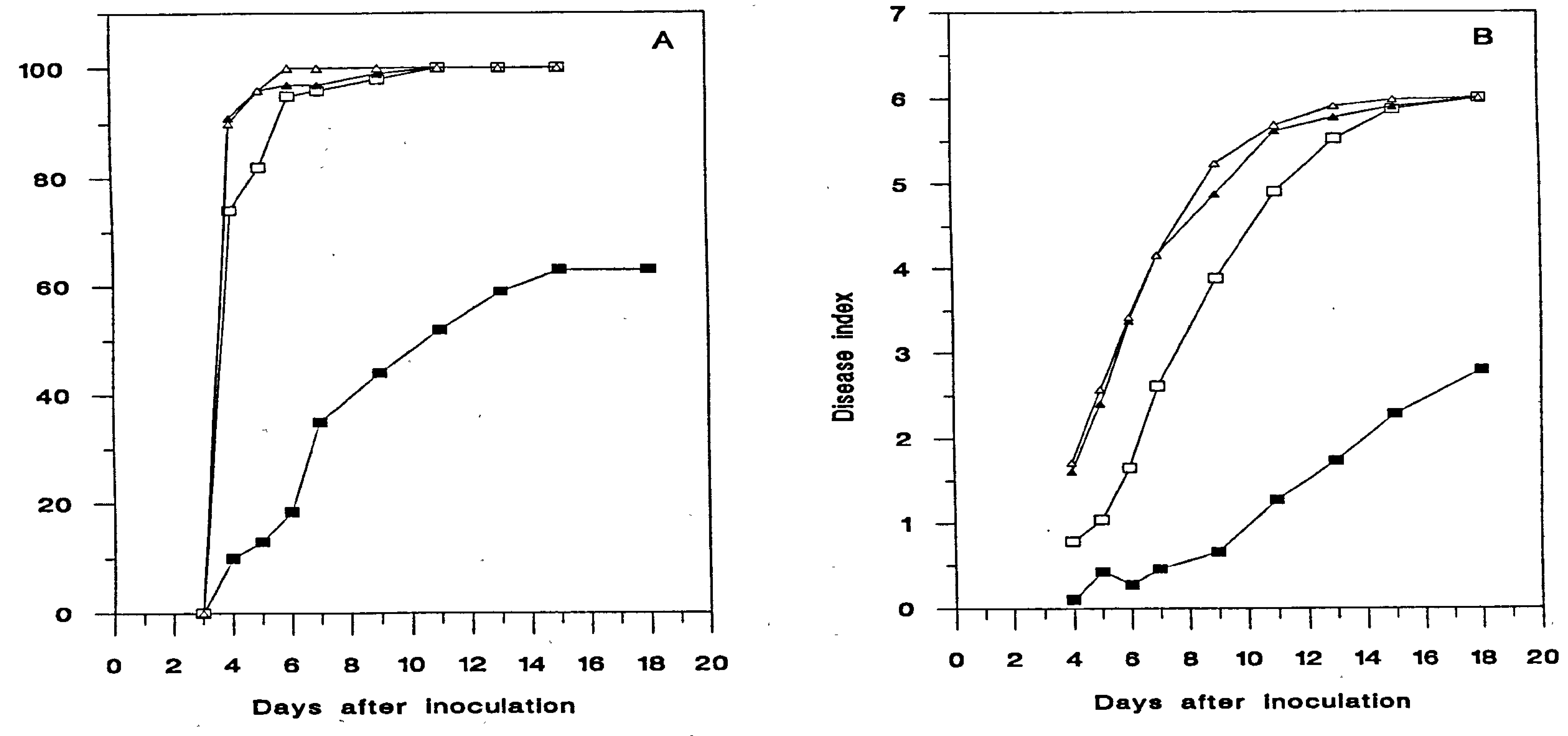



**Figure 5.3.** Incidence (%) (A) and severity (disease index) (B) of cotyledons with symptoms on linseed plants (cv. Antares) inoculated with four different inoculum concentrations of *A. linicola* conidia : (■) : 1 x $10^3$, (□) : 1 x $10^4$, (▲) : 5 x $10^4$ and (△) : 1 x $10^5$ conidia $ml^{-1}$. The disease index was calculated by using the equation 5.1 (section 5.3.5). Disease incidence : SED (12 d.f.) = 3.6; Disease severity : SED (12 d.f.) = 0.2

with 1 x $10^3$ and 1 x $10^4$ conidia $ml^{-1}$, respectively (Fig. 5.3B). When higher inoculum concentrations (5 x $10^4$ or 1 x $10^5$ conidia $ml^{-1}$) were used, the area on the cotyledons showing symptoms was 10% 4 days after inoculation, but increased to more than 80% 18 days after inoculation (Fig. 5.3B).

The first symptoms appeared on the leaves 6 days after inoculation, 2 days later than on the cotyledons (Fig. 5.4). No symptoms developed on leaves when the plants were inoculated with the lowest conidial concentration (1 x $10^3$ conidia $ml^{-1}$), even 18 days after inoculation (Fig. 5.4). When higher inoculum densities were used, the incidence of the disease on leaves increased with increasing inoculum concentration and with time, but did not reach 100% by 18 days after inoculation (Fig. 5.4).

Lesions first appeared on the hypocotyledons, just below the cotyledons, and at various heights on the stems of plants inoculated with 1 x $10^4$, 5 x $10^4$ and 1 x $10^5$ conidia $ml^{-1}$ by 12 days after inoculation, 8 days later than on the cotyledons (Fig. 5.5); some of these lesions developed into cankers. The linear regressions fitted the data quite well (percentages of variance accounted for $>75\%$). No lesions or cankers formed either on the hypocotyledons or on the stems of plants inoculated with the lowest inoculum concentration (1 x $10^3$ conidia $ml^{-1}$) (Fig. 5.5). The disease incidence on stems and hypocotyledons increased with increasing inoculum density. Generally, a higher percentage of plants developed lesions on the stems than on the hypocotyledons, but more of these lesions developed into cankers on the hypocotyledons than on the stems.

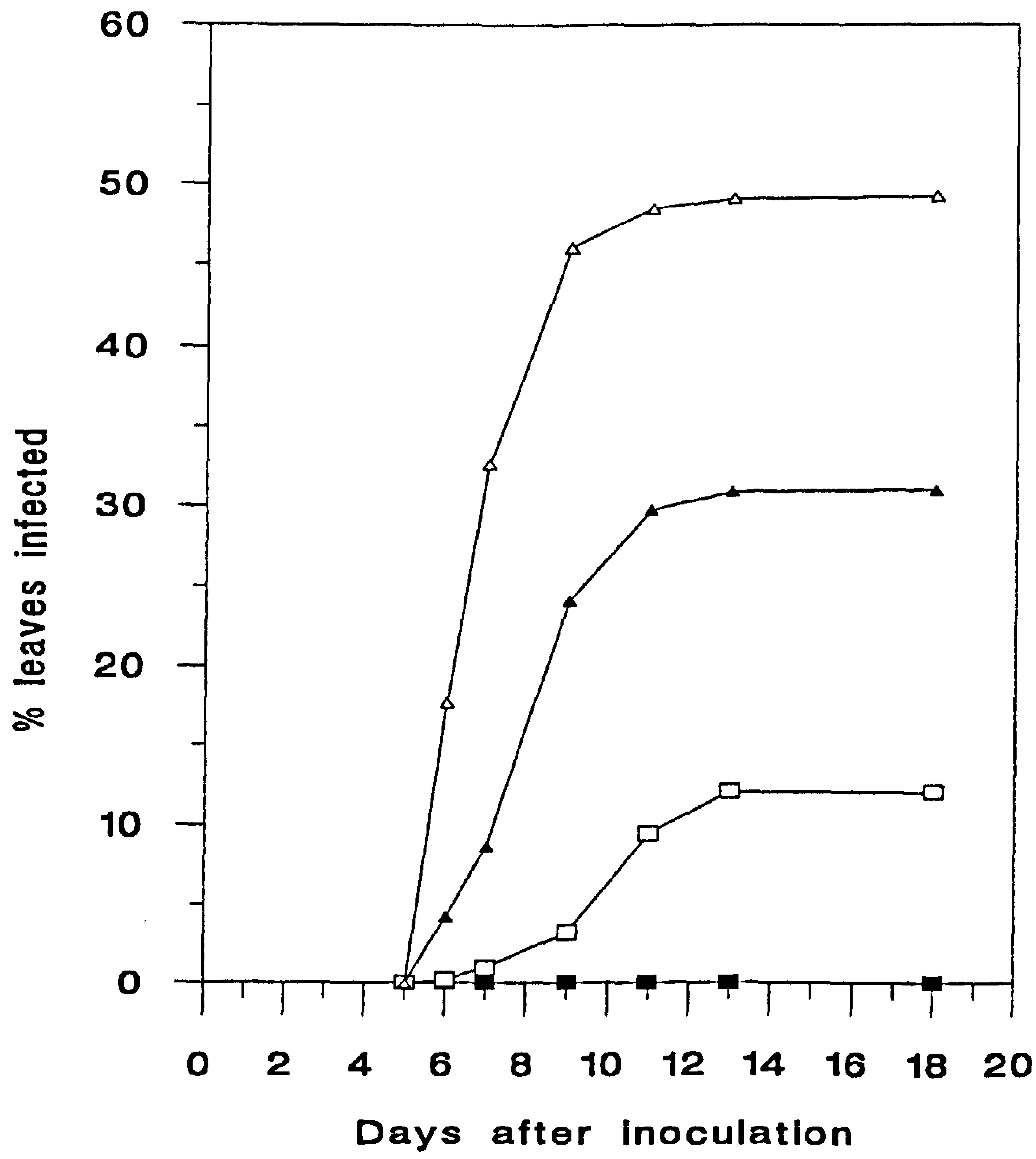



**Figure 5.4.** Incidence (%) of leaves with symptoms on linseed plants (cv. Antares) inoculated with four different inoculum concentrations of *A. linicola* conidia : (■) : 1 x $10^3$, (□) : 1 x $10^4$, (▲) : 5 x $10^4$ and (△) : 1 x $10^5$ conidia $ml^{-1}$. SED (8 d.f.) = 4.2

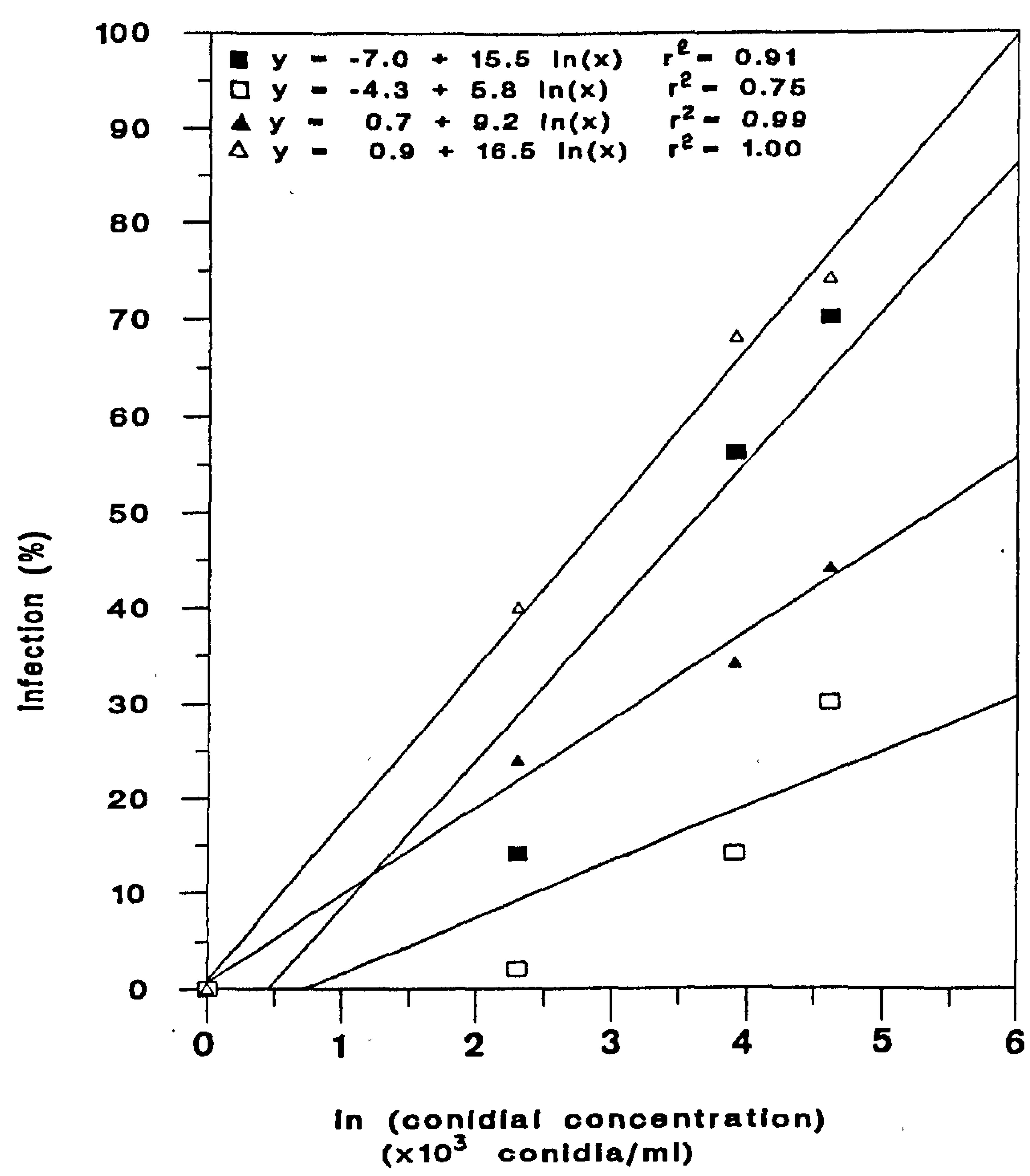



**Figure 5.5.** Effect of inoculum concentration on the development of lesions (■,▲) and cankers (□,△) on stems (■,□) and hypocotyledons (▲,△) of linseed plants (cv. Antares) inoculated with 1 x $10^3$, 1 x $10^4$, 5 x $10^4$ or 1 x $10^5$ conidia $ml^{-1}$ of *A. linicola* and assessed 18 days after inoculation.

**5.4.3. Effects of temperature and leaf wetness duration on infection and symptom development**

Experiment I. No symptoms developed on plants exposed to leaf wetness periods of up to 8 h and up to 6 h at 15°C and 25°C, respectively (Fig. 5.6). However, there was a rapid increase in the percentage of plants with symptoms (from 0% to 96%) when the leaf wetness period increased from 8 h to 10 h at 15°C (Fig. 5.6). Similarly, the percentage of plants with symptoms at 25°C increased rapidly from 0% to 100% when the leaf wetness duration increased from 6 h to 8 h, respectively (Fig. 5.6). However, there was a decrease in the incidence of the disease on plants for leaf wetness periods between 10 h and 16 h at 15°C and between 8 h and 12 h at 25°C (Fig. 5.6). The incidence of the disease increased again on plants exposed to leaf wetness periods > 16 h and > 12 h at 15°C and 25°C, respectively (Fig. 5.6).

The incidence of the disease on leaves (% leaves with symptoms) was affected by the length of the leaf wetness period in a similar way to the incidence of disease on plants at both 15°C and 25°C (Fig. 5.7A). However, the percentage of leaves with symptoms was lower at 15°C than that at 25°C (Fig. 5.7A). After a 72-h period of leaf wetness, only 60% of the leaves developed symptoms at 15°C, whereas at 25°C approximately 100% of the leaves developed symptoms after a 20-h exposure to leaf wetness (Fig. 5.7A).

The severity of the disease was also lower at 15°C than that at 25°C for all leaf wetness periods tested (Fig. 5.7B). At 15°C, the percentage of 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 (Fig. 5.7B). When the control plants were examined for symptom development,

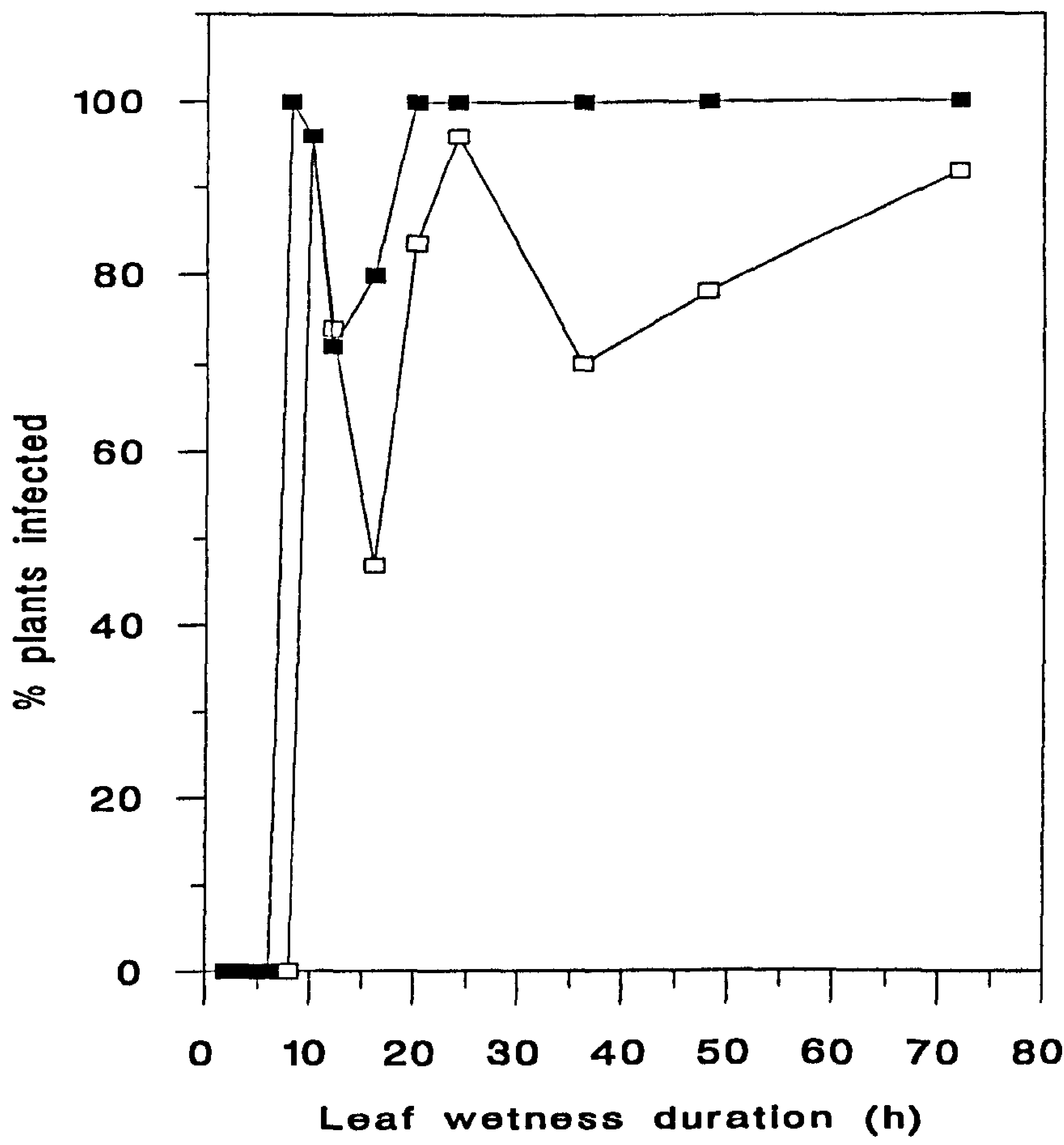
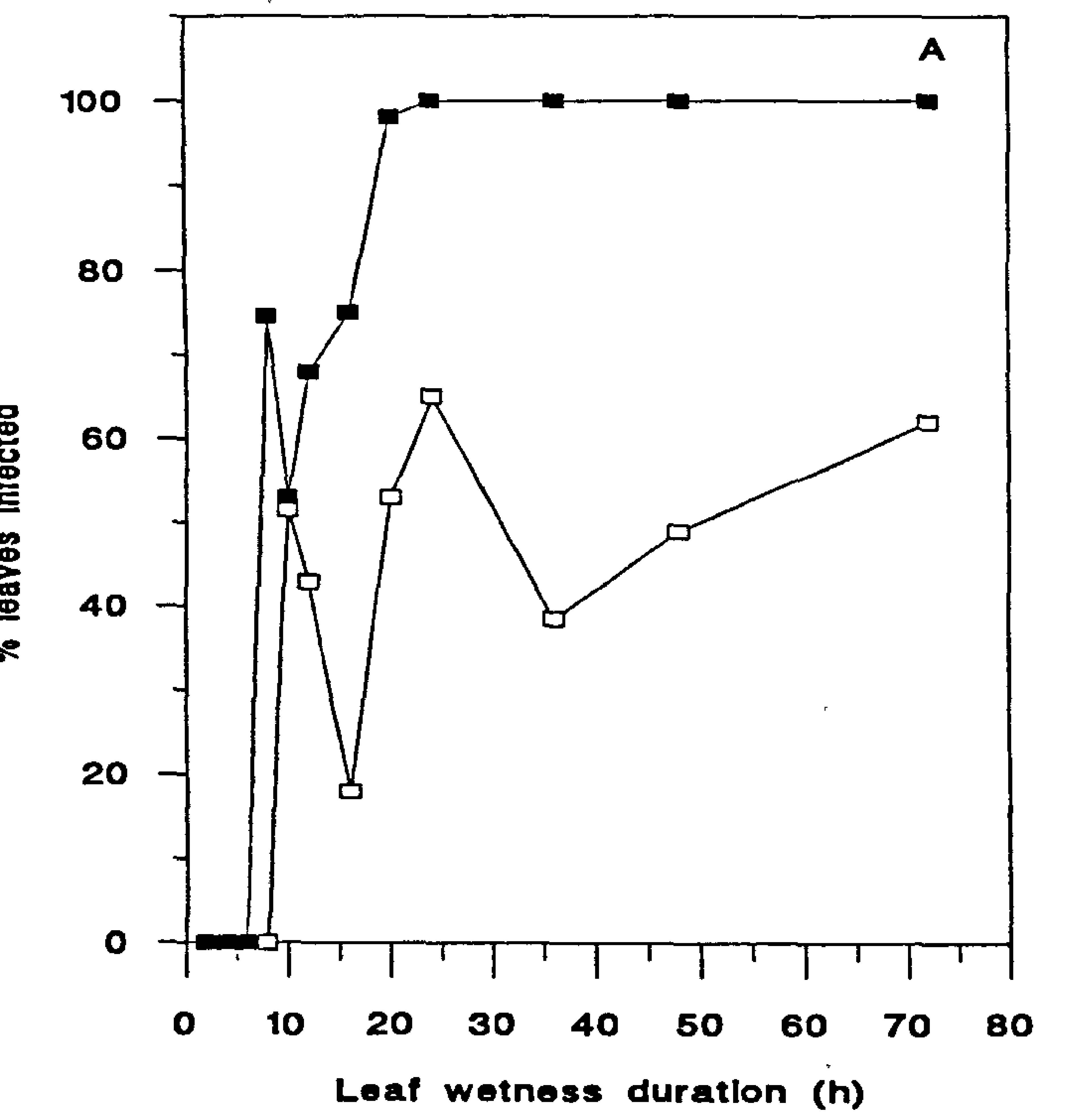



**Figure 5.6.** Effects of leaf wetness duration on the incidence (%) of linseed plants (cv. Antares) with symptoms at 15°C ( ■ ) or 25°C ( □ ). Plants were inoculated with *A. linicola* conidia (3 x $10^4$ conidia $ml^{-1}$) and assessed 4 days after inoculation. SED (56 d.f.) = 9.7



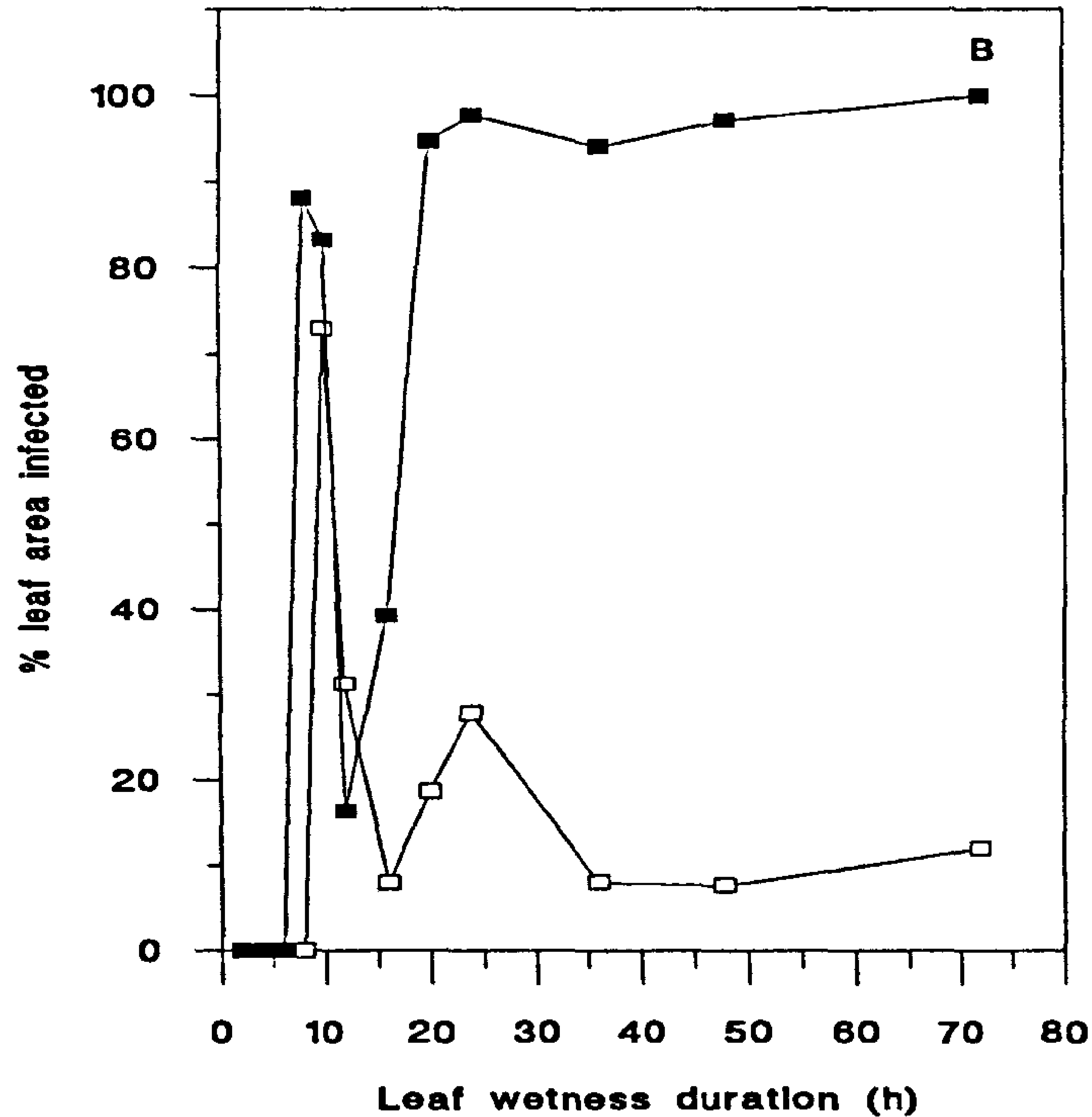




**Figure 5.7**. Effects of leaf wetness duration on the incidence (%) (A) and severity (% area) (B) of leaves with symptoms on linseed plants (cv. Antares) at 15°C (□) and 25°C (■). Plants were inoculated with *A. linicola* conidia (3 x $10^4$ conidia $ml^{-1}$) and assessed 4 days after inoculation. Disease incidence : SED (56 d.f.) = 12; Disease severity : SED (56 d.f.) = 7.9

only those plants which were exposed to 72 h of leaf wetness had developed symptoms at 15°C and 25°C (incidence of the disease on cotyledons 100%). No symptoms developed on the plants in the absence of leaf wetness (24 h dry period) at either of the temperatures tested.

Experiment II. At 15°C, the incidence of the disease on cotyledons and on leaves and the severity of the disease on leaves increased with increasing leaf wetness duration (Fig. 5.8). However, the incidence of the disease on cotyledons was much higher than that on leaves. After 8 h of exposure of the plants to leaf wetness, 36% of the cotyledons and only 3% of the leaves had developed symptoms (Fig. 5.8). The percentage of cotyledons and leaves which developed symptoms increased with increasing leaf wetness duration and reached 92% and 60%, respectively, after exposure to 24 h of leaf wetness (Fig. 5.8). However, after exposure to 8 h of leaf wetness 19% of the leaf area developed symptoms and this percentage increased further to 77% for a 24-h period of leaf wetness. When the control plants were examined for symptom development 24 h after inoculation, only the plants which were exposed to a 24-h period of leaf wetness showed symptoms (incidence of the disease on cotyledons 100%). No symptoms were observed on the plants in the absence of leaf wetness (24 h dry period) at either of the temperatures tested.

### 5.4.4. Effects of interrupted leaf wetness on infection and symptom development

#### 5.4.4.1. Interruption by one dry period

The effects of interrupting a continuous leaf wetness period by a 12-h dry period during the germination and infection process on the subsequent development of symptoms

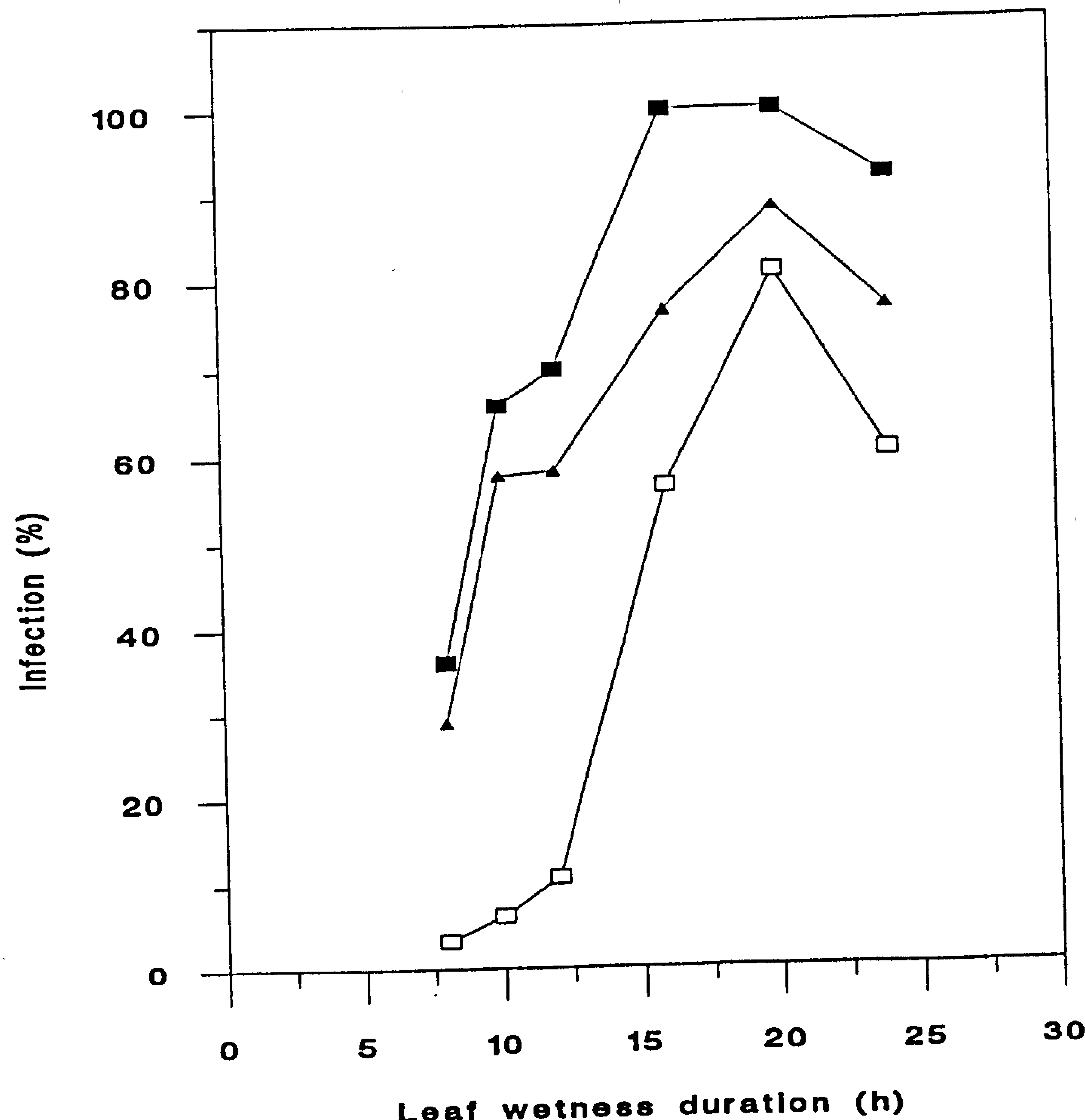


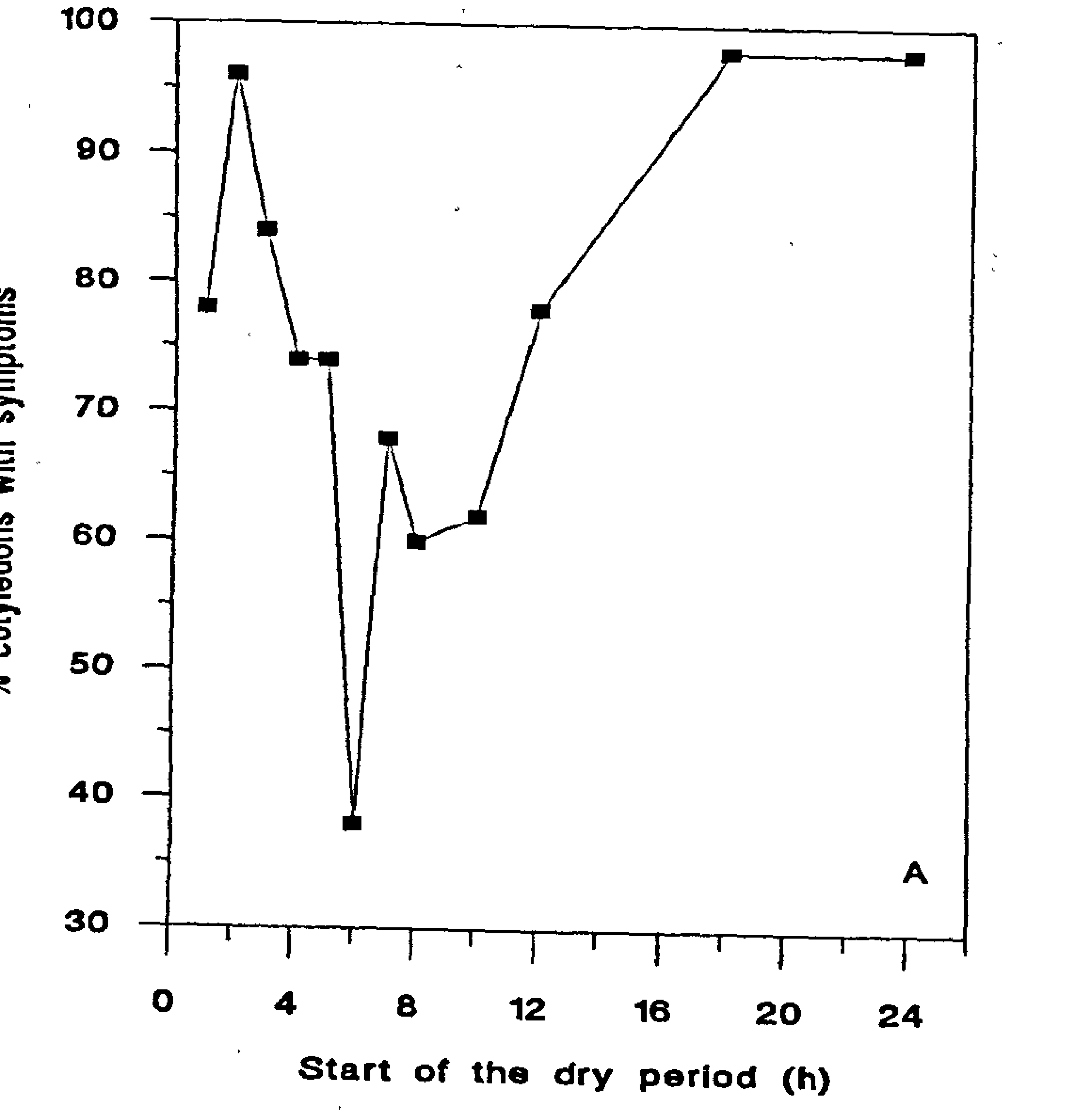

**Figure 5.8.** Effects of leaf wetness duration on the incidence (%) of plants [■ , SED (20 d.f.) = 12.5] or leaves [□ , SED (20 d.f.) = 15.7] with symptoms and on the severity (% area) of leaves with symptoms [▲, SED (20 d.f.) = 13.5] of linseed plants (cv. Antares) at 15°C. Plants were inoculated with *A. linicola* conidia (3 x $10^4$ conidia $ml^{-1}$) and assessed 4 days after inoculation.

on linseed plants by *A. linicola* depended on the time at which the dry interruption occurred (Fig. 5.9). A 12-h dry period applied 1 h after inoculation decreased the percentage of cotyledons which developed symptoms by 20% (disease incidence 80%) compared with the disease incidence (100%) on plants incubated for 24 h under continuous leaf wetness (control plants) (Fig. 5.9A). A dry period applied 2 h after inoculation did not significantly affect the development of symptoms (Fig. 5.9A). A 12-h dry period applied at any time between 3 h and 12 h after inoculation decreased the percentage of cotyledons which developed symptoms, especially when the dry period occurred 6 h after inoculation (60% decrease) (Fig. 5.9A). However, dry periods applied 18 h or 24 h after inoculation had no effect on the percentage of cotyledons which developed symptoms (Fig. 5.9A).

The effects of interruption of a 24-h leaf wetness period by a 12-h dry period at various times after inoculation on the severity of the disease on cotyledons were similar to those on disease incidence (Fig. 5.9B). Generally, a dry period applied at any time between 1 h and 24 h after inoculation decreased the severity of the disease on cotyledons. The greatest decrease (approximately 100%) was observed when the dry period was applied 6 h after inoculation (Fig. 5.9B). However, a 12-h dry period applied 1 h after inoculation decreased the severity of the disease on cotyledons more than a dry period applied 2 h after inoculation (Fig. 5.9B). There was no development of symptoms on plants in the absence of leaf wetness (24 h dry period - control plants).

**5.4.4.2. Interruption by several dry periods**

Conidia of *A. linicola* were able to use successive 12-h periods of leaf wetness



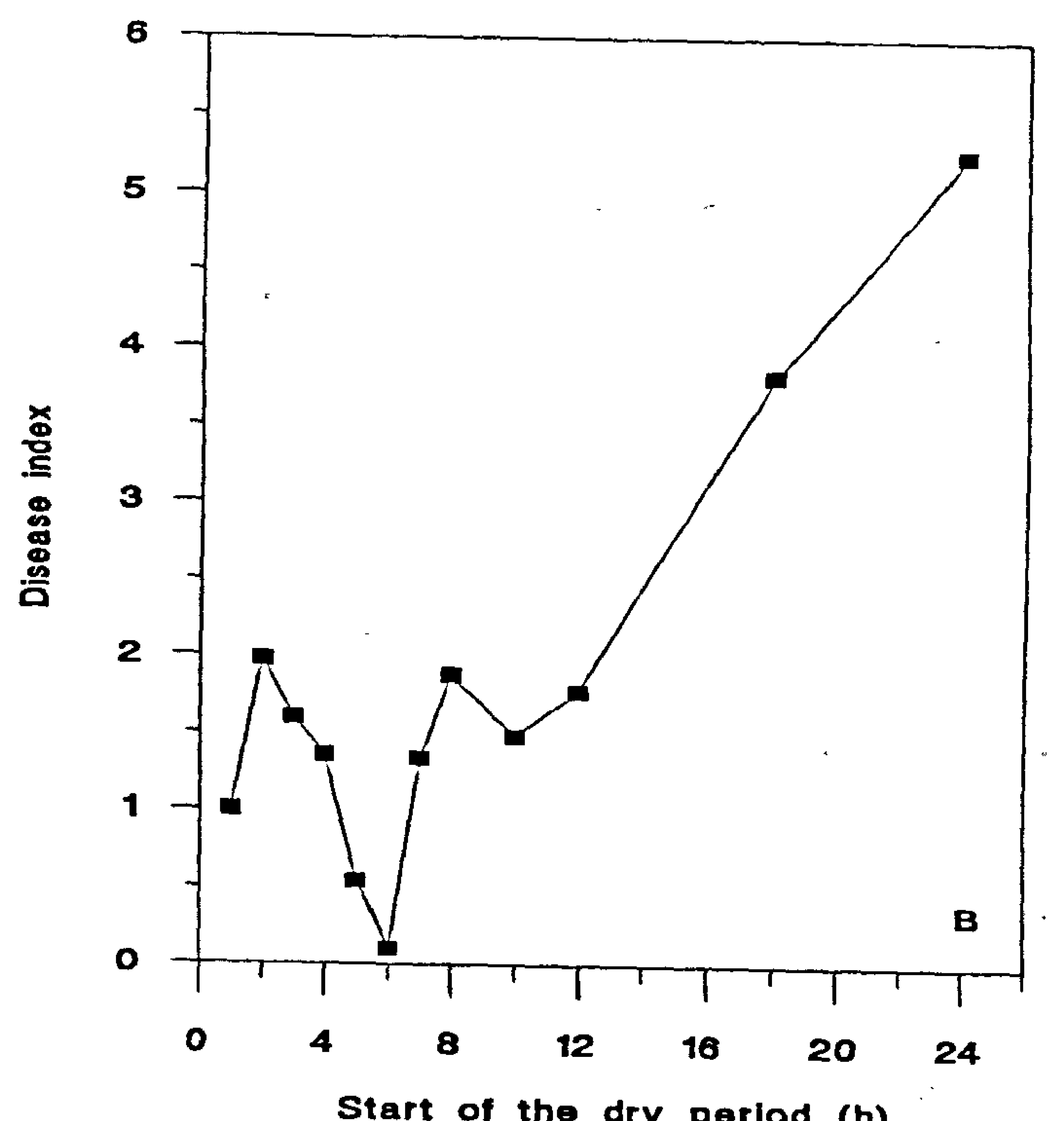




**Figure 5.9.** Effects of interrupting a continuous leaf wetness period by a 12-h dry period at various times after inoculation on the incidence (%) (A) and severity (disease index) (B) of symptoms on cotyledons of linseed plants (cv. Antares) at 15°C. Plants were inoculated with *A. linicola* conidia (3 x $10^4$ conidia $ml^{-1}$) and assessed 4 days after inoculation. The disease index was calculated by using the equation 5.1 (section 5.3.5) Disease incidence : SED (44 d.f.) = 12.4; Disease severity : SED (44 d.f.) = 0.4

cumulatively to infect linseed plants. The percentage of cotyledons with symptoms on the 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 (Fig. 5.10A). When a 24-h period of leaf wetness was interrupted 12 h after inoculation by a 12-h dry period, it resulted in a 30% decrease in the percentage of cotyledons with symptoms (Fig. 5.10A). The decrease in the incidence of the disease was less (approximately 5%) when longer leaf wetness periods (36 or 48 h) were interrupted by dry periods (Fig. 5.10A).

However, the severity of the disease on leaves (% area with symptoms) 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 (Fig. 5.10B).

**5.4.5. Effects of light regime on infection and symptom development**

There were no significant differences in the incidence of the disease on cotyledons between plants exposed for 72 h to different light treatments immediately after inoculation (Table 5.3). However, a slightly greater percentage of cotyledons (91%) developed symptoms on plants exposed to continuous darkness for 72 h than on those exposed to continuous light for 72 h (Table 5.3). The incidence of the disease on stems was positively correlated ($r^2 = 0.93$) with the length of the initial period in darkness (Fig. 5.11), but it was negatively correlated ($r^2 = 0.81$) with the length of the initial period in light (Fig. 5.11). 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 5.3).

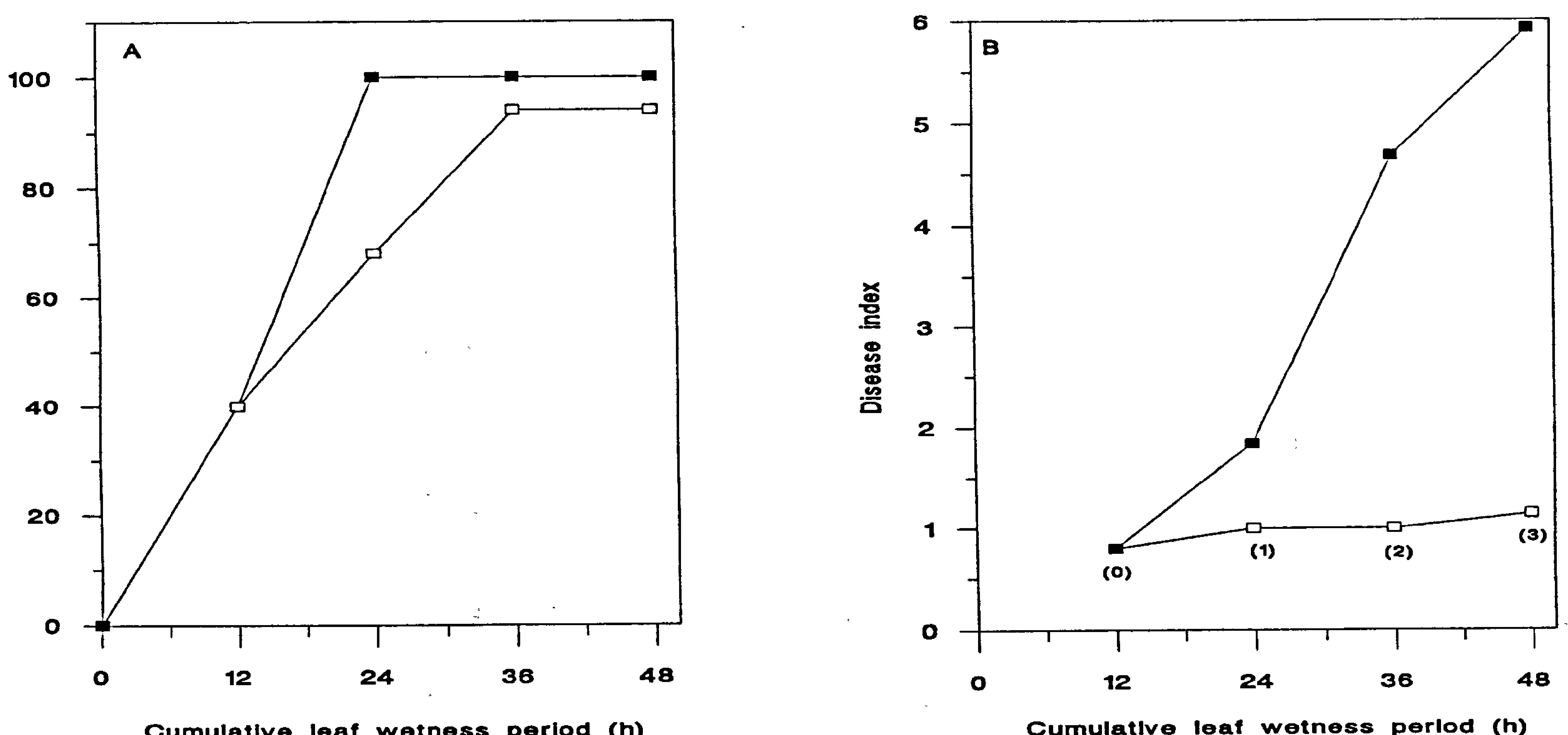



**Figure 5.10.** Effects of continuous (■) or interrupted (□) leaf wetness periods on the incidence (%) (A) and severity (disease index) (B) of symptoms on cotyledons of linseed plants (cv. Antares) at 15°C. Plants were inoculated with *A. linicola* conidia ($3 \times 10^4$ conidia $ml^{-1}$) and assessed 6 days after inoculation. The disease index was calculated by using equation 5.1 (section 5.3.5). Figures in parentheses are the numbers of dry periods (12 h each) interrupting the leaf wetness period. Disease incidence : SED (12 d.f.) = 7.9; Disease severity : SED (12 d.f.) = 5.8

**Table 5.3.** 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 ($3 \times 10^4$ conidia $ml^{-1}$) under controlled environment conditions.

| | Light regimes | Cotyledons with symptoms (%) | Stems with symptoms (%) | Leaf disease index[1] |
|---|---|---|---|---|
| 1 | Continuous L (72 h) | 73.0[2] | 4 | 0.41 |
| 2 | Continuous D (72 h) | 91.0 | 92 | 3.54 |
| 3 | Alternating : 12 h L/12 h D | 94.5 | 28 | 1.15 |
| 4 | Alternating : 12 h D/12 h L | 83.5 | 18 | 0.79 |
| 5 | 12 h L - 60 h D | 97.5 | 80 | 2.86 |
| 6 | 12 h D - 60 h L | 82.0 | 18 | 0.66 |
| 7 | 24 h L - 48 h D | 98.5 | 88 | 3.16 |
| 8 | 24 h D - 48 h L | 95.5 | 50 | 1.44 |
| 9 | 48 h L - 24 h D | 97.5 | 24 | 1.50 |
| 10 | 48 h D - 24 h L | 98.5 | 83 | 2.66 |
| | SED (36 d.f.) | 5.07 | 16.27 | 0.29 |

[1] Disease index 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 the equation 5.6 (section 5.3.8).

[2] Mean of five replicates.

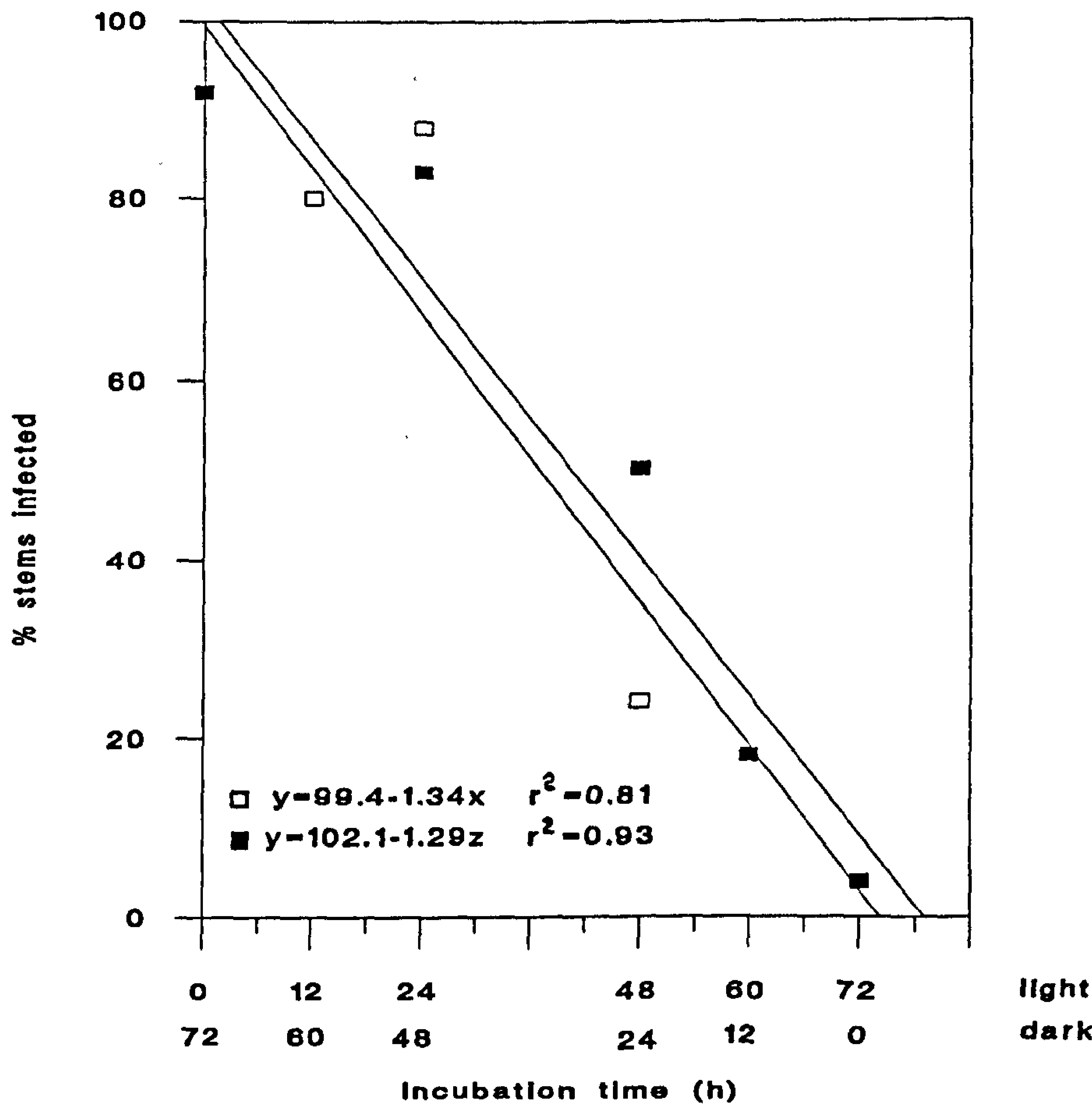



**Figure 5.11.** The relationship between the period of light (□) [or the period of darkness (■)] and the incidence (%) of stems with symptoms on linseed plants (cv. Antares) at 15°C. Plants were inoculated with *A. linicola* conidia (3 x $10^4$ conidia $ml^{-1}$) and assessed 3 days after inoculation.

A high percentage (> 80%) of the stems developed symptoms when the plants were exposed to one of the following treatments : a) 12 h light followed by 60 h darkness, b) 24h light followed by 48 h darkness or c) 48 h darkness followed by 24 h light. For the other treatments the percentage of stems which developed symptoms was ≤ 50% and did not differ significantly from that of the continuous light treatment (Table 5.3).

The severity of the disease on leaves was also positively correlated ($r^2$ = 0.99) with the length of the initial dark period (Fig. 5.12), but it was negatively correlated ($r^2$ = 0.94) with the length of the initial light period (Fig. 5.12). The smallest percentage of leaf area with symptoms was observed on plants exposed to continuous light for 72 h, whereas the highest percentage of leaf area with symptoms was observed on plants exposed to continuous darkness for 72 h (Table 5.3).

**5.5. Discussion**

The results of this study showed that under controlled environment conditions the incidence of the disease on cotyledons, leaves, stems and hypocotyledons increased with increasing inoculum concentration from 1 x $10^3$ to 1 x $10^5$ conidia $ml^{-1}$. This suggests that numbers of conidia which were retained, germinated and penetrated the tissues were probably greater on plants inoculated with high inoculum densities than those on plants inoculated with low inoculum densities. The lowest inoculum concentration of 1 x $10^3$ conidia $ml^{-1}$ was not sufficient to initiate the development of symptoms on leaves, stems or hypocotyledons. However, symptoms developed on the cotyledons when this inoculum concentration was used, but the disease

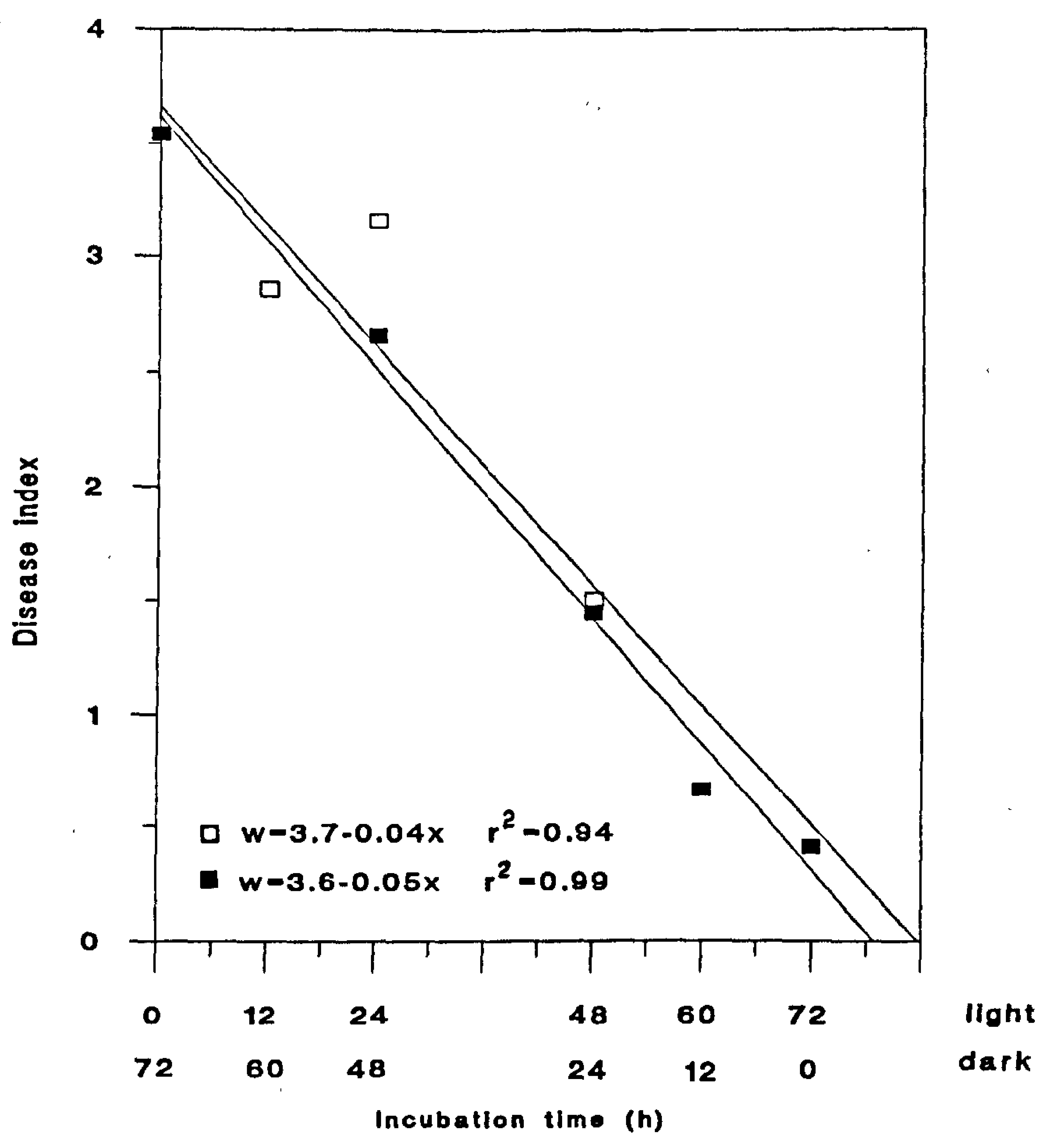



**Figure 5.12.** The relationship between the period of light (□) [or the period of darkness (■)] and the disease index on leaves of linseed plants (cv. Antares) at 15°C. Plants were inoculated with *A. linicola* conidia (3 x $10^4$ conidia $ml^{-1}$) and assessed 3 days after inoculation. The disease index was calculated by using the equation 5.6 (section 5.3.8).

incidence remained relatively low (65%) even 18 days after inoculation. With higher inoculum concentrations, all the cotyledons (100%) 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 first appearance of symptoms) was not affected by the inoculum concentration tested. The first symptoms appeared on the cotyledons 4 days after inoculation, even when the lowest inoculum concentration ($1 \times 10^3$ conidia $ml^{-1}$) was used.

The severity of the disease increased with increasing inoculum concentration from $1 \times 10^3$ to $5 \times 10^4$ conidia $ml^{-1}$. However, approximately the same severity of the disease was observed on plants inoculated with $5 \times 10^4$ and $1 \times 10^5$ conidia $ml^{-1}$. These results suggest that probably at high inoculum concentrations there might be a self-inhibition in the germination of *A. linicola* conidia, especially when the conidia are clumped together (Rotem, 1981). Symptom development depends on the inoculum concentration not only for *Alternaria* species but also for other fungi. In artificial inoculations of safflower plants with *A. carthami* the first symptoms appeared after 67 h and 52 h, when inoculum concentrations of 500 and $4 \times 10^3$ conidia $ml^{-1}$, respectively, were used (Singh & Chand, 1982). The number of lesions on leaves of wheat seedlings increased with increasing concentration of *Septoria nodorum* conidia used for the artificial inoculations (Jeger *et al.*, 1985). Increasing inoculum concentration from $4 \times 10^4$ to $1 \times 10^7$ conidia $ml^{-1}$ increased the severity of the disease caused by *Ascochyta rabiei* on chickpea (*Cicer arientinum* L) (Trapero-Casas & Kaiser, 1992).

There was an interaction between the effects of leaf wetness duration and those of temperature on the incubation period and development of symptoms of

*A. linicola* on cotyledons and leaves of linseed plants. Eight hours of leaf wetness were sufficient to initiate the disease at 25°C, but not at 15°C, when a longer period of 10 h was needed. When the experiment was repeated at 15°C 8 h of leaf wetness period were sufficient for the development of symptoms. Increasing the length of the leaf wetness period from 8 h to 16 h increased the incidence of the disease on plants (from 35% to 100%, respectively), the disease incidence on leaves (from 30% to 75%, respectively) and the disease severity (from 3% to 60%), respectively. These differences in the results between the two experiments might have been due to the different conditions under which the plants were incubated after the end of each leaf wetness period and before the appearance of symptoms. In one of the experiments and at the end of the designated leaf wetness periods, the plants were dried and incubated at 18°C/13°C day/night temperatures and 16 h photoperiod for an additional period of 24 h. Moreover, the plants were transferred to these conditions during the light period. Therefore it is possible that the dry light during the period between 8 h and 10 h and between 10 h and 16 h after inoculation at 25°C and 15°C, respectively, stopped the infection process by affecting the germination and the formation of appressoria by the *A. linicola* conidia (see section 4.5.3.2). However, in the other experiment, the plants were incubated in darkness at 15°C for 24 h immediately after drying.

Most *Alternaria* species require high relative humidity or surface wetness to infect their hosts. The incubation period of many *Alternaria* species ranges from 3 h to 72 h, depending on the species and the temperature. Four hours of leaf wetness were sufficient for the development of lesions of *A. longipes* on

tobacco plants with the number of lesions increasing with increasing length of the leaf wetness period (Norse, 1973; Stavely & Slana, 1975). The first symptoms caused by *A. solani* on potato plants were observed after 8 h of leaf wetness (Rotem & Reichert, 1964). At optimum temperatures (27-31°C), *A. brassicicola* needed at least 18 h of leaf wetness to initiate infection on brassica leaves, whereas *A. brassicae* required only 6 h of leaf wetness for symptom development on the same host at 20-24°C (Rangel, 1945; Degenhardt *et al.*, 1982). Few lesions developed on tomato plants inoculated with *A. tomato* after 24 h of leaf wetness at 10°C, whereas at 26°C lesions developed after 3 h of leaf wetness (Paulus & Pound, 1955).

The results of this study also showed that under controlled environment conditions interruption of a continuous period of leaf wetness by a 12-h dry period may affect the development of *A. linicola* symptoms, depending on the time during the germination and infection process at which the interruption occurs. The disease incidence was decreased on the cotyledons of plants exposed to a 12-h dry period occurring at any time between 3 h and 12 h after inoculation. However, the severity of symptoms on the leaves was affected more by the dry period than the incidence of symptoms. A dry period occurring at any time between 1 h and 12 h after inoculation decreased the percentage of leaf area with symptoms by more than 50%. However, interrupting the leaf wetness period 1 h after inoculation decreased the incidence and severity of the disease more than interrupting it after 2 h. This difference was probably because the germinating conidia were more susceptible to drying during the water imbibition phase (< 2h) than during the germ tube initiation phase (≥ 2 h) (see section

4.5). The results of the experiments on the effects of dry interruptions of a continuous leaf wetness period on conidial germination of *A. linicola* (see section 4.4.2.2) suggest that a 12-h dry period applied at any time during the germination process and before the penetration of the plant tissues stops the germination of the conidia and the germ tube elongation. Furthermore, these conidia do not recover, irrespective of the length of the wet period that may follow the dry period. Therefore, it seems that the negative effects of the dry period on the further development of symptoms on linseed plants were due to the effects of this dry period on conidial germination.

These controlled environment studies suggest that *A. linicola* conidia are able to use successive 12-h periods of leaf wetness cumulatively to infect linseed plants, although the disease incidence and severity were lower under interrupted than under continuous leaf wetness. Previous studies (see section 4.4.2.2) have shown that at 15°C under controlled environment conditions, 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 h 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 (see section 4.4.2.2). Therefore, it may delay the formation of appressoria, the penetration and subsequently the infection of plants by *A. linicola.*

*A. dauci* on carrots (Strandberg, 1988), *A. helianthi* on sunflowers (Allen *et al.*, 1983), *A. brassicae* and *A. brassicicola* on cabbage (Humpherson-Jones *et al.*, 1983), *A. alternata* on tobacco (Stavely & Slana, 1975) and *A. cassiae* on sicklepod (Walker & Boyette, 1986) can all successfully use both interrupted and

continuous leaf wetness periods to infect their hosts. However, interrupting the leaf wetness period decreased the incidence of the disease on brassica plants inoculated with *A. brassicae* or *A. brassicicola* compared with the continuous leaf wetness period (Humpherson-Jones *et al.*, 1983).

The results of this study suggest that light is another environmental factor which can affect the development of symptoms caused by *A. linicola* on linseed plants and that the effects of light on infection can be as important as those of leaf wetness duration. 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 (see section 4.4.3) have shown that wet light periods of 12 h 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 on leaves through effects on the

germination process.

Exposure of wheat plants to light during the period between 6 h and 24 h after inoculation inhibited the infection of the plants by *Puccinia recondita* f.sp. *triticina* (Zadoks, 1967). Light was also a critical environmental factor decreasing the severity of infection of wheat by *Septoria tritici* (Benedict, 1971), of barley by *Rhynchosporium secalis* (Ryan & Clare, 1975), of sunflowers by *Puccinia helianthi* (Sood & Sackston, 1972) and of Bermuda grass (*Cynodon* spp.) by *P. cynodontis* (Vargas et al., 1967). Moreover, it has been demonstrated that toxins produced by *Alternaria* species during the germination of their conidia are involved in the pathogenesis of these species (Otani *et al.*, 1975; Kohmoto *et al.*, 1976; Scheffer, 1976) and that light inhibits the production of these toxins (Haggblom & 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 the production of these toxins.

The results of this study also showed that the length of the incubation period and the further development of symptoms differ between different linseed plant tissues. Leaves appear to be more resistant to *A. linicola* infection than the cotyledons, since the disease incidence on leaves was much less than that on the cotyledons for the same inoculum concentration used. Moreover, there was a 2-day delay in the appearance of symptoms on the leaves compared with that on the cotyledons. The difference in the susceptibility to *A. linicola* infection between cotyledons and leaves might have been related to differences in tissue maturity with the cotyledons being older, more senescent and as consequence more susceptible than the younger and physiologically more active leaves. It is also possible that the

retention of *A. linicola* conidia on the cotyledons may have been greater than that on the leaves because of differences between them in the wax content of their surfaces.

Differences in the wax content between cotyledons and leaves might also explain the absence of symptoms on leaves, but not on the cotyledons, when the lowest concentration (1 x $10^3$ conidia $ml^{-1}$) was used, if no conidia were retained on the leaves. The leaf epicuticular wax of canola (*Brassica napus* or *B. campestris*) decreased susceptibility to *A. brassicae* in at least three ways : a) by creating a hydrophobic surface that decreased the retention of water-borne conidia, b) by decreasing germination of *A. brassicae* conidia and c) by decreasing the number of germ tubes produced (Conn & Tewari, 1989). Symptoms caused by *A. macrospora* on cotton plants developed to a much greater extent on the cotyledons than on the leaves (Bashi *et al.*, 1983).

Differences in the development of symptoms were also observed between linseed stems and hypocotyledons for the same inoculum concentration used. The appearance of more severe symptoms on the hypocotyledons than on the stems (a higher percentage of the lesions developed into cankers on the hypocotyledons than on the stems) might have been due to differences in the anatomy between these two plant parts, although this was not investigated in this study.

In this study the effects of leaf wetness duration and light regime on infection and symptom development of *A. linicola* on linseed plants were studied under constant temperatures. However, under field conditions and at fluctuating temperatures not only the infection but also the development of symptoms might have been different. Moreover, it is possible that the 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. Therefore, it is necessary to confirm the results of this study under field conditions.

# CHAPTER VI. DETECTION OF *ALTERNARIA LINICOLA* ON SEED AND EFFICIENCY OF SEED TRANSMISSION

## 6.1. Introduction

Survival on seed is a very effective mechanism whereby pathogens can persist during the period between harvest and sowing of the following crop. The extent to which pathogens, especially fungi, survive on seeds depends on their ability to produce propagules such as resting hyphae, chlamydospores, xerophilic conidia, sclerotia or microsclerotia, which can survive under the extremely dry conditions in seed as a carrier. The establishment and development of disease on seedlings growing from infected seed is the final stage in the process of seed transmission, since many fungi may be seed-borne and yet not seed-transmitted.

Infected seed is the main route by which diseases caused by *Alternaria* species are introduced in new areas (Neergaard, 1977; Soteros, 1979; Maude & Humpherson-Jones, 1980b; Herr & Lipps, 1982; Rotem, 1994). Richardson (1979) listed 59 *Alternaria* species which are both seed-borne and seed-transmitted. Most *Alternaria* species can survive on seed for many years of storage (Neergaard, 1977) and seed treatment with fungicides is the only effective method used worldwide for the control of seed-borne *Alternaria* diseases.

*A. linicola*, an important seed-borne pathogen of linseed in the UK, is the main reason for the failure of the seed to reach the UK certification standards which require that less than 5% of the seed in total is infected (Mercer *et al.*,

1991a). The fungus survives on the seed coat as resting mycelium. Under favourable conditions the resting hyphae are activated, colonize tissues and kill the young seedlings before or soon after their emergence. The pathogen can decrease emergence and yield, and it can also affect oil quantity and quality (Mercer *et al.*, 1989). Iprodione had been successfully used as a seed treatment for the control of the seed-borne phase of *A. linicola.* However, this fungicide has been replaced by prochloraz, since iprodione-resistant strains of the pathogen were detected in 1986 (Mercer *et al.*, 1988). Although seed is considered to be the main source of primary inoculum for the disease caused by *A. linicola* on linseed crops, there is no information on the extent to which this inoculum is transferred from the infected seed to the seedlings.

## 6.2. Objectives

1. To examine the mycoflora of linseed seeds and to assess the incidence of *A. linicola* on seed samples from different origins.
2. To examine the efficiency of transmission of *A. linicola* from infected linseed seeds to seedlings.

## 6.3. Materials and Methods

### 6.3.1. Detection of *A. linicola* and other fungi on seed

Twenty seed samples, each consisting of 300 seeds, were tested (Table 6.1). Ten of these samples were taken from commercial seed of four cultivars (Antares, Barbara,

**Table 6.1.** Information about the seed samples tested.

| Seed sample | Cultivar | Year of harvest | Source[1] | Storage[2] | Date of seed treatment | Date tested |
|---|---|---|---|---|---|---|
| 1 | Antares 1-T[3] | 1990 | ISP | Room | Mar91 | Apr91 |
| 2 | Antares 1 | 1990 | ISP | Room | - | Apr91 |
| 3 | Barbara 1-T | 1990 | ISP | Room | Mar91 | Apr91 |
| 4 | Barbara 1 | 1990 | ISP | Room | - | Apr91 |
| 5 | McGregor 1-T | 1990 | SIS | Room | Mar91 | Apr91 |
| 6 | McGregor 1 | 1990 | SIS | Room | - | Apr91 |
| 7 | Norlin-T | 1990 | SIS | Room | Mar91 | Apr91 |
| 8 | Norlin | 1990 | SIS | Room | - | Apr91 |
| 9 | Antares 2-T | 1991 | Dalgety | Room | * | Apr92 |
| 10 | Antares 2 | 1991 | Dalgety | Room | * | Apr92 |
| 11 | Antares 3 | 1991 | RF | 4°C | - | Apr92 |
| 12 | Antares 4 | 1991 | RF | 4°C | - | Apr92 |
| 13 | Antares 5 | 1992 | NI | Room | - | Nov93 |
| 14 | Antares 6-T | 1992 | RF | 4°C | Dec92 | May93 |
| 15 | Antares 6 | 1992 | RF | 4°C | - | May93 |
| 16 | Atalante | 1992 | NI | Room | - | Nov93 |
| 17 | Barbara 2 | 1992 | NI | Room | - | Nov93 |
| 18 | Mystic | 1992 | NI | Room | - | Nov93 |
| 19 | Norman | 1992 | NI | Room | - | Nov93 |
| 20 | Royale | 1992 | NI | Room | - | Nov93 |

[1] ISP : International Seed Producers, Bury St Edmund's; SIS : Sharpes International, Sleaford, Lincs; RF : Rothamsted Farm; NI : Northern Ireland.
[2] Storage conditions during the period between harvest and testing; Room : room temperature.
[3] T : Seed treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem); the treatment was applied by Dr G. Scott at Schering Agriculture, Chesterford Park, Saffron Walden, Essex.
*Not known.
- Not applied.

McGregor and Norlin), harvested in 1990 or in 1991 and either treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem) or left untreated. Six more samples were from Northern Ireland (supplied by P. Mercer). These samples were of the cvs. Antares, Atalante, Barbara, Mystic, Norman and Royale, harvested in 1992 from varietal trials and they were untreated. The remaining four seed samples were of cv. Antares harvested in 1991 or 1992 from linseed crops at Rothamsted Experimental Station. One of the samples was treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem), whereas the remaining three were untreated. All seed samples treated with prochloraz were washed thoroughly in tap water before testing.

For detection of the mycoflora on seeds an agar plate method was used (Neergaard, 1977). V-8 juice agar, prepared by the method described in section 2.1.7 was used as a nutrient medium (20 ml of medium per plate). One hundred seeds from each sample were immersed for 1 min in 200 ml of 1% NaOCl (containing 0.01 ml of 0.01% Tween 80 as a wetting agent) to eliminate superficial contamination of the seeds. Seeds were then rinsed in two changes of sterile distilled water and allowed to dry on sterile filter paper (Whatman No1) before placing them on V-8 agar plates (10 seeds per plate). The total incidence of seed-borne inoculum was estimated by using unsterilized seeds. One hundred unsterilized seeds from each sample were placed on V-8 agar plates (10 seeds per plate). All the plates were sealed with parafilm and incubated for 7 days under diurnal NUV-light [12 h NUV-light (365 nm)/12 h darkness] at 20°C. After this period, fungal colonies emerging from the seeds were examined under a stereo-microscope (x 40 magnification); identification of the fungi was based on their conidial morphology.

Seed samples were tested for germination by the standard blotter method used for the detection of pathogens on seed (Maude *et al.*, 1966; Scott *et al.*, 1973; Neergaard, 1977). One hundred unsterilized seeds from each sample were placed (10 seeds per plate) on two layers of moistened sterile filter paper (Whatman No1) lining the bottom of Petri dishes (9 cm in diameter). The dishes were sealed with parafilm and incubated at room temperature for 7 days before assessment. No statistical analyses were done on the data as there were no replicates.

### 6.3.2. Seed transmission of *A. linicola*

6.3.2.1. Glasshouse experiments

The transmission of *A. linicola* from the seed to the seedlings was tested on three untreated samples of linseed seed (cv. Antares) naturally infected by *A. linicola* and on one sample treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem). The incidence of *A. linicola* infection on these samples, tested on surface-sterilized seed by the method described in section 6.3.1, was 1, 4, 17 and 2%, respectively.

Five hundred seeds from each sample were sown (depth 0.5 cm) in plastic trays (35 x 26 x 7 cm) containing a soil-less compost with a slow release fertilizer [Croxden compost, produced by Nursery trades (Lea valley) Ltd]. The trays were placed in a heated glasshouse (temperature range 15 - 25°C) with an additional light (7 h) provided by two 400 W high pressure sodium plant radiators with integral control (Thermoforce Ltd., Camplex Plantcare Division, Tetbury, Glos., UK). Ten days after sowing, when the seedlings had one pair of true leaves (GS 3, Fig. 1:2), the first lesions were observed on the cotyledons. To identify the fungus, the trays were covered with polyethylene bags sprayed inside with water (100% r.h.) for another

10 days to induce sporulation. One hundred seedlings per seed sample, collected at random, were assessed for *A. linicola* symptoms. Seedlings with brown lesions on their cotyledons were examined under a stereo-microscope (x 40 magnification) for the presence of *A. linicola* conidia. No statistical analyses were done on the data as there were no replicates.

6.3.2.2. Controlled environment experiments

To study the transmission of *A. linicola* from the seed to the seedlings under controlled environment conditions, ten untreated linseed seed samples naturally infected by *A. linicola* from different origins were used (Table 6.3). The incidences of *A. linicola* infection on these samples tested on surface-sterilized or unsterilized seeds by the method described in section 6.3.1 are given in Table 6.3.

Four hundred unsterilized seeds from each sample were sown in plastic trays (35 x 26 x 7 cm) containing the soil mixture described in section 6.3.2.1. The seeds were sown in 6 rows at a depth of 1 cm. Three trays were used as replicates for each seed sample. The trays were watered, covered with plastic transparent lids to maintain 100% relative humidity and they were randomized on the floor of two controlled environment cabinets (see section 2.1.4) designed at Rothamsted Experimental Station. The cabinets were set at 10$^{0}$C and light was provided in each cabinet by 18 fluorescent lamps placed 70 cm above the trays. The daylength was 16 h (from 24:00 h to 16:00 h) and the light intensity at the level of the trays (measured by the method described in section 2.1.4) was 120 - 160 $\mu$Einsteins m$^{-2}$ sec$^{-1}$. The first seedlings emerged approximately 7 days after sowing, but the final emergence of the plants was recorded 6 days later, when no more seedlings were emerging. The incidence of *A. linicola* on the emerged seedlings was assessed 20

days after sowing. All the seedlings with symptoms (damping-off or brown lesions on the cotyledons) were collected and incubated for 5 days by the method described in section 2.2.1 to induce sporulation. After this period, the seedlings were examined under a stereo-microscope (x 40 magnification) for the presence of *A. linicola* conidia.

6.3.2.3. Statistical analyses

For analyzing the data on the seed to seedling transmission of *A. linicola* under controlled environment conditions, the linear regression used was :

$$y = a + b x \qquad (6.1)$$

in which y is the percentage of emerged seedlings infected when the estimated incidence of *A. linicola* on the seed is x, a is the intercept on the y-axis and b is the slope of the line. For analyzing the relationship between the incidence of *A. linicola* on the seed and the percentage of seedlings which emerged, the linear regression used was:

$$z = a - b x \qquad (6.2)$$

in which, z is the percentage of seedlings emerged from seed with x incidence of seed-borne inoculum of *A. linicola*, a is the intercept on the z-axis and b is the slope of the line.

## 6.4. Results

### 6.4.1. Detection of *A. linicola* and other fungi on seed

*A. linicola* was detected on 12 of the seed samples tested (Table 6.2 & Fig. 6.1). The incidence of *A. linicola* on infected seeds ranged from 1% to 81% on surface-sterilized seeds and from 1% to 78% on unsterilized seeds. Neither prochloraz as a seed treatment nor surface sterilization of the seeds with NaOCl greatly affected

**Table 6.2.** Incidence of seed-borne fungi on the seed of eight linseed cultivars.

| Seed No | Cultivar[1] | Germ.[2] (%) | Seed infection (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Al[3] | Ai | Aspp | Bc | St | Cl | Fs | Ep |
| 1 | Ant 1-T* | 99 | 0[4](0)[5] | 1(0) | 3(10) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| 2 | Ant 1 | 100 | 0(0) | 0(3) | 0(8) | 0(2) | 0(2) | 0(2) | 0(0) | 0(2) |
| 3 | Barb 1-T | 93 | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| 4 | Barb 1 | 93 | 0(0) | 7(18) | 20(25) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| 5 | McG-T | 99 | 0(0) | 1(0) | 0(1) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| 6 | McG | 100 | 0(0) | 0(0) | 0(3) | 0(0) | 0(0) | 0(1) | 0(0) | 0(0) |
| 7 | Norl-T | 90 | 0(0) | 0(0) | 3(4) | 0(0) | 0(0) | 0(0) | 0(0) | 0(0) |
| 8 | Norl | 73 | 0(0) | 0(1) | 1(9) | 0(0) | 0(0) | 0(11) | 0(0) | 0(0) |
| 9 | Ant 2-T | 91 | 2(1) | 15(11) | 8(16) | 0(0) | 1(16) | 0(0) | 0(0) | 3(15) |
| 10 | Ant 2 | 90 | 1(2) | 1(11) | 0(0) | 0(0) | 0(2) | 0(0) | 0(0) | 0(0) |
| 11 | Ant 3 | 75 | 17(14) | 68(78) | 22(25) | 0(1) | 6(6) | 0(0) | 0(0) | 1(2) |
| 12 | Ant 4 | 84 | 4(5) | 70(87) | 16(19) | 0(3) | 2(6) | 0(0) | 0(0) | 0(0) |
| 13 | Ant 5 | 95 | 71(75) | 0(0) | 0(7) | 0(0) | 0(0) | 0(0) | 1(0) | 0(0) |
| 14 | Ant 6-T | 96 | 4(3) | 15(2) | 63(72) | 0(0) | 10(29) | 0(0) | 2(8) | 1(3) |
| 15 | Ant 6 | 94 | 4(15) | 13(7) | 73(87) | 1(2) | 5(6) | 0(0) | 6(13) | 1(2) |
| 16 | Atal | 93 | 81(78) | 1(3) | 4(15) | 0(0) | 0(2) | 0(0) | 0(0) | 0(0) |
| 17 | Barb 2 | 92 | 53(66) | 6(4) | 17(30) | 0(0) | 9(5) | 0(0) | 0(2) | 0(0) |
| 18 | Myst | 90 | 64(65) | 4(7) | 4(12) | 0(0) | 4(6) | 0(0) | 0(3) | 0(1) |
| 19 | Norm | 100 | 68(67) | 2(5) | 3(18) | 0(0) | 2(6) | 0(0) | 0(3) | 0(0) |
| 20 | Roy | 99 | 63(66) | 3(8) | 3(8) | 0(0) | 3(6) | 0(0) | 0(1) | 0(0) |

[1] Ant : Antares; Bar : Barbara; McG : McGregor; Norl : Norlin; Atal : Atalante; Myst : Mystic; Norm : Norman; Roy : Royale.
[2] Percentage germination tested with the blotter method.
[3] Al : *A. linicola;* Ai : *A. infectoria;* Aspp : *Alternaria* spp.; Bc : *Botrytis cinerea;* St : *Stemphylium* spp.; Cl : *Cladosporium* spp.; Fs : *Fusarium* spp.; Ep : *Epicoccum* spp.
[4] On surface sterilized seed (1% NaOCl for 1 min).
[5] On unsterilized seed.
*T : Seed treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem).

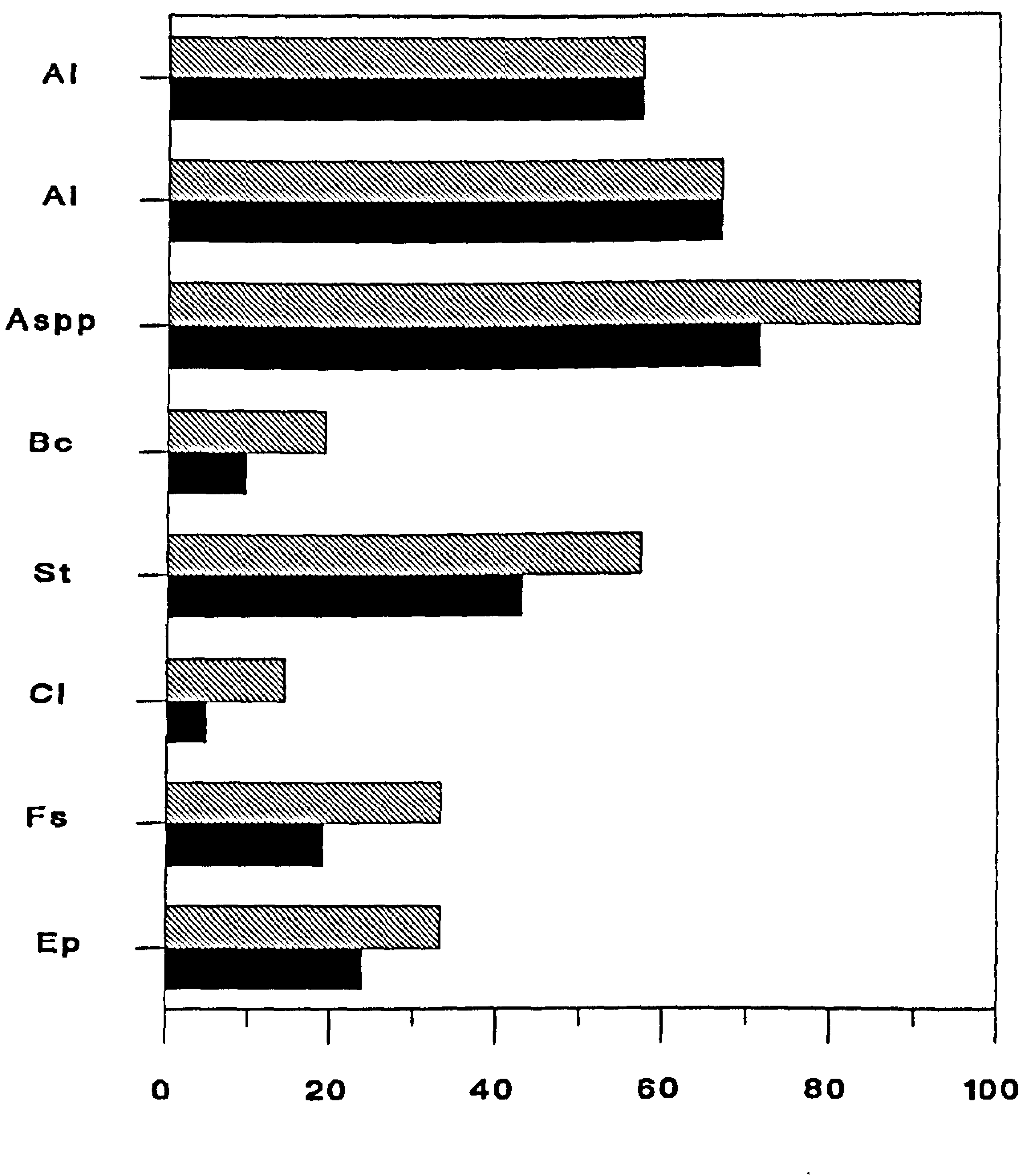



**Figure 6.1.** Frequency (% seed samples infected) of seed-borne fungi on surface-sterilized ( ■ ) and unsterilized ( ▧ ) linseed seed tested on V-8 agar medium. Al : *A. linicola;* Ai : *A. infectoria*; Aspp. : *Alternaria* spp; Bc : *B.cinerea;* St : *Stemphylium* spp.; Cl : *Cladosporium* spp.; Fs : *Fusarium* spp.; Ep : *Epicoccum* spp.

the percentage of seeds on which *A. linicola* was detected (Table 6.2). Surface sterilization slightly increased the incidence of *A. linicola* on the seed in some samples, but slightly decreased the incidence in other samples (Table 6.1). A greater incidence of *A. linicola* infection was detected on the seed samples from Northern Ireland (incidence 53% to 81% on surface-sterilized seed) than on the seed samples from the SE of England (incidence 1% to 17% on surface-sterilized seed) (Table 6.1 & Table 6.2).

*Botrytis cinerea,* another seed-borne pathogen of linseed, was detected on 9% and 19% of the surface-sterilized and unsterilized seed samples, respectively (Table 6.2 & Fig. 6.1). All these seed samples were untreated samples of cv. Antares. The incidence of *B. cinerea* infection on these seed samples was small (1 - 3%) and was decreased to 0% by surface sterilization of the seed (Table 6.2).

Other fungi detected on the seed samples included *A. infectoria, Alternaria* spp., *Stemphylium* spp., *Cladosporium* spp., *Fusarium* spp. and *Epicoccum* spp. The incidence of these fungi on the seed differed between the samples; in general, they were isolated more frequently from the untreated or unsterilized than from the treated or surface-sterilized seed samples (Table 6.2 & Fig. 6.1). However, *A. infectoria* and other *Alternaria* species were the dominant fungi on the seed samples tested, followed by *Stemphylium* spp., *Fusarium* spp., *Epicoccum* spp. and *Cladosporium* spp. (Fig. 6.1).

The majority of the seed samples (85%) germinated well (germination ≥90%) (Table 6.2). However, the percentage germination of three samples (one commercial and two from a linseed crop at Rothamsted Experimental Station) ranged from 73% to 84% (Table 6.2). Prochloraz as a seed treatment had no effect

on the percentage germination of seed (Table 6.2)

### 6.4.2. Seed transmission of *A. linicola*

6.4.2.1. Glasshouse experiments

*A. linicola* was effectively transmitted from infected seeds to seedlings (Fig. 6.2). When seed samples with 1, 2, 4 or 17% incidence of *A. linicola* infection were sown, the percentages of disease incidence on the emerged seedlings were 2, 2, 40 and 38%, respectively (Fig. 6.2).

6.4.2.2. Controlled environment experiments

Transmission of *A. linicola* from seeds to seedlings also occurred at 10°C. Two types of symptoms were observed on the emerged seedlings : a) seedlings which died immediately after emergence and were covered with mycelium and conidia of the pathogen (Fig. 6.3) and b) seedlings which emerged and had brown lesions on their cotyledons; these lesions were mainly observed beneath the remains of the seed coat (Fig. 6.3).

The efficiency of transmission from seed to seedling depended on the incidence of *A. linicola* infection on the seed used for sowing. In general, the greater the incidence of *A. linicola* infection on the seed, the greater was the percentage of infected seedlings (Table 6.3 & Fig. 6.4). The ratio of seed to seedling transmission ranged from 1 : 0.4 to 1 : 0.7 for seed samples with an incidence of *A. linicola* infection (on surface-sterilized seed) which ranged from 28% to 82% (Table 6.3). However, for seed samples with a lower incidence of *A. linicola* infection (1 - 16%) the ratio of seed to seedling transmission ranged from 1 : 0.1 to 1 : 0.2 (Table 6.3).

Regression analysis showed that infection of seedlings was closely correlated

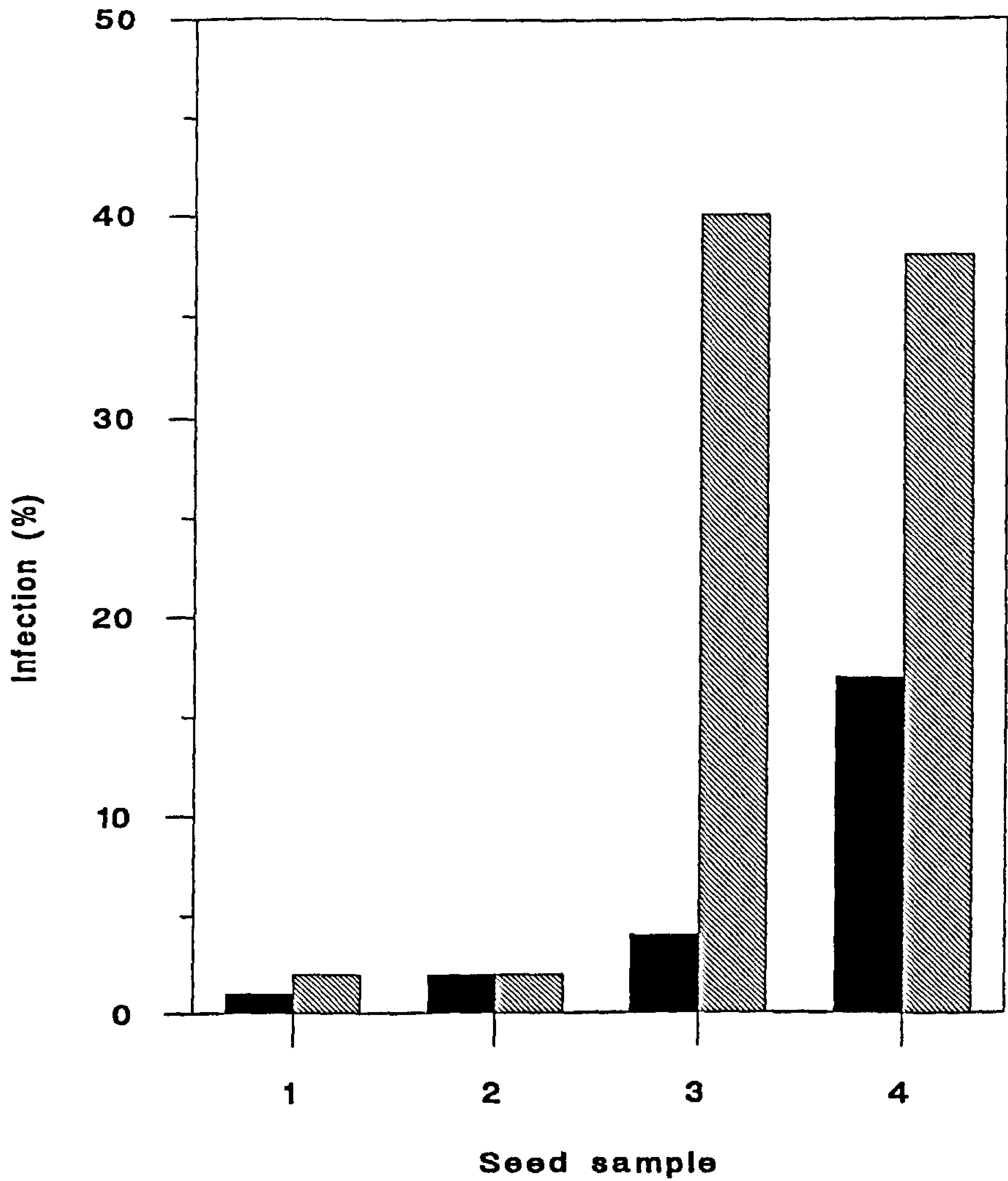



**Figure 6.2.** Effects of seed-borne *A. linicola* inoculum ( ■ ) on the percentage of emerged seedlings with symptoms ( ▧ ) tested on four linseed seed samples (cv. Antares) harvested in 1991. 1 : seed treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem); 2, 3 & 4 : untreated seed.

**Figure 6.3.** Symptoms of *A. linicola* on seedlings emerged from infected seed (cv. Antares) 20 days after sowing at 10°C : a) seedlings covered with hyphae and conidia of the pathogen and b) seedlings with brown lesions on their cotyledons; the lesions were initially observed at the tips of the cotyledons where the remains of the seed coat were.

**Table 6.3.** Effects of seed-borne *A. linicola* inoculum on the percentage of seedlings which emerged and on the percentage of seedlings with brown lesions on their cotyledons.

| Cultivar | Source | Seed infection [1] (%) | | Emergence (%) | Seedlings infected (%) | Seed : seedling infection | |
|---|---|---|---|---|---|---|---|
| | | SS | NS | | | SS | NS |
| Atalante | Northern Ireland | 82 | 78 | 22.9 | 58.2 | 1:0.7 | 1:0.7 |
| Antares | Northern Ireland | 71 | 75 | 45.3 | 49.1 | 1:0.7 | 1:0.7 |
| Norman | Northern Ireland | 68 | 69 | 19.3 | 25.2 | 1:0.4 | 1:0.4 |
| Barbara | Northern Ireland | 67 | 69 | 26.5 | 33.6 | 1:0.5 | 1:0.5 |
| Mystic | Northern Ireland | 64 | 65 | 12.7 | 32.5 | 1:0.5 | 1:0.5 |
| Royale | Northern Ireland | 63 | 66 | 20.1 | 25.8 | 1:0.4 | 1:0.4 |
| Antares | Rothamsted Farm | 28 | 30 | 73.3 | 11.0 | 1:0.4 | 1:0.4 |
| Antares | Rothamsted Farm | 16 | 14 | 66.6 | 3.93 | 1:0.2 | 1:0.3 |
| Antares | Rothamsted Farm | 4 | 4 | 63.3 | 0.26 | 1:0.1 | 1:0.1 |
| Antares | Rothamsted Farm | 1 | 1 | 48.9 | 0 | 1:0 | 1:0 |
| SED | | - | - | (18 d.f.) = 3.96 | (16 d.f.) = 4.46 | | |

[1] SS : surface sterilized seed (1% NaOCl for 1 min); NS : unsterilized seed.
- Statistical analyses were not done on the data as there were no replicates.

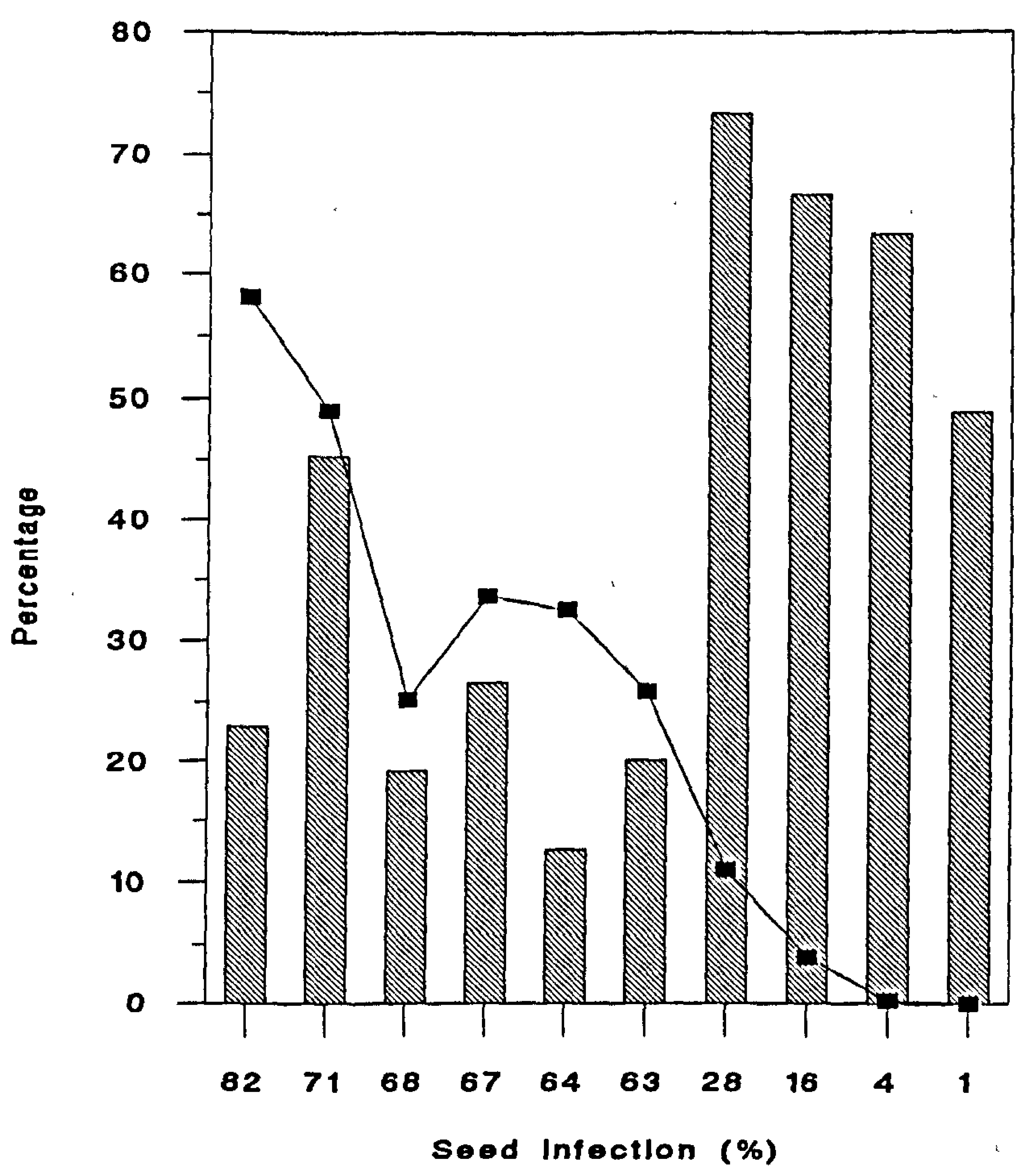



**Figure 6.4.** Effects of seed-borne inoculum of *A. linicola* on the percentage of seedlings which emerged [ ▧ , SED (18 d.f.) = 3.96] and on the percentage of seedlings with brown lesions on their cotyledons [■ , SED (16 d.f.) = 4.46], 20 days after sowing at 10°C .

($r^2$ = 0.86) with the amount of seed-borne *A. linicola* inoculum (Fig. 6.5). The percentage of seedlings which emerged was negatively correlated ($r^2$ = 61) with the incidence of *A. linicola* infection on the seed (Fig. 6.6). In general, more seedlings emerged from seed samples with a low incidence (1 - 28%) of *A. linicola* infection than from those with a greater incidence (63 - 82%) of infection. However, the percentage of seedlings which emerged from a seed sample with a high incidence of *A. linicola* infection (cv. Antares, with incidence 71%) was approximately the same as that from a seed sample with a low incidence of *A. linicola* infection (cv. Antares, with incidence 1%) (Table 6.3 & Fig. 6.4).

**6.5. Discussion**

These results show that *A. linicola* is a seed-borne pathogen of linseed. Although a small number (20) of seed samples was tested, *A. linicola* was detected on 57% of these samples with an average of 36% of seeds infected. The Plant Pathology Division of the Department of Agriculture for Northern Ireland, which is responsible for the health certification of most of the flax and linseed seed in the UK, records the incidence of seed-borne pathogens in more than 1000 seed samples every year. According to these records, the incidence of *A. linicola* on unsterilized seed, tested by the Ulster method [2% malt agar at 22°C under alternating NUV-light (16 h NUV-light/8 h darkness) for 7 days] can be as great as 90% (Mercer *et al.*, 1985).

At least 59 *Alternaria* species can colonize the seed of their host plants and are considered to be seed-borne pathogens (Neergaard, 1977; Richardson, 1979). Reports on seed tests from many countries indicate that the incidence of *Alternaria*

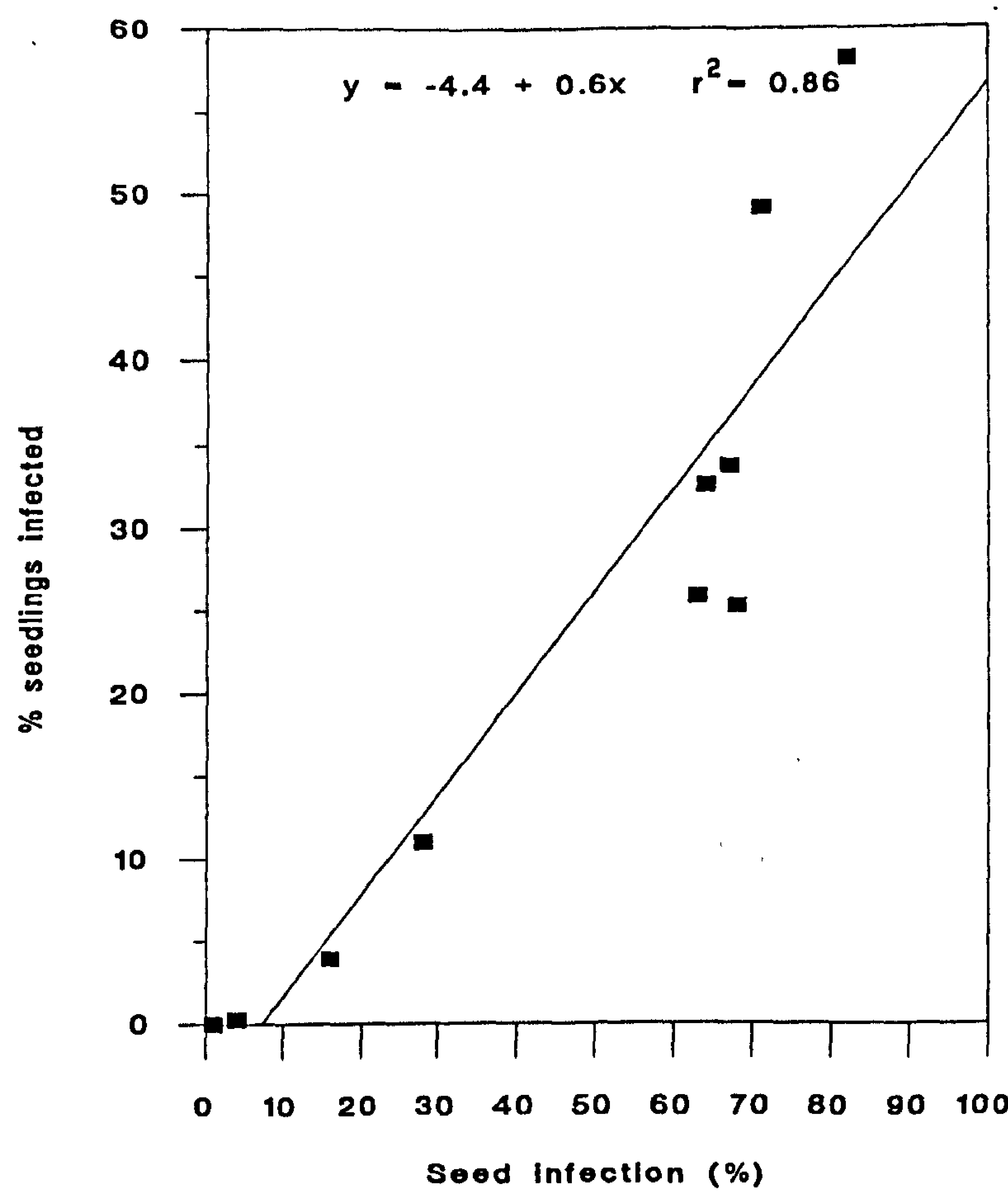



**Figure 6.5.** Relationship between the incidence of *A. linicola* infection on the seed and the percentage of emerged seedlings with symptoms, 20 days after sowing at 10°C.

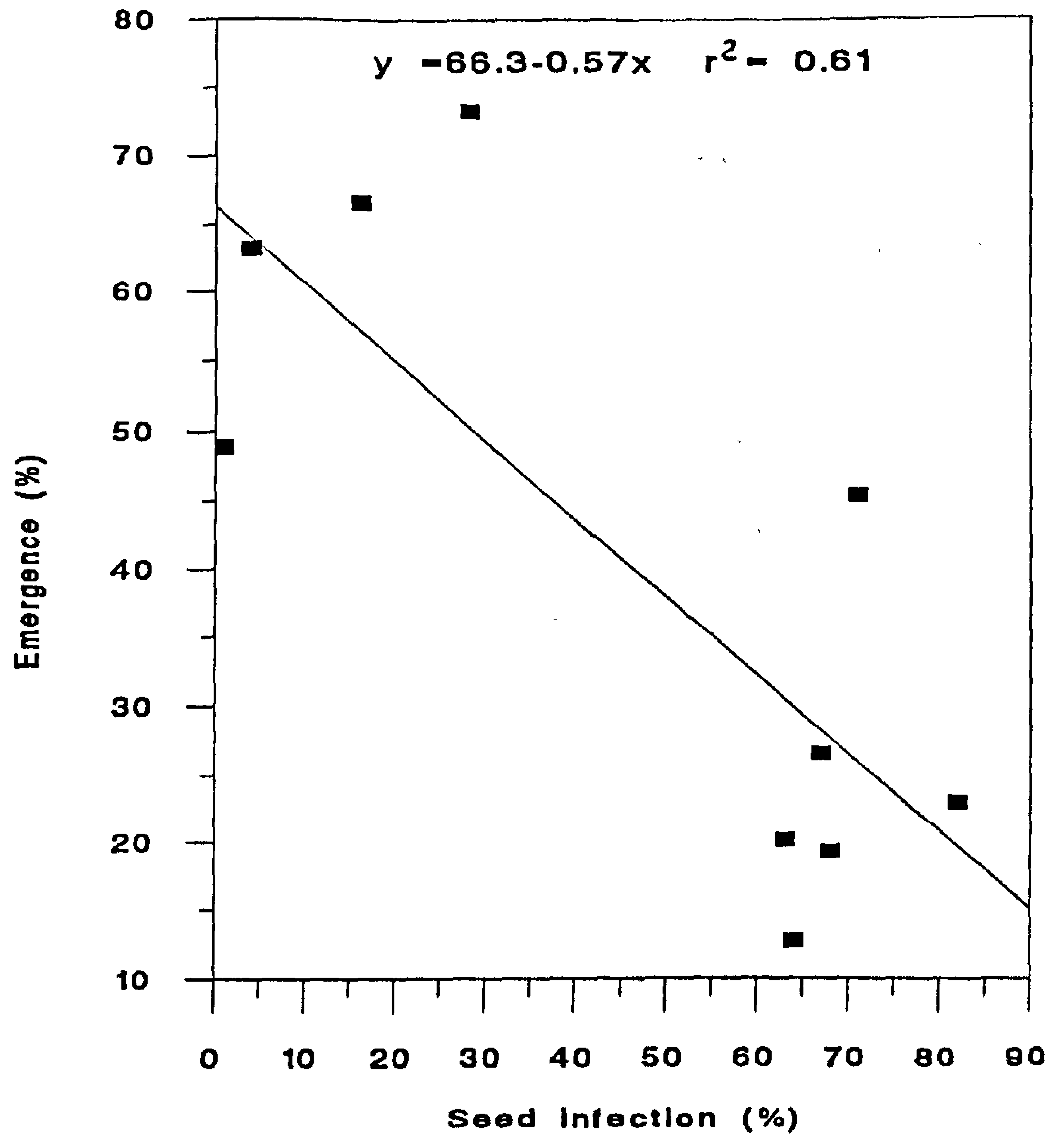



**Figure 6.6.** Relationship between the incidence of *A. linicola* on the seed and the percentage of seedlings which had emerged, 20 days after sowing at 10°C.

species on seeds of different crops can be very high (Neergaard, 1945; Bashan, 1984; Humpherson-Jones, 1985; Stovold *et al.*, 1987). Infection and colonization of linseed seeds by *A. linicola* seems to be influenced by the same environmental conditions that favour the disease in the crop, especially during the period between flowering and harvest (July - September). The highest incidences (53 - 82%) of *A. linicola* were detected on the seed samples from Northern Ireland. *A. linicola* seems to be one of the most serious diseases of linseed in Northern Ireland every year, due to the wet weather during the entire growing season (P. Mercer, personal communication). In contrast, seed samples from the SE of England were either free or had a low incidence (1 - 17%) of *A. linicola* infection, depending on the year the seed was harvested. Although a relatively high incidence (1 - 17%) of *A. linicola* infection was detected on the samples of seed harvested in 1991 or 1992, no *A. linicola* infection was detected on the samples harvested in 1990. In 1991 and 1992 the relatively wet weather in July and August (total rainfall 118 mm and 176 mm in 1991 and 1992, respectively compared with only 64 mm of rain in 1990) may have favoured the spread of the disease in the crop (see section 9.4.3) and subsequently the infection of the seed by *A. linicola.*

The effect of wet weather during the period between flowering and seed development on the infection of seed crops by *Alternaria* species has been demonstrated in the past not only for *A. linicola* on linseed seeds (Mercer & Hardwick, 1991; Fitt & Vloutoglou, 1992), but also for *A. brassicae* and *A. brassicicola* on brassica seeds (Neergaard, 1969; Humpherson-Jones, 1985).

The incidence of *A. linicola* infection on the seed samples tested was not greatly affected by the surface sterilization of the seeds with NaOCl, suggesting that

the inoculum was present inside the seed rather than on the surface. The observation that the incidence of *A. linicola* infection on four out of the 20 seed samples tested was slightly greater on the surface-sterilized than on the unsterilized seeds suggests that surface sterilization may have suppressed fungi which were saprophytic or antagonistic to *A. linicola.* Such fungi often obscure the conidia of *A. linicola* and make the identification of the pathogen difficult in culture. Moreover, the suppression of the antagonistic microorganisms on the seed surface by the surface sterilization might have also favoured the growth of the pathogen located inside the seed as was observed for *A. radicina* on carrot seeds (Tylkowska, 1992).

The location of propagules (conidia or hyphae) of other *Alternaria* species on or in the seed of their host depends on the species, the structure of the seed or the time at which the infection of the seed occurs. In some cases, conidia of *Alternaria* species may be carried on the seed surface (contaminated seed), whereas in other cases hyphae growing from the infected fruit tissue penetrate the seed coat (infected seed). Frequently, both external contamination of the seed and deep penetration of the seed by the hyphae of the pathogen occur together (Rotem, 1994).

*A. brassicicola* was carried on brassica seeds both externally and internally (Maude & Humpherson-Jones, 1980b). *A. radicina* hyphae have been observed in the inner layers of the pericarp of carrot seeds and occasionally in the testa, but never in the endosperm or embryo (Tylkowska, 1992). Conidia of *A. dauci* were carried on the surface of carrot seeds (Hewett, 1964; Netzer & Kenneth, 1969; Soteros, 1979; Strandberg, 1983), while hyphae were present in the inner layer of the pericarp (Netzer & Kenneth, 1969). However, in shrivelled carrot seeds infected early in the season *A. dauci* hyphae colonized both the endosperm and embryo (Strandberg,

1983). In sesame seed, the inner cuticle of the seed coat and the outer thick cuticle of the endosperm both appear to inhibit deeper penetration of the seed by hyphae of *A. sesamicola* (Singh *et al.*, 1980). Internal infections of oilseed rape seeds by *A. brassicicola* occurred as a result of early attack of pods by the pathogen (while seeds were still developing), whereas superficial contamination resulted from later infections of the seed (Humpherson-Jones & Maude, 1982a).

In these experiments, 25% of the seed samples tested were infected by *B. cinerea*, which is another seed-borne pathogen of linseed (Mercer *et al.*, 1991a). By contrast with *A. linicola, B. cinerea* inoculum was eliminated by surface sterilization of the seed, suggesting that the pathogen was carried on the surface of the seeds. If conidia or hyphae of *B. cinerea* are carried only on the surface of the seeds, this may explain the rapid decline in the incidence of *B. cinerea* on linseed seed during storage (Neergaard, 1977; Mercer *et al.*, 1991a). While surface sterilization of seeds greatly decreased the incidence of saprophytes such as *Stemphylium* spp., *Cladosporium* spp., *Epicoccum* spp. or *Fusarium* spp., it had only a slight effect on the incidence of *A. infectoria* and other *Alternaria* spp. on the seeds. The method used for surface sterilization of the seed (1% NaOCl for 1 min) was probably too mild to kill these *Alternaria* species, at least on the seed samples that were heavily colonized by these fungi.

This study demonstrates for the first time the significance of the seed-borne phase of *A. linicola* as a source of primary inoculum.The pathogen was effectively transmitted from the seeds to the seedlings. Infected seedlings were either killed before or immediately after emergence or they emerged with brown lesions on their cotyledons. However, transmission of *A. linicola* from seeds to seedlings was influenced

by the temperature and the number of infected seeds used for sowing. *A. linicola* was more effectively transmitted at high temperatures (temperature range 15 - 25°C) than at low temperature (10°C). The transmission of seed-borne *A. brassicicola* was also temperature-dependent with little seedling disease developing at temperatures below 20°C (Chirco & Harman, 1979; Bassey & Gabrielson, 1983; White, 1988). By contrast, the efficiency of *A. dauci* transmission from infected carrot seeds to seedlings was greater at 13°C than at 25°C (Tahvonen, 1978).

In the present study, the efficiency of transmission of *A. linicola* from seeds to seedlings was studied in compost either in the glasshouse or in controlled environment cabinets. However, this efficiency might have been different under field conditions or in soil (sterilized or not), as has been demonstrated for other *Alternaria* species. The transmission rate for *A. brassicicola* was 19% in glasshouse experiments, but it was only 6% under field conditions (Maude & Humpherson-Jones, 1980b). *A. macrospora* was transmitted from infected cotton seeds to 4% of seedlings in a growth chamber and to 1% of seedlings in the field (Rotem, 1994).

The first lesions of *A. linicola* infection on the cotyledons of the seedlings appeared beneath the remains of the seed coat, suggesting that the route by which *A. linicola* is transferred from infected seed to the seedlings is similar to that of other seed-borne and seed-transmitted *Alternaria* species. During germination, in most cases the pathogen is carried passively either on the cotyledons or on the seed coat and sooner or later penetrates the host tissues (Neergaard, 1977).

It is difficult to make conclusions on the effects of seed-borne inoculum of *A. linicola* on seed germination and emergence from the results of experiments on germination *in vitro* (Petri dishes) and emergence in trays with compost due to the

small number of seed samples tested. The low percentage germination (75% & 84%) *in vitro* of two out of the 12 seed samples infected by *A. linicola* cannot be entirely attributed to the presence of the pathogen, as another sample (cv. Norlin) with no detectable *A. linicola* infection also had a low percentage germination (73%). Similarly, controlled environment experiments showed that in trays with compost seed-borne *A. linicola* inoculum may decrease emergence, but a low emergence is not always attributed to the presence of the pathogen on the seed. Therefore, the low percentage germination of seed in these seed samples may have been due to other factors such as environmental conditions during storage. Neergaard (1977) and Tahvonen (1978) reported that infection by *A. dauci* caused a significant decrease in germination of carrot seeds, whereas Soteros (1979) did not observe any effect of the same pathogen on seed germination. The decrease in the number of seedlings which emerged from carrot seeds infected by *A. radicina* was due to the damping-off symptoms caused by the pathogen carried on the seed (Neergaard, 1945; Maude, 1966; Ellis & Holliday, 1972; Tahvonen, 1978). A negative correlation between the incidence of infection on carrot seeds and seedling emergence was also observed for *A. dauci* (Neergaard, 1945; Hewett, 1964; Netzer & Kenneth, 1969).

The results of this study showed that prochloraz as a seed treatment decreased the incidence of *A. linicola* infection on the unsterilized seed. This suggests that the application of this fungicide to the linseed seed may suppress the superficial but not the deep-seated inoculum of the pathogen in the seed. Glasshouse experiments have shown that at temperatures ranging between 15°C and 25°C the efficiency of transmission of *A. linicola* from seeds treated with prochloraz to the seedlings may have been as great as 100%. However, more

seed samples need to be tested to confirm this hypothesis. Mercer *et al.* (1988) reported that no *A. linicola* was detected by the Ulster method on seed samples treated with prochloraz, although the incidence of the pathogen on the untreated seed was 40%. However, it seems likely that prochloraz as a seed treatment has a fungistatic rather than fungitoxic effect on *A. linicola* (Mercer *et al.*, 1989).

The most effective method for controlling the seed-borne phase of other *Alternaria* species seems to be treatments with fungicides (benomyl, iprodione, prochloraz, thiram, etc) or hot water applied to the seed (Maude & Humpherson-Jones, 1980; Jeffrey *et al.*, 1985; Maude & Bambridge, 1991, Strandberg, 1992; Maude *et al.*, 1992; Aveling *et al.*, 1993). Attempts to control the seed-borne phase of *Alternaria* species with antagonistic fungi have shown some promising results, although the biological agents were effective in controlling the superficial rather than the internal seed-borne inoculum (Wu & Lu, 1984; Tahvonen & Avikainen, 1987).

The results of this study suggest that chemical treatment of the linseed seed with prochloraz used by the seed industry probably does not completely eliminate the deep-seated propagules of the pathogen in the seed, which are able to initiate the disease early in the growing season. Moreover, seed treatment will probably be less effective in areas where other sources of primary inoculum (infected debris, volunteers or alternate hosts) are present in the field (see section 8.4). The application of prophylactic fungicide sprays to the linseed crops to prevent seed infection by *A. linicola* cannot be greatly recommended for two reasons : a) the effectiveness of the fungicide applications depends on the weather conditions and on the incidence of the disease in the crop and b) linseed is a low input break crop and sprays with

fungicides are often considered to be uneconomic. Furthermore, unlike other seed-borne pathogens (*Septoria apiicola, Botrytis cinerea, Fusarium graminearum, Sclerospora sorghi, Colletotrichum gloeosporioides,* etc.), which can survive for only up to 2-3 years on the seed of their hosts, *Alternaria* species are considered to be long-lived fungi (> 5 years) during storage of the seed (Neergaard, 1977; Maude & Humpherson-Jones, 1980b; Hewett, 1987). According to Mercer *et al.* (1991a), the incidence of *A. linicola* infection on a seed sample tested decreased from 100% to 20% after 5 years of storage at room temperature. This suggests that storing the infected seed for some years before using it for sowing is unlikely to control the seed-borne phase of *A. linicola*.

# CHAPTER VII. DISPERSAL OF *ALTERNARIA LINICOLA* CONIDIA; SEASONAL AND DIURNAL PERIODICITY AND DISPERSAL GRADIENTS

## 7.1. Introduction

Conidia of *Alternaria* species are a common component of the air-spora and therefore they have been used as a model in epidemiological studies of plant diseases (Rotem, 1964; Meredith, 1966; Schenk, 1968; Strandberg *et al.*, 1977; Everts & Lacy, 1990; Bashan *et al.*, 1991) or in allergic studies (Stakman *et al.*, 1923; Pady & Kapica, 1953). The dispersal of conidia of many *Alternaria* species follows seasonal and diurnal periodicities. The highest concentrations of air-borne *Alternaria* conidia are often observed late in the growing season and coincide with the increases in disease incidence in senescent crops (Rotem, 1964; 1991; Datar & Mayce, 1982; Humpherson-Jones & Maude, 1982a; Suhag *et al.*, 1985). However, the daily diurnal pattern of dispersal is more closely related to changes in the relative humudity, temperature and wind speed (Gregory, 1973). The maximum numbers of *Alternaria* conidia are often dispersed at midday when the humidity decreases and the temperature and wind speed increase (Rotem, 1964; Meredith, 1966; Pearson & Hall, 1975; Langenberg *et al.*, 1977; Strandberg, 1977; Humpherson-Jones & Maude, 1982a; Allen *et al.*, 1983).

Once *Alternaria* conidia become air-borne they can be dispersed over distances ranging from a few centimetres within a crop to many kilometres between continents

(Gregory, 1968; Aylor, 1978). However, studies have demonstrated that the distance over which *Alternaria* conidia are transported by the wind within crops depends on the local wind direction, the inoculum source and the direction of the rows of the plants. Most of the conidia are transported only short distances from the foci of their production and therefore can create only local epidemics (Humpherson-Jones, 1982a; Bashan *et al.*, 1992). In the case of *A. linicola* conidia, the seasonal dispersal pattern seems to be like that of other *Alternaria* species. Fitt & Vloutoglou (1992) reported that in 1989 and 1990 the greatest concentrations of air-borne conidia above linseed crops were observed in July and August and coincided with the increase in the disease incidence in the crop. However, there is no information on the diurnal dispersal pattern of *A. linicola* conidia, or on the distances over which the conidia are transported by the wind within a linseed crop. Moreover, it is not known if conidia dispersed from an inoculum source can create disease epidemics during the same growing season.

**7. 2. Objectives**

1. To study whether the dispersal of *A. linicola* conidia above linseed crops follows seasonal and diurnal periodicities and which environmental factors influence these periodicities.

2. To investigate the inoculum potential of *A. linicola* in a linseed crop by using bait plants.

3. To study the *A. linicola* disease gradients and spore dispersal gradients from point or line inoculum sources.

## 7.3. Materials and Methods

### 7.3.1. Seasonal and diurnal dispersal of *A. linicola* conidia

In 1991, 1992 and 1993 the seasonal dispersal of *A. linicola* conidia was studied by using a 7-day recording volumetric spore sampler (Burkard Manufacturing Co.Ltd., Woodcock Hill, Industrial Estate, Rickmansworth, Herts, UK) (Hirst, 1952) (Fig. 7.1). In 1991 the spore sampler was operated in an untreated linseed crop situated approximately 100 m north of the experimental plots (Field B, Fig. 9.1.). In 1992 and 1993 the sampler was operated within the experimental area (Field A, Fig. 9.1). Each year the sampler was placed in the middle of the linseed crop in an area (2 m diameter) free from plants with the orifice 40 cm above ground level (Fig. 7.1). In 1991, sampling began on 14 June, 65 days after the emergence of the crop and finished on 10 October when the crop was harvested. However, in 1992 and 1993 the sampler was operated continuously. Sampling started on 21 April 1992 when the first crop was sown and finished on 10 October 1993, 23 days after the harvest of the second crop. The numbers of *A. linicola* conidia collected daily with the spore sampler were counted according to the method described in section 2.2.2. The mean daily concentration of the conidia was estimated by using the equation 2.1 (see section 2.2.2). The hourly changes in the concentration of *A. linicola* conidia in the air above the linseed crop were monitored only in 1992 by selecting six days during which the greatest numbers of conidia were collected by the Burkard spore sampler. The hourly dispersal of *A. linicola* conidia each day was calculated by using the equation 2.3 (see section 2.2.2). The hourly concentration of *A. linicola* conidia above a linseed crop was calculated as the average number of conidia collected hourly

**Figure 7.1.** Burkard spore sampler used to monitor the concentrations of air-borne *A. linicola* conidia in linseed crops in 1991, 1992 and 1993.

over these six days.

Records of daily rainfall, mean temperature and hourly wind speed were obtained from a meteorological station situated 0.5 - 1 km from the experimental site.

**7.3.2. Inoculum potential of *A. linicola***

In 1992 and 1993, to investigate if infection of linseed crops by *A. linicola* could have taken place early in the growing season by means of air-borne or splash-dispersed conidia, the method of exposing young linseed seedlings (bait plants) within the crop at weekly intervals was used. Linseed seed [cv. Antares, treated with prochloraz (4 g $kg^{-1}$ seed, Prelude 20LF, Agrichem)] free of *A. linicola* infection, was sown in six plastic trays (35 x 26 x 7 cm) each time. The trays contained a mixture of soil-less compost with a slow release fertilizer [Croxden compost produced by Nursery trades (Lea valley) Ltd]. The trays were placed in a heated glasshouse (temperature range 15 - 25$^{0}$C) with additional light (7 h) provided by two high pressure sodium plant irradiators with integral control (Thermoforce Ltd, Camplex Plantcare Division,Tetbury Glos., UK). Ten days after sowing, when the seedlings had one pair of true leaves (GS 2, Fig. 1.2), four of the trays were transferred to the field (Fig. 9.1, Field A). Two of the trays (replicates) were placed at ground level and the other two (replicates) at approximately 1 m above ground within the linseed crop (Fig. 7.2). The remaining two trays (controls) were transferred to an unheated glasshouse (temperature range 10 - 20$^{0}$C). After one week of exposure, all the trays with the bait plants were brought into the unheated glasshouse and were covered with polyethylene bags (100% r.h.) (including the trays used as controls) for 5 days.

**Figure 7.2.** Bait plants used to study the inoculum potential of *A. linicola* early in the growing season in a linseed crop in 1992 and 1993; (a) bait plants placed at ground level and (b) bait plants placed 1 m above ground level.

Each time 100 seedlings per tray were sampled at random and assessed for the presence of disease symptoms. The plant tissues (cotyledons or leaves) showing symptoms were incubated by the method described in section 2.2.1 and assessed under a stereo-microscope (x 40 magnification) for the presence of *A. linicola* conidia. The disease incidence (% plants infected) on the bait plants each week was compared with the number of air-borne *A. linicola* conidia collected by the Burkard spore sampler that same week. Bait plants were exposed to the linseed crops continuously from April 1992 until November 1993.

### 7.3.3. Disease gradients from a point inoculum source - 1992 field experiment

7.3.3.1. Husbandry and site details

In order to study the *A. linicola* disease gradients from a point inoculum source, a field experiment was sown on the Rothamsted farm (Garden Plots). The site was located approximately 2 km south of the nearest linseed crop and the previous crop was lupins. The experimental area was approximately 0.01 ha and the linseed seed (cv. Antares, untreated) was sown on 14 April at a rate of 600 seeds $m^{-2}$. Before sowing the seed was tested *in vitro* by the method described in section 6.3.1 and was found to be free of *A. linicola* infection. Nitram (34.5% nitrogen, ICI Agrochemicals Ltd) was applied on 14 April at 220 kg $ha^{-1}$. The herbicides bentazone (960 g a.i. $ha^{-1}$, Basagran, BASF) and bromoxynil + clopyralid (240 : 50 g a.i. $ha^{-1}$, Vindex, DowElanco) were applied on 2 June. The crop was irrigated by using an overhead oscillating system on 17, 18, 25 and 29 June, 9 and 28 July with 12 mm of water on each occasion.

7.3.3.2. Emergence

The emergence of the plants was assessed 31, 38 and 45 days after sowing. Each time 10 assessments were made at random on row-lengths selected throughout the field and the number of plants which had emerged in a 0.5 m row-length and the number of plants with brown lesions on their cotyledons were counted. The cotyledons with symptoms were incubated by the method described in section 2.2.1 and assessed under a stereo-microscope (x 40 magnification) for the presence of *A. linicola* conidia.

7.3.3.3. Experimental design - Inoculum source - Sampling method

The experimental area was only one plot, as shown in Figure 7.3. The inoculum source consisted of naturally infected linseed stem debris placed in 0.30 x 1 m nylon net bags. On 8 July four diagonal paths (0.5 x 20 m each) were cut to facilitate sampling (Fig. 7.3) and a sample of 100 plants was collected at random throughout the field in order to assess the incidence of *A. linicola* infection in the crop before the introduction of the artificial inoculum (background infection). On 9 July, approximately 24 days after full flowering, six bags with debris were placed at ground level in the central area (1 x 2 m) of the plot. In order to minimize the dispersal of *A. linicola* conidia from the infected debris during transport through the linseed crop, the net bags with the debris were enclosed in polyethylene bags until they were placed at the central area of the field. After the introduction of the inoculum into the crop no farm machinery entered the field. Samples of plants were collected from all four directions, along the paths at 0, 0.1, 0.3, 0.6, 1, 1.5, 2, 3, 5, 10 and 20 m distance from the inoculum source (central area) during the growing season. At each sampling point two plants were collected (one plant from each side of the path). Samples were taken by proceeding from the least (edge of the field) to the

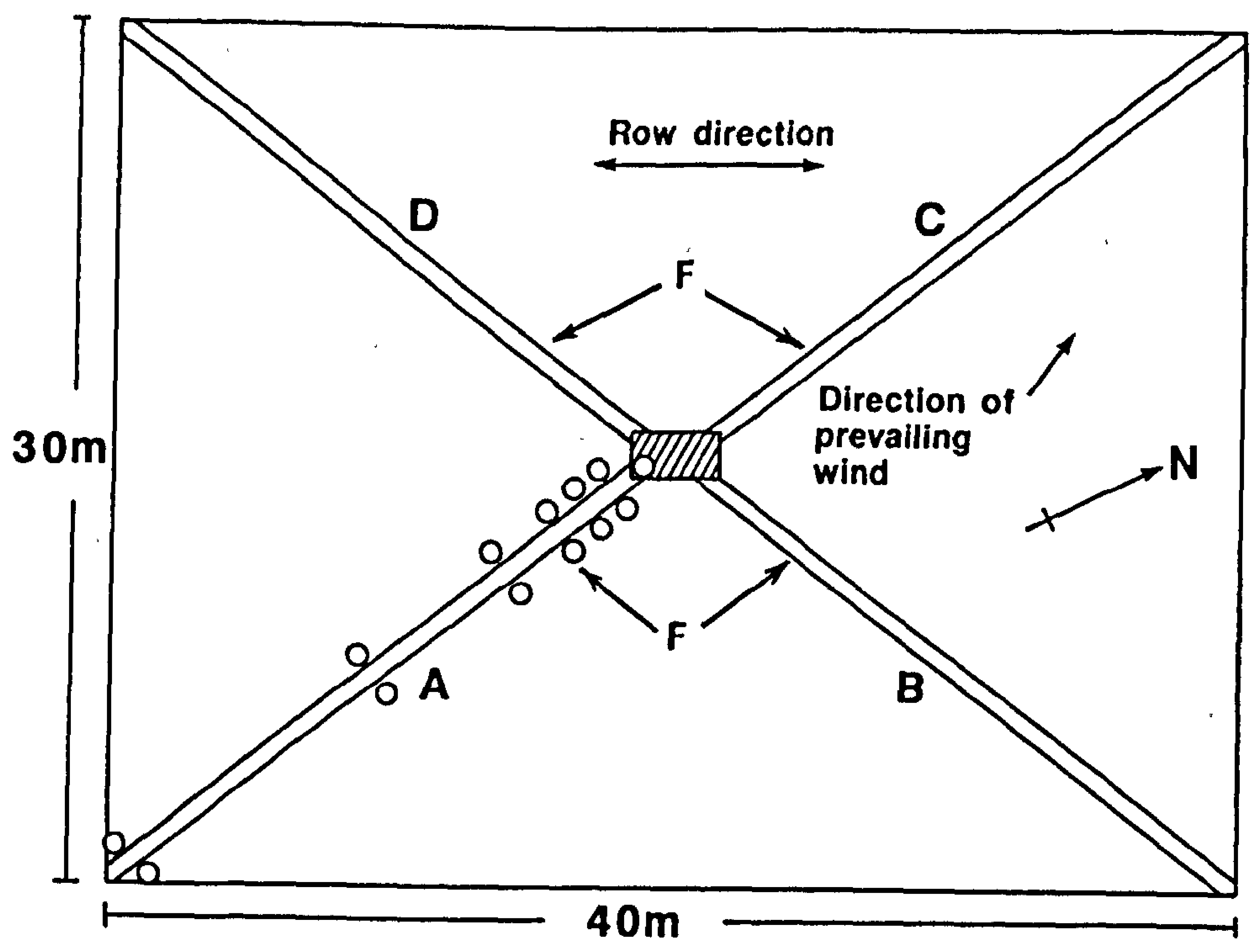



**Figure 7.3.** Plan of the 1992 field experiment for studying the *A. linicola* disease gradients from a point inoculum source. A, B, C, D : sampling directions; F : footpaths (each 0.5 x 20 m); ▨ : inoculum source (1 x 2 m); o : sampling points in one direction.

most severely infected area (central area) and care was taken to avoid brushing against the plants in order to minimize the spread of the inoculum by physical contact. A total of four samples were collected at approximately two-weekly intervals until harvest, starting one week (16 July, GS 9) after the introduction of the inoculum. Growth stages were identified using a key (Turner, 1987) (Fig. 1.2) and the dates on which the samples were taken and the growth stages of the plants are described in Table 7.1. Records of wind direction were obtained from a meteorological station situated 0.5 - 1 km from the experimental site.

7.3.3.4. Disease identification - assessments

It was difficult to distinguish visually between the symptoms caused by *A. linicola* and those caused by *B. cinerea,* natural senescence or by *Septoria linicola* on cotyledons, leaves, stems, buds and sepals of linseed plants. For this reason the method described in section 2.2.1 was used for the identification of *A. linicola* infection. The disease gradients were expressed as the percent of plants infected at increasing distances from the inoculum source, calculated as averages over all four directions.

7.3.3.5. Statistical analyses

Two different empirical models are often used to describe disease or spore dispersal gradients with distance from an inoculum source (Fitt *et al.*, 1987) : a) the inverse power law model (Gregory, 1968) and b) the negative exponential model (Kiyosawa & Shiyomi, 1972). According to the inverse power law model, the amount of disease or the number of conidia (y) is inversely proportional to some power of distance (x) from the inoculum source:

$$y = a\,x^{-b} \qquad (7.1)$$

**Table 7.1.** Growth stages of linseed (cv. Antares) at which samples were taken to study the *A. linicola* disease gradients from a point inoculum source in 1992.

| Date of sampling | Growth stage (GS)[1] |
|---|---|
| 16 July | 9 |
| 13 August | 10-11 |
| 27 August | 11 |
| 12 September | 12 |

[1] Turner, 1987 (Fig. 1.2).

According to the negative exponential model, the amount of disease or the number of conidia (y) is inversely proportional to an exponential function of the distance (x) from the inoculum source :

$$y = c \exp(-dx) \qquad (7.2)$$

In these models, y = amount of disease or number of conidia, x = the distance (m) from the inoculum source, a (equation 7.1) is a constant equal to the value of y at x = 1 m, c (equation 7.2) is equal to the value of y at x = 0 m and the exponents b (equation 7.1) and d (equation 7.2) are measures of the gradients. For more precise comparison of the disease or spore dispersal gradients, equations 7.1 & 7.2 can be made linear by taking natural logarithms of both sides. Therefore, equation 7.1 becomes :

$$\ln(y) = \ln(a) - b \ln(x) \qquad (7.3)$$

and equation 7.2 becomes :

$$\ln(y) = \ln(c) - dx \qquad (7.4)$$

In the equations 7.3 & 7.4 the exponents b and d are the slopes of the linear regressions and they describe the steepness of the gradients. The parameters a, b, c and d in equations 7.3 & 7.4 can be estimated by plotting the linear regressions of ln (y) on ln (x) and of ln (y) on x, respectively. To test the goodness of fit of each model to data for the *A. linicola* disease or spore dispersal gradients, the percentage variance accounted for ($r^2$) was calculated for each linear regression.

### 7.3.4. Disease gradients from a line inoculum source - 1993 field experiment

#### 7.3.4.1. Husbandry and site details

In 1993, to study the *A. linicola* disease and spore dispersal gradients from a line

inoculum source, a field experiment was sown on the Rothamsted farm (Long Hoos, Fields A & C, Fig. 9.1). The previous crops had been linseed for the last two years in field A and oilseed rape in 1991 and potatoes in 1992 in field C. The total experimental area was approximately 0.48 ha (Field A = 0.16 ha & Field C = 0.32 ha) (Fig. 9.1) and the linseed seed (cv. Antares, untreated) was sown on 21 April at a rate of 600 seeds $m^{-2}$. Before sowing, the seed was tested *in vitro* by the method described in section 6.3.1 and was found to be free of *A. linicola* infection. Nitram (34.5% nitrogen, ICI Agrochemicals Ltd) was applied on 12 May at 217 kg $ha^{-1}$. The herbicide metsulfuron-methyl (6 g a.i. $ha^{-1}$, Ally, Du Pont) was applied on 1 June to control the volunteer potato plants. The crop was irrigated by using an overhead oscillating system on 2 and 6 July with 12.5 mm water each time and it was desiccated on 6 September with diquat (120 g a.i. $ha^{-1}$, Stefes Diquat, Stefes). "Vassgro Spreader" (300 ml $ha^{-1}$, Vass) was added to the desiccant as a wetting agent. The crop was combine harvested on 10 October.

7.3.4.2. Emergence

The emergence of the plants was assessed 29, 36 and 43 days after sowing. Ten assessments were made on row-lengths selected throughout each field (Fields A & C) and the number of plants which had emerged in a 0.5 m row-length and the number of plants with symptoms on their cotyledons were counted. The cotyledons with symptoms (lesions or necrotic areas) were incubated by the method described in section 2.2.1 and assessed under a stereo-microscope (x 40 magnification) for the presence of *A. linicola* conidia.

7.3.4.3. Experimental design - Inoculum source - Sampling method

The experimental area was two plots (Field A & Field C) (Fig. 7.4). The inoculum

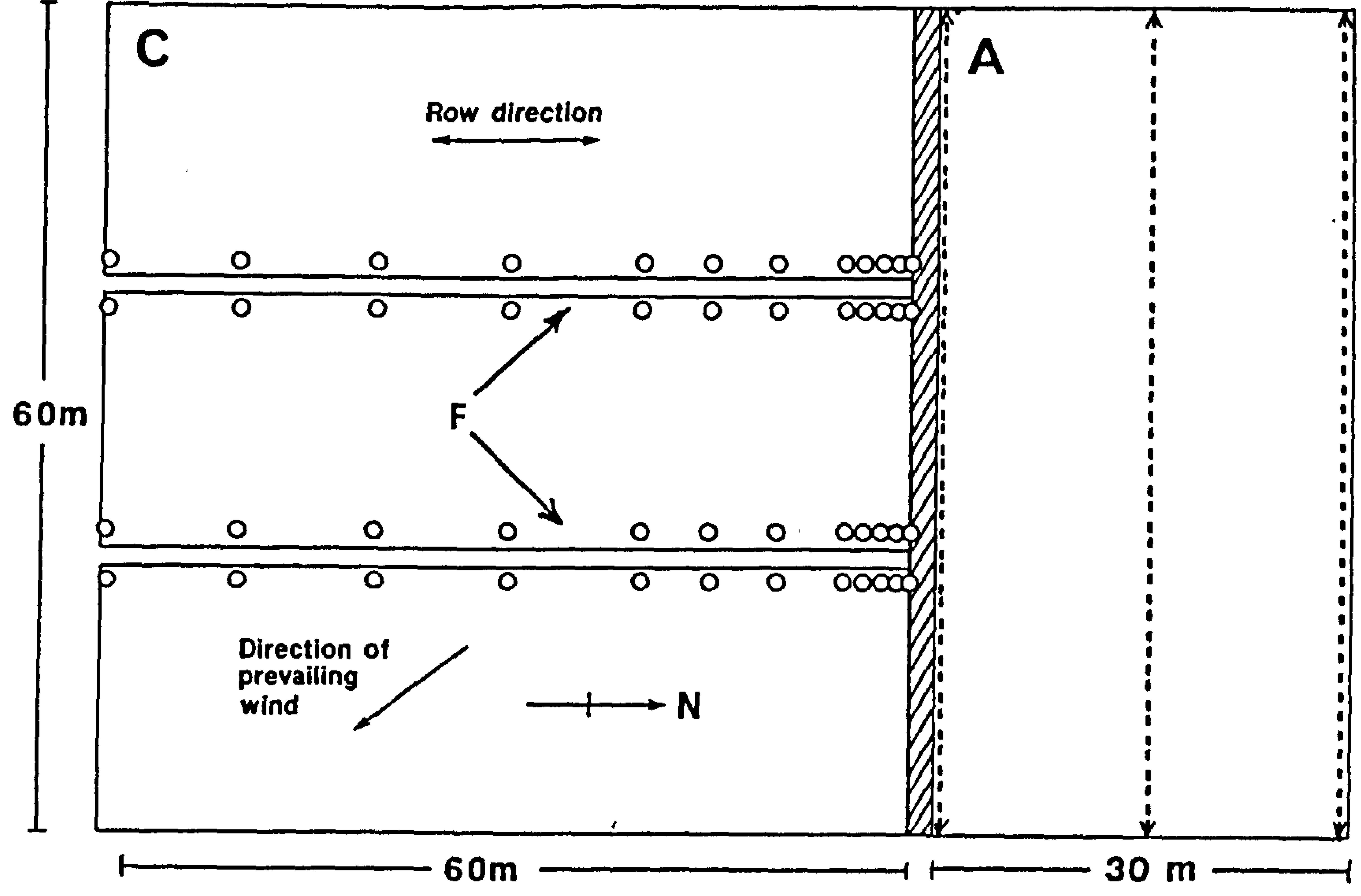



**Figure 7.4.** Plan of the 1993 field experiment for studying the *A. linicola* disease gradients of downwind (field C) and upwind (field A) from a line inoculum source. F : footpaths (each 0.5 x 60 m); ▨ : inoculum source (0.5 x 60 m); o : sampling points in field C; (←----→) : sampling directions in field A.

source consisted of linseed stem debris naturally infected by *A. linicola* placed in a nylon net bag (0.5 x 60 m). On 6 June two paths (0.5 x 60 m each) were cut along the field in order to facilitate sampling (Fig. 7.4) and two samples, of 100 plants each, were collected at random throughout the two fields (one sample per field) to assess the incidence of *A. linicola* in the crop before the introduction of the artificial inoculum (background infection). On 7 June (GS 6), approximately two weeks before the start of flowering, the bag with the debris was placed at ground level between field A and field C (Fig. 7.5) and held in place by wooden poles. To minimize the dispersal of *A. linicola* conidia from the infected debris during transport through the linseed crop, the net bag with the debris was enclosed in a polyethylene sheet until it was placed at the final position between field A and field C. After the introduction of the inoculum into the crop, no farm machinery entered the field.

In field A samples of plants (20 plants per distance and sample) were collected across the field at 0, 15 and 30 m from the inoculum source. In field C samples of 10 plants each were collected from both sides of the paths (five plants from each side) and at different distances (0, 1, 2, 3, 5, 10, 15, 20, 30, 40, 50 and 60 m) from the inoculum source. In field C, samples were taken by proceeding from the least (60 m from the source) to the most severely infected area (next to the inoculum source) and care was taken to avoid brushing against the plants to minimize the spread of inoculum by physical contact. A total of seven samples were collected from each field during the growing season at approximately two-weekly intervals. Growth stages were identified by using a key (Turner, 1987) (Fig. 1.2) and the dates on which the samples were taken and the growth stages of the plants are described in Table 7.2. Records of wind direction were obtained from a meteorological station situated

**Figure 7.5.** Line inoculum source : linseed stem debris naturally infected by *A. linicola* enclosed in a net bag (0.5 x 60 m) and placed between field A and field C (Fig. 7.4) for studying the *A. linicola* disease gradients downwind and upwind from a line inoculum source in 1993.

**Table 7.2.** Growth stages of linseed (cv. Antares) at which samples were taken to study the *A. linicola* disease gradients from a line inoculum source in 1993.

| Date of sampling | Growth stage (GS)[1] |
|---|---|
| 24 June | 6 |
| 8 July | 6-7 |
| 22 July | 7-8 |
| 5 August | 9 |
| 19 August | 10 |
| 2 September | 12 |
| 17 September | 12 |

[1] Turner, 1987 (Fig.1.2).

0.5 - 1 km from the experimental site.

7.3.4.4. Disease identification - assessments

For the identification of *A. linicola* symptoms on the linseed plants, the method described in section 2.2.1 was used. The disease gradients in field C (downwind from the source) were expressed as the average percent of plants infected along the two paths at increasing distances from the inoculum source and in field A (upwind from the source) as the percent of plants infected at 0, 15 and 30 m from the source.

7.3.4.5. Statistical analyses

See section 7.3.3.5.

### 7.3.5. Spore dispersal gradients

The spore dispersal gradients were studied in field C (downwind from the inoculum source) by using five rotorod - type samplers (Perkins, 1957) (Fig. 7.6). The samplers were operated on the first dry day after a period of rain from 10:00 h to 16:00 h. This period of the day was chosen because the greatest numbers of air-borne *A. linicola* conidia were collected by the Burkard spore sampler during this period. The horizontal spore dispersal gradients were studied by placing the samplers 30 cm above ground and at different distances from the inoculum source. For studying the vertical dispersal of *A. linicola* conidia, the samplers were placed in the middle of the field (approximately 30 m from the inoculum source); one of them was located at ground level (height = 0 cm) and the others at 35, 70, 100 and 150 cm above ground level (mean height of the crop 70 cm, GS 12; Fig. 1.2).

The samplings for studying the horizontal spore dispersal gradients began

**Figure 7.6.** Rotorod sampler used to study the *A. linicola* spore dispersal gradients downwind from a line inoculum source in 1993. The samplers were operated during dry days from 10:00 h until 16:00 h at 30 cm above ground and at different distances from the source. The same samplers were used for studying the vertical dispersal of *A. linicola* conidia within and above a linseed crop in 1993.

approximately 2 weeks after the introduction of the inoculum into the field. A total of six samplings was made throughout the growing season. The samplings for studying the vertical dispersal of *A. linicola* conidia started when the plants were at growth stage 12 (Fig. 1.2) and the disease had already spread in the crop. A total of six samplings was made until harvest. The numbers of *A. linicola* conidia collected were counted under a light microscope (x 250 magnification) and the hourly concentration of the conidia was estimated by using the equation 2.4 (see section 2.2.2).

7.3.5.1. Statistical analyses

The data on the horizontal *A. linicola* spore dispersal gradients were analyzed by the method described in section 7.3.3.5. For analyzing the data on the vertical dispersal of *A. linicola* conidia, the linear regression used was :

$$y = a - bx \qquad (7.5)$$

in which y = the number of conidia collected at different heights x within or above the linseed crop, a is the intercept on the y - axis and b is the slope of the line.

## 7.4. Results

### 7.4.1. Seasonal and diurnal dispersal of *A. linicola* conidia

There was considerable seasonal variation in numbers of *A. linicola* conidia collected above linseed crops in 1991, 1992 and 1993. Generally, conidia of *A. linicola* were collected in large numbers on dry days following rainy days, with the numbers collected gradually decreasing during extended dry periods.

In 1991, the concentrations of air-borne conidia of *A. linicola* above a linseed crop remained low throughout the collecting period (Fig. 7.7). Few conidia (mean

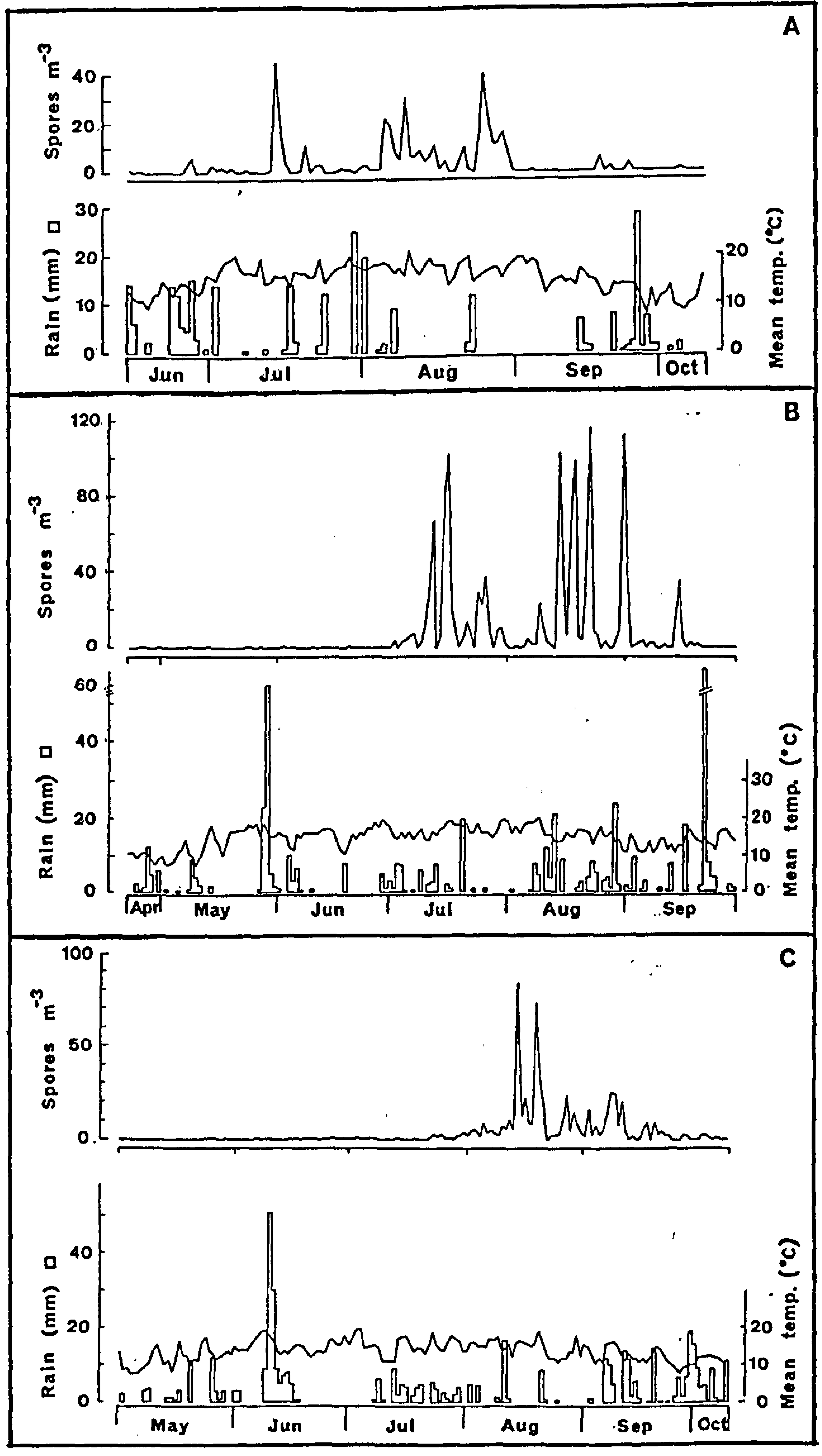



**Figure 7.7.** Mean daily concentrations of air-borne *A. linicola* conidia above a linseed crop in relation to rainfall and mean temperature in 1991 (A), 1992 (B) and 1993 (C).

daily concentration 1-7 conidia $m^{-3}$) were collected during June and at the beginning of July. June was cold (mean temperature 11.1°C) and very wet (total rainfall 100 mm). On 14 July there was a maximum concentration of 46 conidia $m^{-3}$, which decreased rapidly over the next few days. Conidia of *A. linicola* were consistently collected during August with the highest concentrations being observed on 5, 9, 25 and 26 August (22, 31, 40 and 20 conidia $m^{-3}$, respectively) (Fig. 7.7). July and August were generally hot (mean temperatures 16.9°C and 17.4°C, respectively) and dry (total rainfall 73.4 mm and 45.5 mm, respectively) (Fig. 7.7). No *A. linicola* conidia were collected during the first half of September, when the total rainfall was only 9.9 mm. However, a few conidia (mean daily concentration 1-5 conidia $m^{-3}$) were collected during the second half of September, when more rain fell (total rainfall 51.5 mm) (Fig. 7.7). Although the crop was not harvested until 10 October, no *A. linicola* conidia were collected by the Burkard spore sampler during the period between the end of September and 10 October. This period was generally dry and cold with a total rainfall of 3.3 mm and a mean temperature of 11.3°C.

In 1992, the seasonal dispersal of *A. linicola* conidia followed a pattern similar to that in 1991. However, the number of conidia collected during the growing season was greater in 1992 than in 1991 (maximum daily concentrations 46 and 117 conidia $m^{-3}$ in 1991 and 1992, respectively) (Fig. 7.7). Although sampling started earlier in 1992 (21 April) than in 1991 (14 June), few *A. linicola* conidia were collected during May and June 1992 (mean daily concentration 1 conidium $m^{-3}$) (Fig. 7.7). Most of the days in May were dry and although a total rainfall of 103 mm was recorded during this month, most of this rain fell in the last three days (total rainfall from 28 May to 31 May 85.2 mm). June was generally dry (total rainfall 37.5 mm) but

the crop was irrigated three times during this period with a total of 12 mm of water each time. Air-borne *A. linicola* conidia were consistently collected during July and August, with the highest concentrations being observed on 12, 14 and 15 July, 14, 17, 18 and 31 August (67, 81, 102, 113, 98, 117 and 113 conidia $m^{-3}$, respectively) (Fig. 7.7). Both July and August were wet with a total rainfall of 62.2 mm and 114.2 mm, respectively. Although the crop was not harvested until 17 September and 42.9 mm of rain fell during the first two weeks of September, few conidia were collected during this period, with one maximum of 36 conidia $m^{-3}$ on 15 September (Fig.7.7). No *A. linicola* conidia were collected between 22 September 1992 and 24 March 1993.

Although the crop was sown in 1993 on the same date as in 1992, the difference in the weather conditions between these two years during the growing season affected the number of air-borne *A. linicola* conidia collected. Generally, fewer conidia were collected by the Burkard spore sampler in 1993 than in 1992 (Fig. 7.7). Few conidia (mean daily concentration 1-4 conidia $m^{-3}$) were collected during the period between the beginning of May and the end of July 1993. May was drier in 1993 (total rainfall 44.7 mm) than in 1992 (total rainfall 103 mm), but more rain fell during June 1993 (total rainfall 131 mm) than during June 1992 (total rainfall 35.7 mm) (Fig. 7.7). July was generally dry in both years with a total rainfall of 62.2 mm and 58.9 mm in 1992 and 1993, respectively.

*A. linicola* conidia were consistently collected during August and September 1993, with the highest concentrations being observed on 14, 16, 19, 20 and 27 August, 9 and 10 September (84, 23, 74, 34, 24, 25 and 25 conidia $m^{-3}$, respectively) (Fig. 7.7). The concentration of air-borne *A. linicola* conidia decreased rapidly during

the period between mid-September and 10 October when the crop was harvested and no *A. linicola* conidia were collected after that date. August was drier in 1993 (total rainfall 39.3 mm) than in 1992 (total rainfall 114.2 mm), but September was wet in both years (total rainfall 127.5 mm and 114.3 mm in 1992 and 1993, respectively) (Fig. 7.7).

The hourly concentrations of air-borne *A. linicola* conidia collected by the Burkard spore sampler on 6 dry days in 1992 (12, 15 and 24 July, 14, 18 and 23 August) showed a well defined diurnal periodicity (Fig. 7.8). No *A. linicola* conidia were collected between 21:00 h and 07:00 h and few were collected between 07:00 h and 08:00 h, respectively (Fig. 7.8). However, a sharp increase in the number of air-borne conidia was observed between 08:00 h and 12:00 h (from 35 to 514 conidia $m^{-3}$ $h^{-1}$, respectively) as the wind speed increased (Fig. 7.8). The maximum concentration occurred around noon (12:00 h - 13:00 h), after which the concentration of air-borne conidia decreased. The highest concentration of air-borne *A. linicola* conidia occurred approximately 3 h before the maximum wind speed (3.6 m $sec^{-1}$) (Fig. 7.8).

### 7.4.2. Inoculum potential of *A. linicola*

In both 1992 and 1993, it was possible to detect air-borne *A. linicola* conidia above linseed crops much earlier in the growing season by using bait plants than by using the Burkard spore sampler (Fig. 7.9 & Fig 7.10). In both years, conidia of *A. linicola* were present in the field early in the growing season (mid-April 1992 and early April 1993) before the emergence of the crop (Fig. 7.9 & Fig. 7.10). As a result, in both years the incidence of *A. linicola* infection on the bait plants placed at ground level

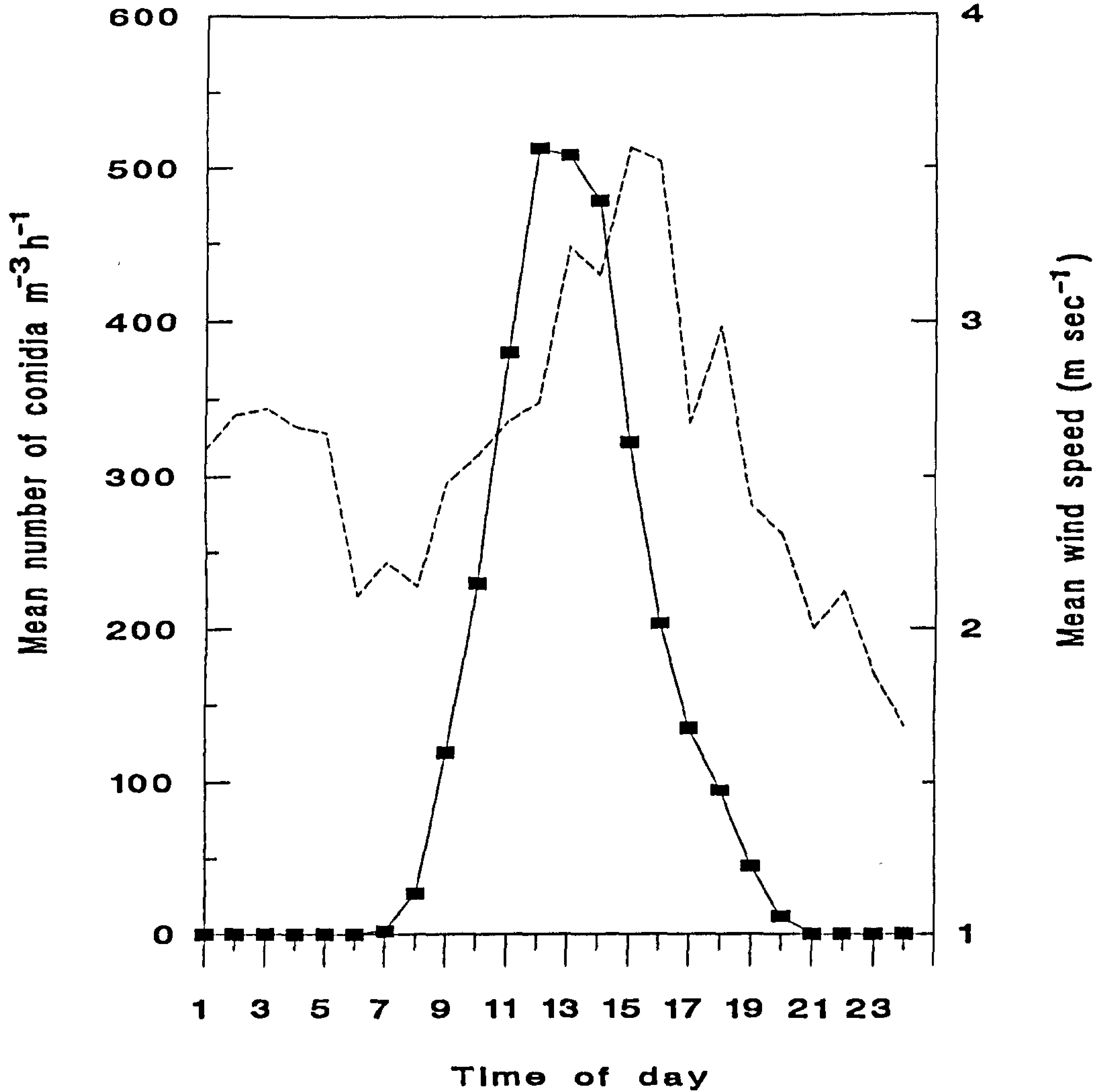



**Figure 7.8.** Hourly concentrations of air-borne *A. linicola* conidia ( ■ , average of 6 days) recorded by the Burkard spore sampler and hourly wind speed (----- ,average of 6 days) above a linseed crop on 12 June, 15 June, 24 June, 14 August, 18 August and 22 August 1992.

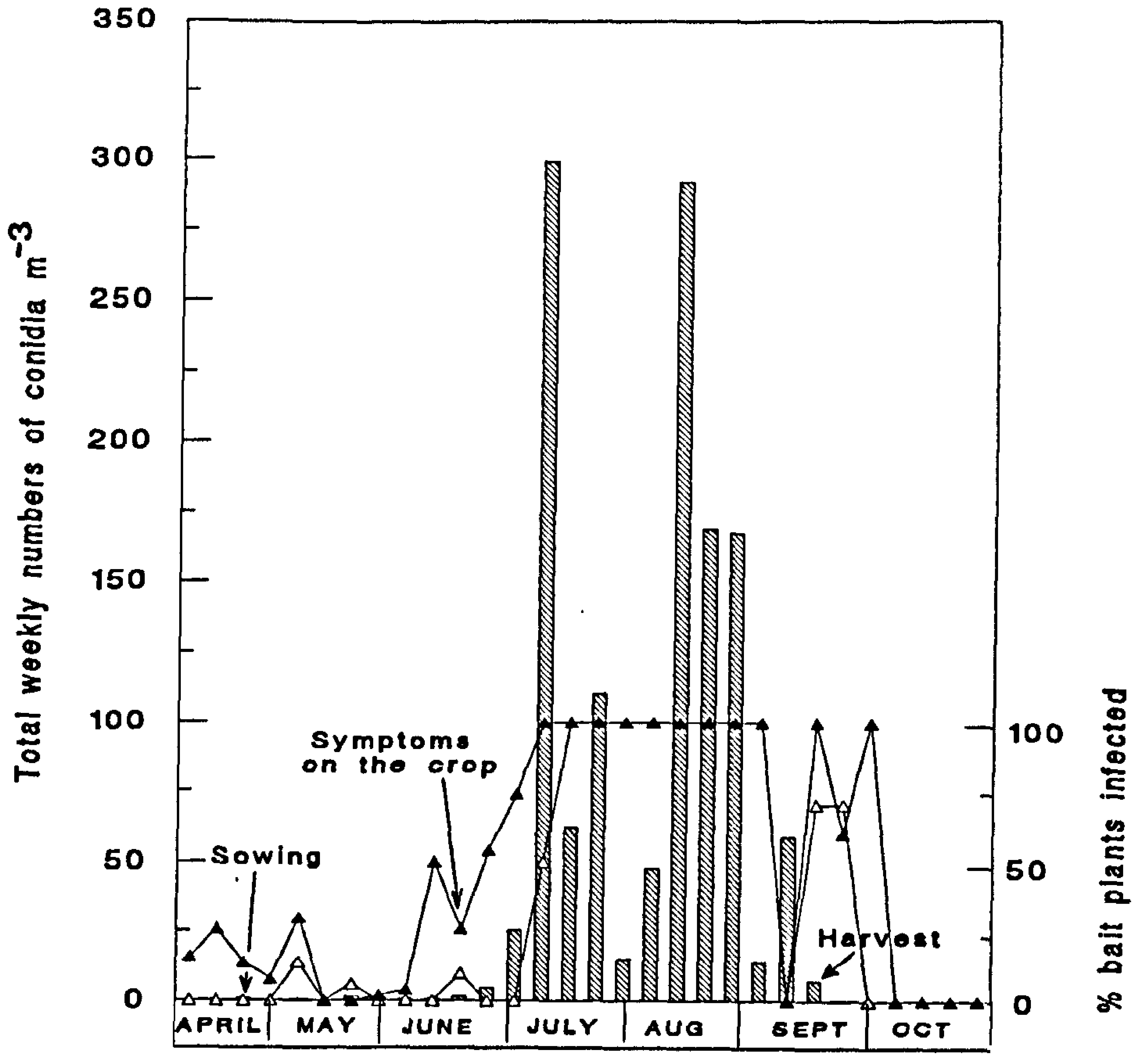



**Figure 7.9.** Total weekly numbers of air-borne *A. linicola* conidia (▧) collected by the Burkard spore sampler above a linseed crop and disease incidence (%) on bait plants exposed in the same crop either at ground level (▲) or 1 m above ground (△) during the period April - October 1992.

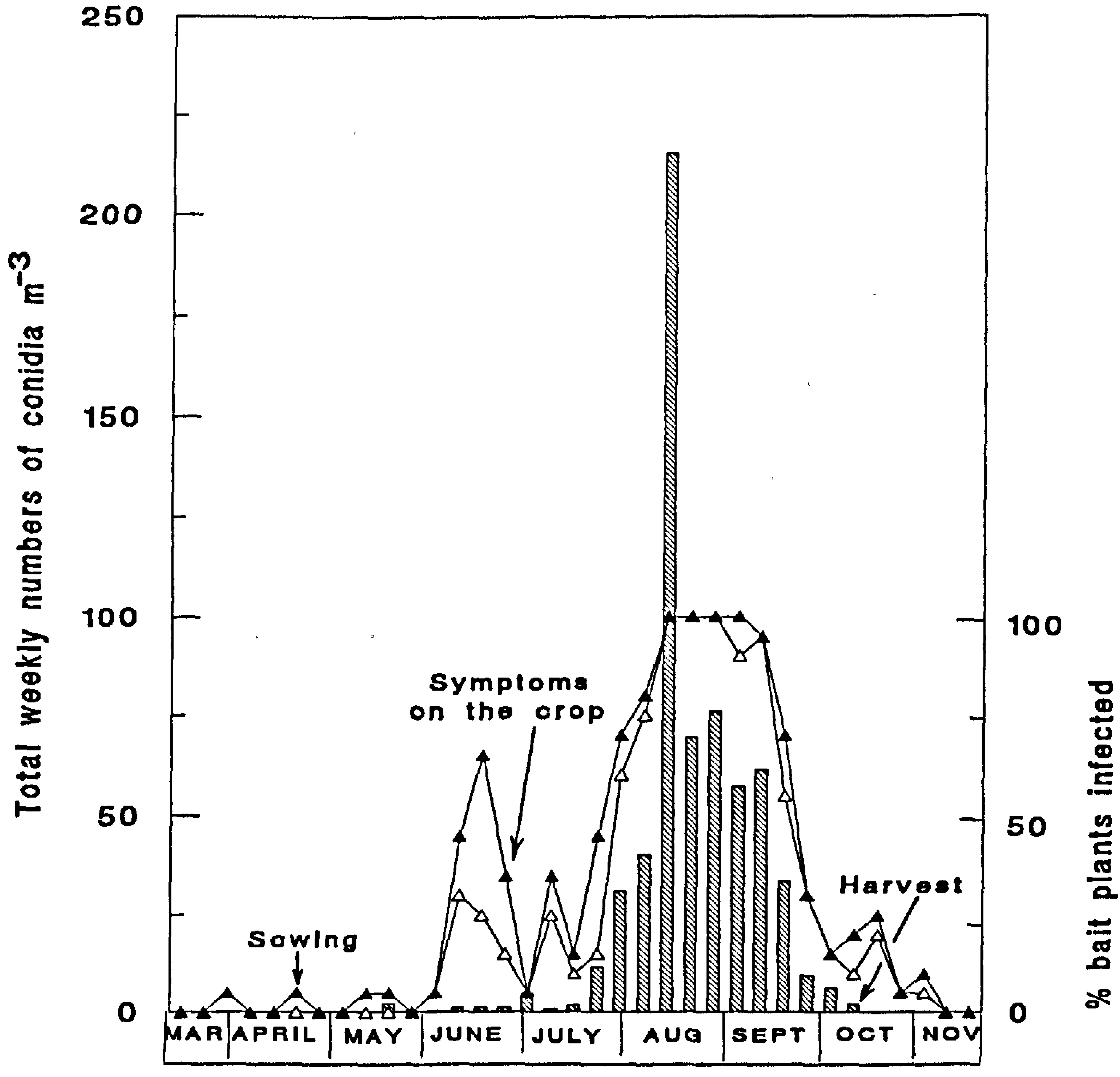



**Figure 7.10.** Total weekly numbers of air-borne *A. linicola* conidia ( ▧ ) collected by the Burkard spore sampler above a linseed crop and disease incidence (%) on bait plants exposed in the same crop either at ground level ( ▲ ) or 1 m above ground ( △ ) during the period 15 March to 15 November 1993.

was greater than on those placed 1 m above ground. This may have been because a slightly greater number of conidia was deposited on the bait plants placed at ground level than on those placed 1 m above ground, at least at the beginning of the growing season (April-July 1992 and March-July 1993) (Fig. 7.9 & Fig. 7.10). The highest incidences (100%) of *A. linicola* infection on the bait plants (either on those placed at ground level or on those placed 1 m above ground) were usually correlated with periods when the greatest numbers of air-borne conidia were collected by the Burkard spore sampler (between July and early September in 1992 and between August and early October in 1993) (Fig. 7.9 & Fig. 7.10).

In both years, no *A. linicola* conidia were collected by the Burkard spore sampler after harvest of the crop (harvest dates 17 September and 10 October in 1992 and 1993, respectively) (Fig. 7.9 & Fig. 7.10). However, conidia produced on the infected linseed stem debris left on the ground after harvest, were deposited on the bait plants for two or three weeks after harvest in 1992 and 1993, respectively (Fig. 7.9 & Fig. 7.10). Although bait plants were exposed in the field during the period between October 1992 and March 1993 and the Burkard spore sampler was operated continuously during the same period, no *A. linicola* conidia were either deposited on the bait plants or collected by the sampler. Data collected during this period (October 1992 - March 1993) has not been plotted. No *A. linicola* infection was detected during the experimental period April 1992 to November 1993 on the bait plants not exposed in the linseed crops (controls).

### 7.4.3. Disease gradients from a point inoculum source - 1992 field experiment

7.4.3.1. Emergence

In 1992, the maximum emergence was 60% by 29 May (45 days after sowing), with 100% of the emerged seedlings showing flea beetle damage on their cotyledons (Fig. 7.11). No *A. linicola* infection was detected on the seedlings during the emergence counts.

7.4.3.2. Disease gradients

In 1992, when the *A. linicola* disease gradients from a point inoculum source were studied, the percent of plants infected decreased with increasing distance from the source. The inverse power law model (Gregory, 1968) fitted better than did the negative exponential model (Kiyosawa & Shiyomi, 1972) for all four sets of data. On average, the linear regressions of ln (y) on ln (x) (inverse power law model) accounted for 78% of the variance and the regressions of ln (y) on x (negative exponential model) accounted for 46% of the variance (Table 7.3). Although the direction of the local wind varied constantly during the experimental period (9 July -12 September), there were 27 days when the direction of the wind was SW. The disease was first detected downwind on 16 July, one week after the introduction of the inoculum into the linseed crop (Fig. 7.12). Relatively steep disease gradients were observed on 16 July ($b = -0.29$) and on 13 August ($b = -0.26$) up to 1.5 m and 2 m from the source, respectively (Fig. 7.12). On 16 July, there was a 90% decrease in the disease incidence on the plants between the inoculum source ($x = 0$ m) and 1.5 m from the source.

Approximately one month after the introduction of the inoculum (13

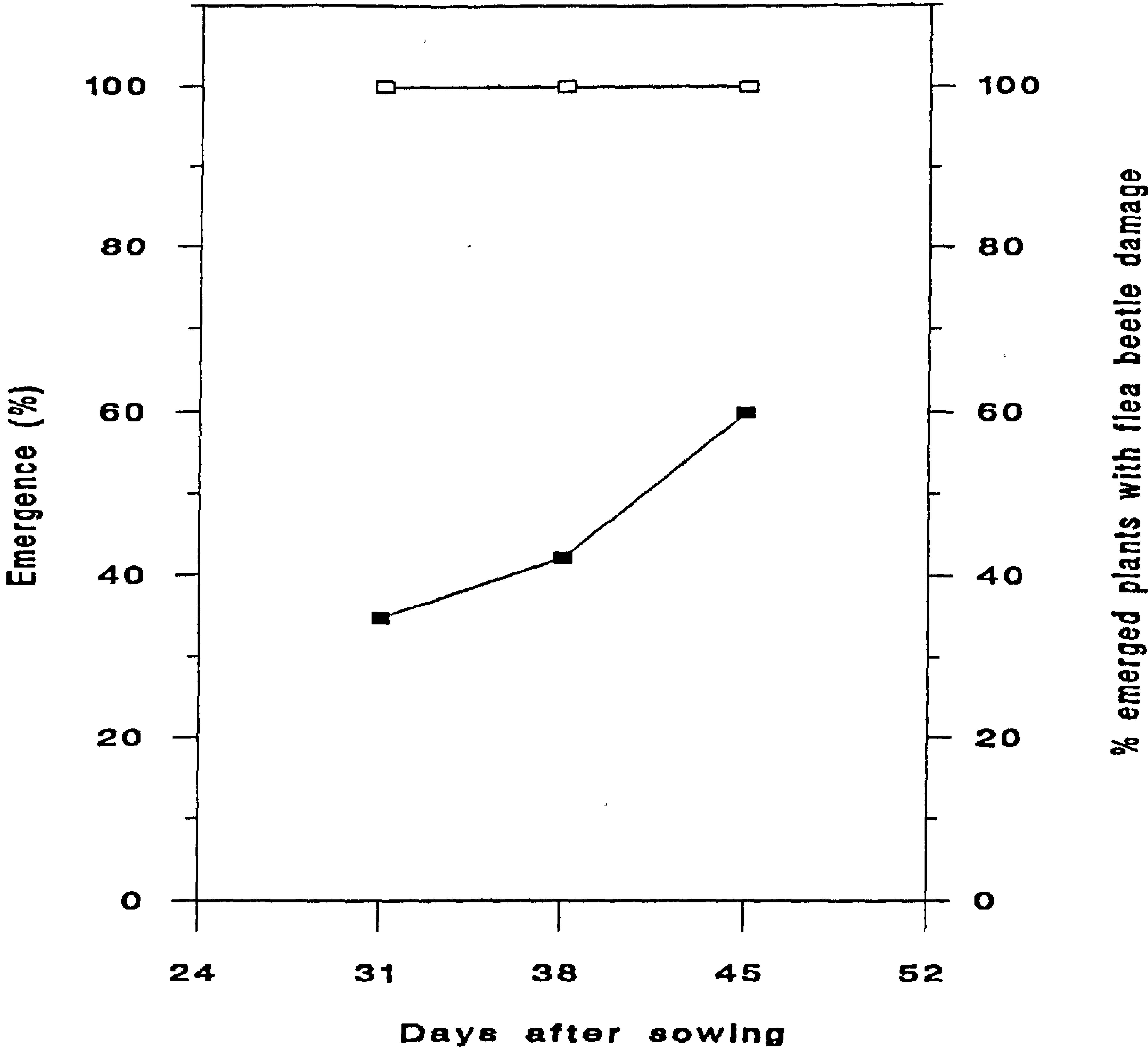



Figure 7.11. Emergence (%) of linseed (■) and percentage of emerged plants with flea beetle damage on their cotyledons (□) in field (Garden Plots) sown with untreated seed (cv. Antares) on 14 April and used for studying the *A. linicola* disease gradients from a point inoculum source in 1992.

**Table 7.3.** Parameters estimated for the inverse power law model [ln (y)* = ln (a) - b ln (x)‡] and the negative exponential model [ln (y) = ln (c) - dx] used to describe the *A. linicola* disease gradients from a point inoculum source in 1992.

| Sampling date | Inverse power law model | | | Negative exponential model | | |
|---|---|---|---|---|---|---|
| | ln (a)† | b¶ | $r^2$††† | ln (c)†† | d¶ | $r^2$††† |
| 16 July | 2.13 | -0.29 | 82.9 | 3.28 | -1.11 | 57.4 |
| 13 August | 2.86 | -0.26 | 85.1 | 3.80 | -0.74 | 55.3 |
| 27 August | 4.08 | -0.16 | 82.7 | 4.24 | -0.06 | 61.4 |
| 12 September | 4.20 | -0.06 | 60.0 | 4.26 | -0.01 | 10.1 |

* y : disease incidence (%).
‡ x : distance from the inoculum source (m).
† a : constant equal to the value of y at x = 1 m.
¶ b & d : slopes of the linear regressions.
†† c : constant equal to the value of y at x = 0 m.
††† $r^2$ : % variance accounted for.

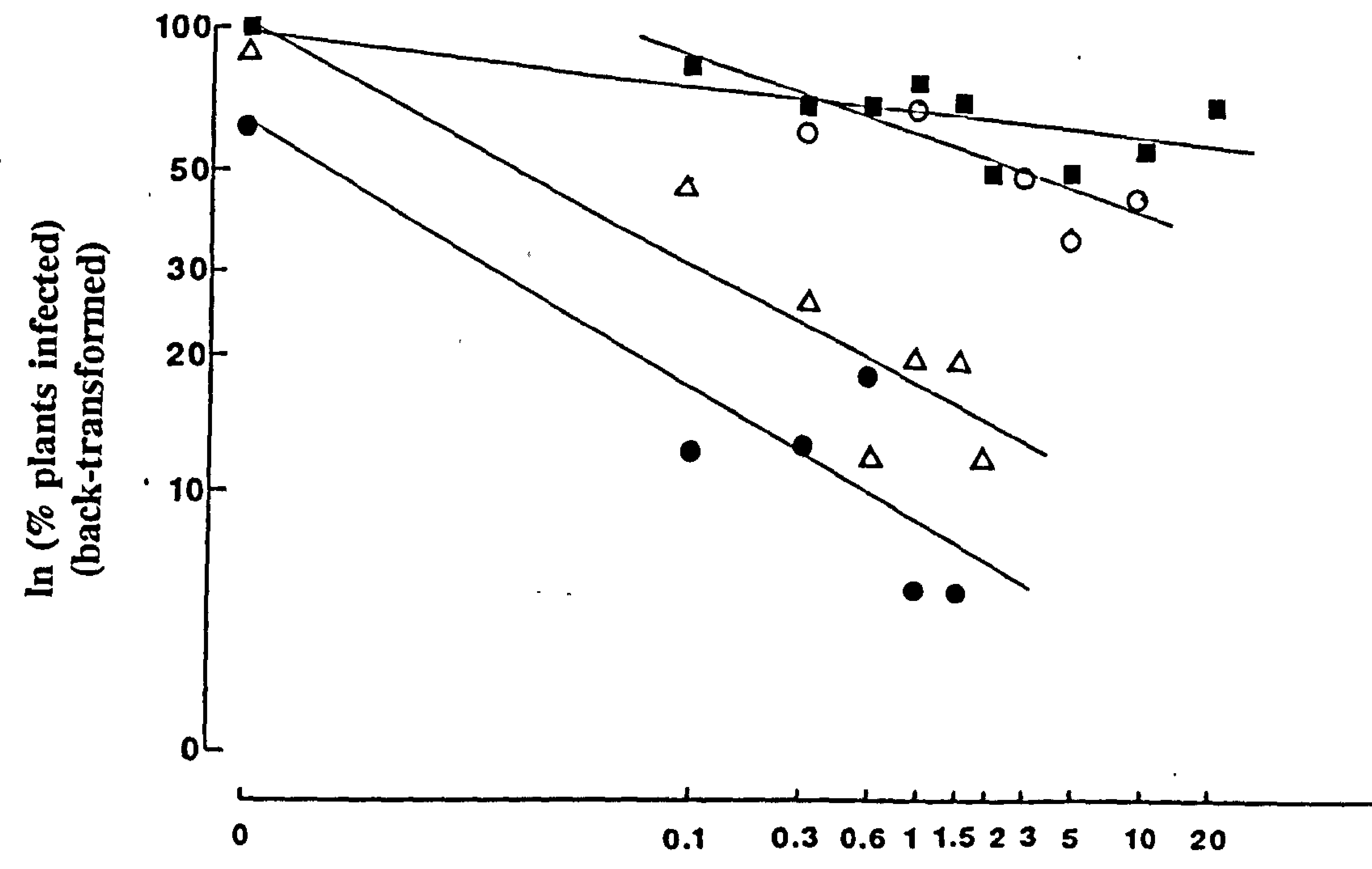



**Figure 7.12.** *A. linicola* disease gradients from a point inoculum source (average of 4 directions around the source) on 16 July (●), 13 August (△), 27 August ( ○ ) and 12 September ( ■ ) 1992. The gradients were fitted by the inverse power law model [ln (y) = ln (a) - b ln (x)]; the slopes of the regression lines (b) and the % variance accounted for ($r^2$) are given in Table 7.3.

August) the disease was detected in all four directions and the percent of plants infected decreased by 87% between the inoculum source and 2 m from the source (Fig. 7.12). Gradients became flatter with time and values of the slopes increased (became less negative) (Table 7.3) due to the secondary spread of the disease. On 29 August and 12 September (45 and 65 days after the introduction of the inoculum into the crop, respectively) plants infected by *A. linicola* were detected up to 10 m and 20 m from the source, respectively (Fig. 7.12). On 27 August, the percent of plants infected decreased by 50% between the source and 10 m from the source, whereas on 12 September, the decrease in the percent of infected plants between the source and 20 m from the source was only 31% (Fig. 7.12).

In 1992, there was no background infection in the crop as no *A. linicola* infection was detected on the plants collected on the date when the inoculum source was introduced into the crop. Although symptoms of *A. linicola* were initially observed only on leaves, by the end of the growing season (12 September) symptoms appeared on stems, sepals and capsule cases.

**7.4.4. Disease gradients from a line inoculum source - 1993 field experiment**

7.4.4.1. <u>Emergence</u>

The maximum emergence was 52% in field A (cropped with linseed for the previous three years) (Fig. 7.13) and 70% in field C (cropped with linseed for the first time) (Fig. 7.14) by 3 June, approximately 43 days after sowing (Fig. 7.13 & Fig. 7.14). By 3 June 100% of the emerged seedlings showed flea beetle damage on their cotyledons in both fields (Fig. 7.13 & Fig. 7.14). No *A. linicola*

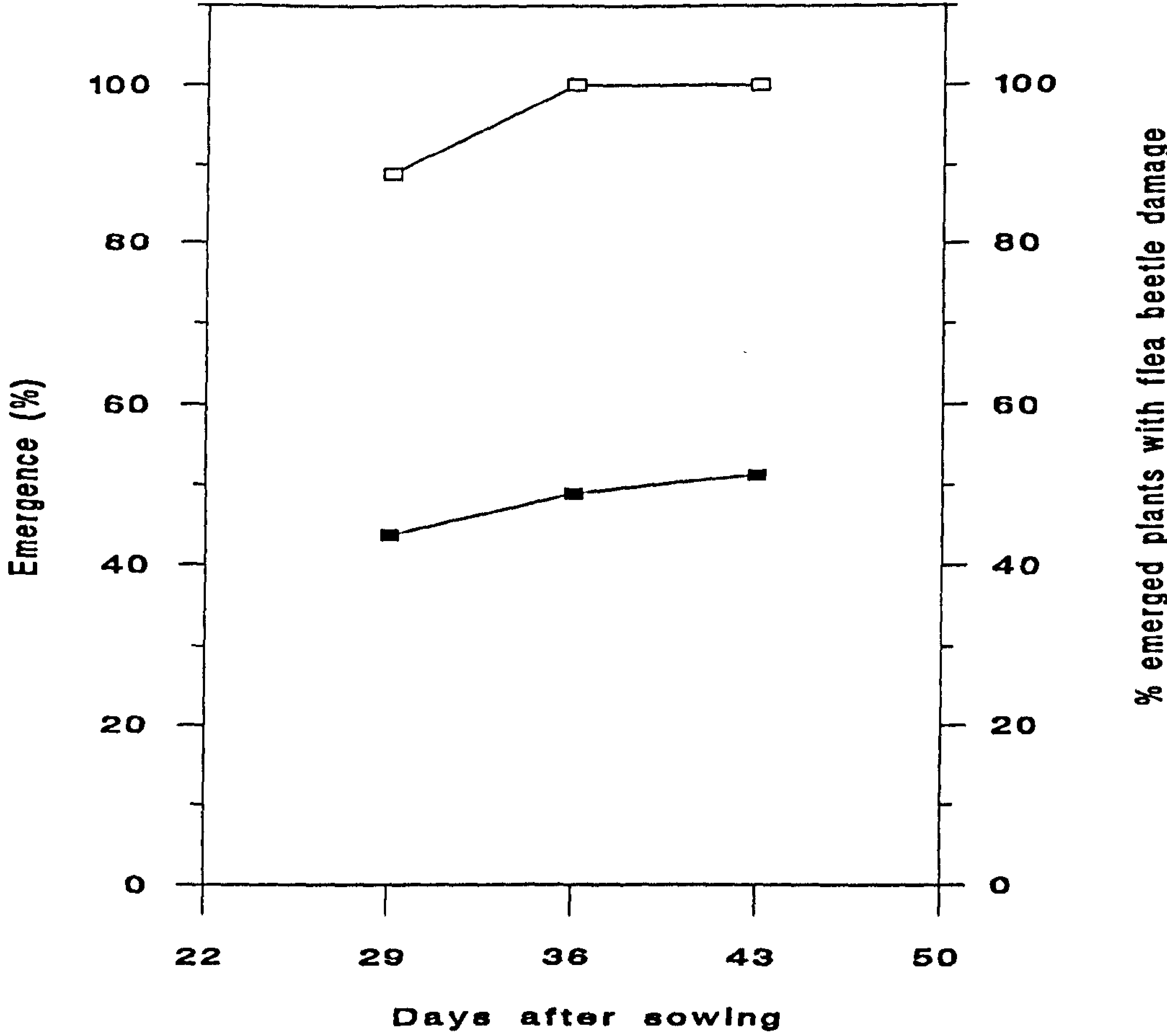



**Figure 7.13.** Emergence (%) of linseed ( ■ ) and percentage of emerged plants with flea beetle damage on their cotyledons (□) in field A (Long Hoos) sown with untreated seed (cv. Antares) on 21 April and used for studying the *A. linicola* disease gradients upwind from a line inoculum source in 1993.

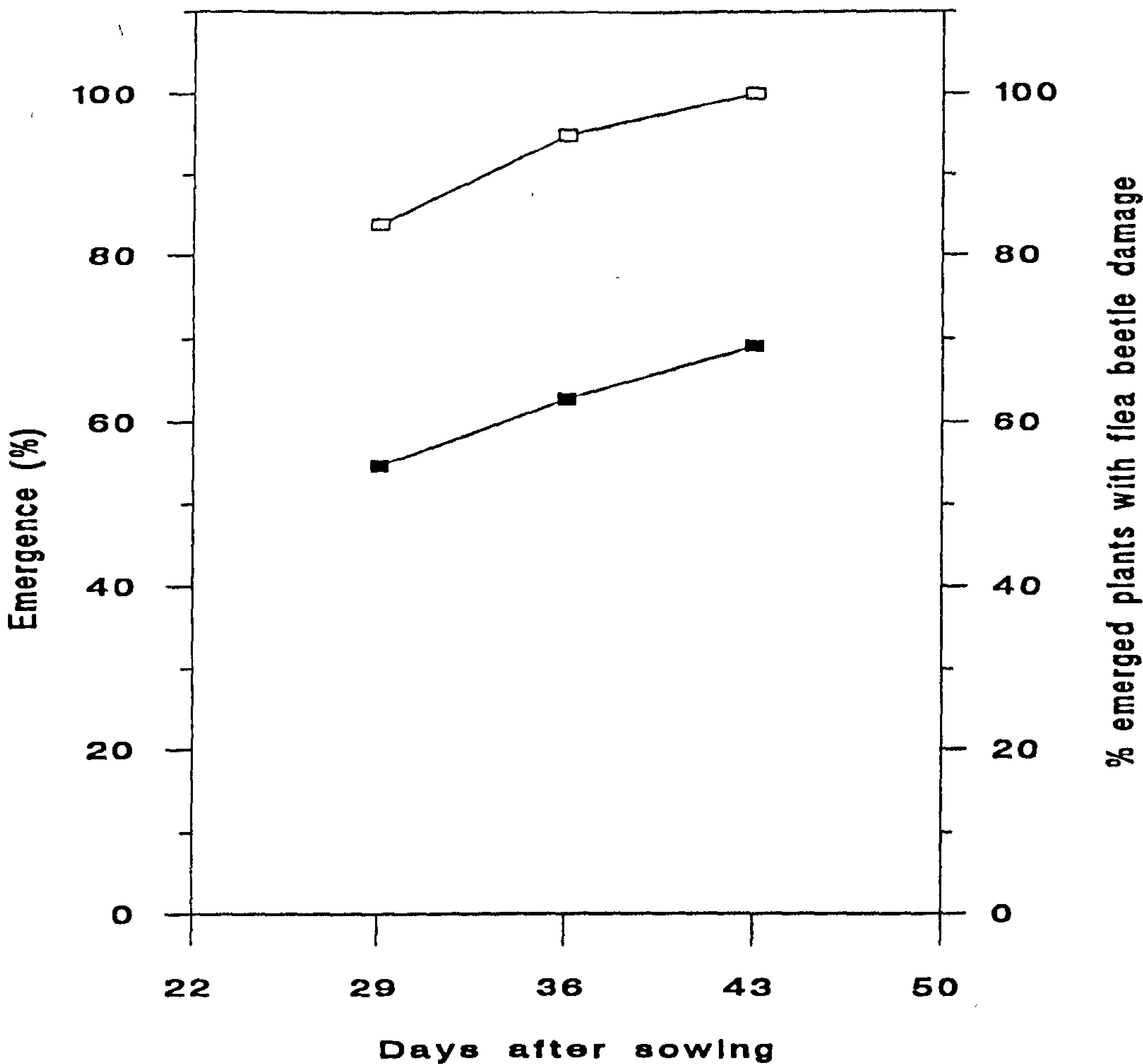



**Figure 7.14.** Emergence (%) of linseed ( ■ ) and percentage of emerged plants with flea beetle damage on their cotyledons (□) in field C (Long Hoos) sown with untreated seed (cv. Antares) on 21 April and used for studying the *A. linicola* disease gradients downwind from a line inoculum source in 1993.

infection was detected on the seedlings during the emergence counts.

7.4.4.2. Disease gradients

In 1993, when the *A. linicola* disease gradients were studied downwind (Field C, Fig. 7.4) and upwind (Field A, Fig. 7.4) from the inoculum source the percent of plants infected generally decreased with increasing distance from the inoculum source. In 1993, although the wind direction varied constantly during the experimental period (7 June - 17 September), there were 53 days with the wind direction being NW.

Disease gradients downwind from the source

The negative exponential model (Kiyosawa & Shiyomi, 1972) gave a better fit than did the inverse power law model (Gregory, 1968) to the data for the *A. linicola* disease gradients. On average, the linear regressions of ln (y) on x (negative exponential model) and of ln (y) on ln (x) (inverse power law model) accounted for 92% and 49% of the variance ($r^2$), respectively (Table 7.4). The disease was first detected on 24 June (approximately 2 weeks after the introduction of the inoculum into the linseed crop) and steep disease gradients ($b = -0.95$) were observed up to 3 m from the source (Fig. 7.15). On 24 June the decrease in the percent of plants infected between the source ($x = 0$ m) and 3 m from the source was 95%. Between 3 m and 30 m from the source the disease gradients appeared to be flatter (regression line parallel to the x - axis) with 5% disease incidence on plants (Fig. 7.15). This was probably because of the 5% background infection which was detected on the plants sampled just before the introduction of the inoculum source to the crop.

One month (8 July) after the introduction of the inoculum to the crop, the disease gradients were steep ($b = -0.64$) up to 3 m from the source, but became

**Table 7.4.** Parameters estimated for the negative exponential model [ln (y)* = ln (c) - dx‡] and the inverse power law model [ln (y) = ln (a) - b ln (x)] used to describe the *A. linicola* disease gradients downwind from a line inoculum source in 1993.

| Sampling date | Negative exponential model | | | Inverse power law model | | |
|---|---|---|---|---|---|---|
| | ln(c)† | d¶ | $r^2$††† | ln(a)†† | b¶ | $r^2$††† |
| 24 June (1)** | 4.32 | -0.95 | 95.0 | 2.58 | -0.33 | 63.0 |
| 24 June (4)** | 1.61 | 0.00 | 100 | 1.61 | 0.00 | 100 |
| 8 July (2)** | 4.38 | -0.64 | 94.3 | 3.34 | -0.18 | 57.2 |
| 8 July (5)** | 2.30 | 0.00 | 100 | 2.30 | 0.00 | 100 |
| 22 July (3)** | 4.42 | -0.20 | 95.6 | 3.97 | -0.09 | 50.0 |
| 22 July (6)** | 3.17 | -0.19 | 79.6 | 2.47 | -0.17 | 51.1 |
| 5 August | 4.36 | -0.04 | 96.6 | 3.89 | -0.19 | 45.3 |
| 19 August | 4.48 | -0.03 | 91.7 | 4.15 | -0.14 | 41.0 |
| 2 September | 4.51 | -0.02 | 86.6 | 4.32 | -0.08 | 39.3 |
| 17 September | 4.50 | -0.01 | 96.0 | 4.37 | -0.06 | 46.7 |

* y : disease incidence (%).
‡ x : distance from the inoculum source (m).
† c : constant equal to the value of y at x = 0 m.
¶ b & d : slopes of the linear regressions.
†† a : constant equal to the value of y at x = 1 m.
††† $r^2$ : % variance accounted for.
** : disease gradients on 24 June, 8 July and 22 July described by two regression lines each (Fig. 7.15); (1), (2) & (3) show the primary disease gradients; (4) & (5) show the background infection and (6) shows the effect of the background infection (5%) on the disease gradients.

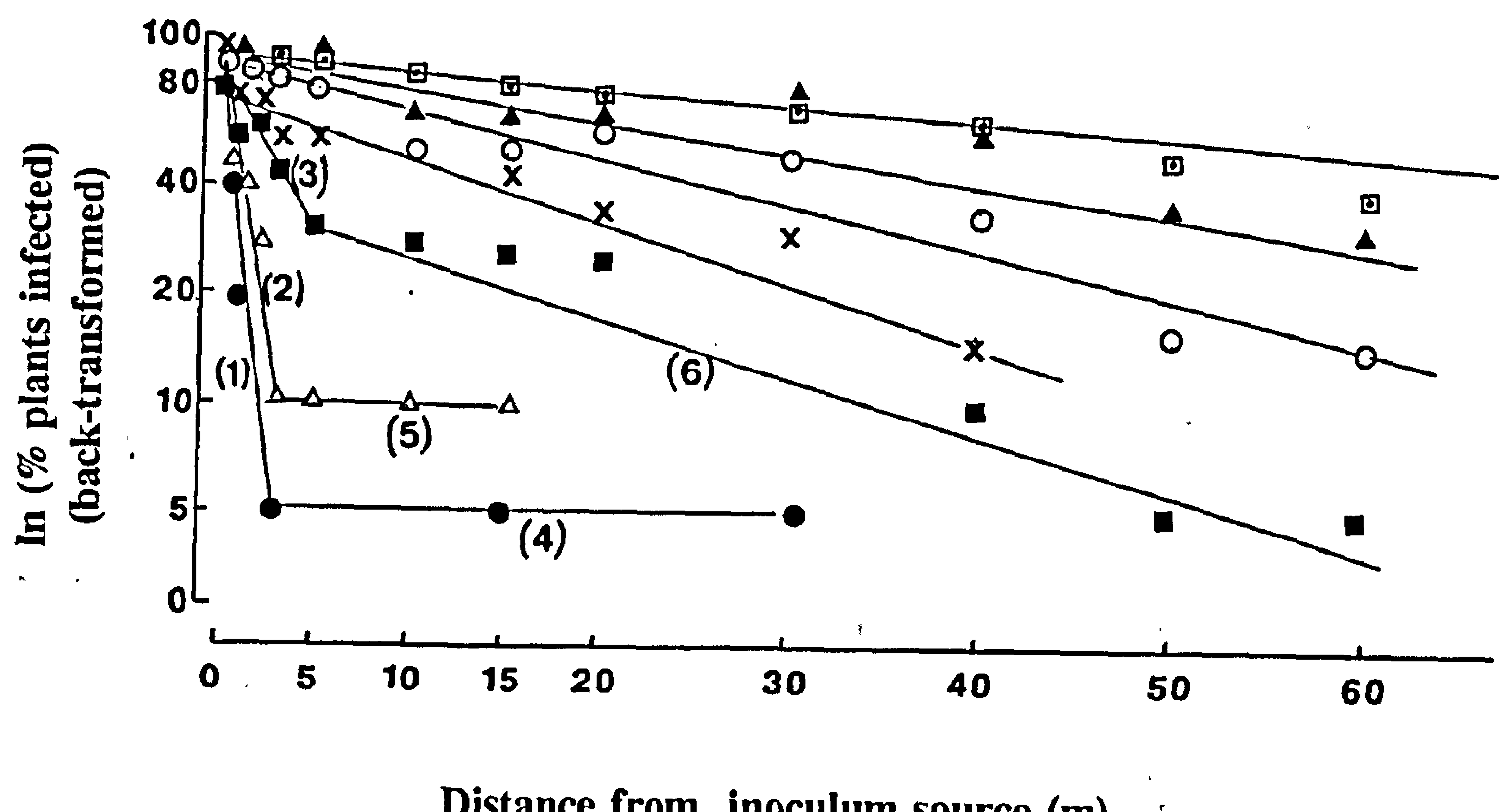



Figure 7.15. *A. linicola* disease gradients downwind from a line inoculum source on 24 June (●), 8 July (△), 22 July (■), 5 August (×), 19 August (○), 2 September (▲) and 17 September (⊡) in 1993, fitted by the negative exponential model [ln (y) = ln (c) - dx]. The regression lines (1), (2) and (3) show the primary disease gradients, the lines (4) and (5) show the background infection, whereas the line (6) shows the effect of the background infection on the disease gradients. The slopes of the regression lines (d) and the % variance accounted for ($r^2$) are given in Table 7.4.

flatter between 3 m and 15 m from the source (Fig. 7.15). On 22 July, the disease was detected 60 m from the source. Disease gradients were relatively steep up to 5 m from the source (b = - 0.20), but they became flatter beyond this distance up to 60 m (Fig. 7.15). Gradients also became flatter with time and values of the slopes increased (became less negative) (Table 7.4) due to the secondary spread of the disease (Fig. 7.15). By the end of the growing season (17 September), approximately one month before harvest, the disease gradients were very flat (b = - 0.01) with the regression line being almost parallel to the x - axis (Fig. 7.15).

Disease gradients upwind from the source

The disease gradients in field A (upwind from the inoculum source) were fitted equally well by the inverse power law model (Gregory, 1968) and negative exponential model (Kiyosawa & Shiyomi, 1972). The average percentage of the variance accounted for ($r^2$) by each model was 93% (Table 7.5) and for this reason only the relationship ln (y) on x (negative exponential model) was plotted (Fig. 7.16). The disease was first detected up to 30 m from the inoculum source at a very low incidence (1%) on the same date (24 June) as in the neighbouring field C, where the disease gradients downwind from the source were studied. Although the gradients were steep at the beginning of the growing season, they became flatter with time and values of the slopes increased (became less negative) (Table 7.5), due to secondary spread of the disease (Fig. 7.16). Incidences of the disease (% plants infected) were approximately the same downwind and upwind at 0, 15 and 30 m from the inoculum source.

**Table 7.5.** Parameters estimated for the negative exponential model [ln (y)* = ln (c) - dx‡] and the inverse power law model [ln (y) = ln (a) - b ln (x)] used to describe the *A. linicola* disease gradients upwind from a line inoculum source in 1993.

| Sampling date | Negative exponential model | | | Inverse power law model | | |
|---|---|---|---|---|---|---|
| | ln(c)† | d¶ | $r^2$††† | ln(a)†† | b¶ | $r^2$††† |
| 24 June | 4.02 | -0.14 | 98.2 | 1.84 | -0.35 | 89.3 |
| 8 July | 4.01 | -0.11 | 89.4 | 2.21 | -0.31 | 98.2 |
| 22 July | 4.26 | -0.08 | 86.7 | 3.01 | -0.22 | 99.1 |
| 5 August | 4.34 | -0.06 | 95.0 | 3.33 | -0.17 | 94.4 |
| 19 August | 4.44 | -0.04 | 84.5 | 3.87 | -0.10 | 99.6 |
| 2 September | 4.50 | -0.03 | 95.6 | 4.05 | -0.07 | 93.7 |
| 17 September | 4.61 | -0.02 | 99.8 | 4.25 | -0.05 | 76.5 |

* y : disease incidence (%).
‡ x : distance from the inoculum source (m).
† c : constant equal to the value of y at x = 0 m.
¶ b & d : slopes of the linear regressions.
†† a : constant equal to the value of y at x = 1 m.
††† $r^2$ : % variance accounted for.

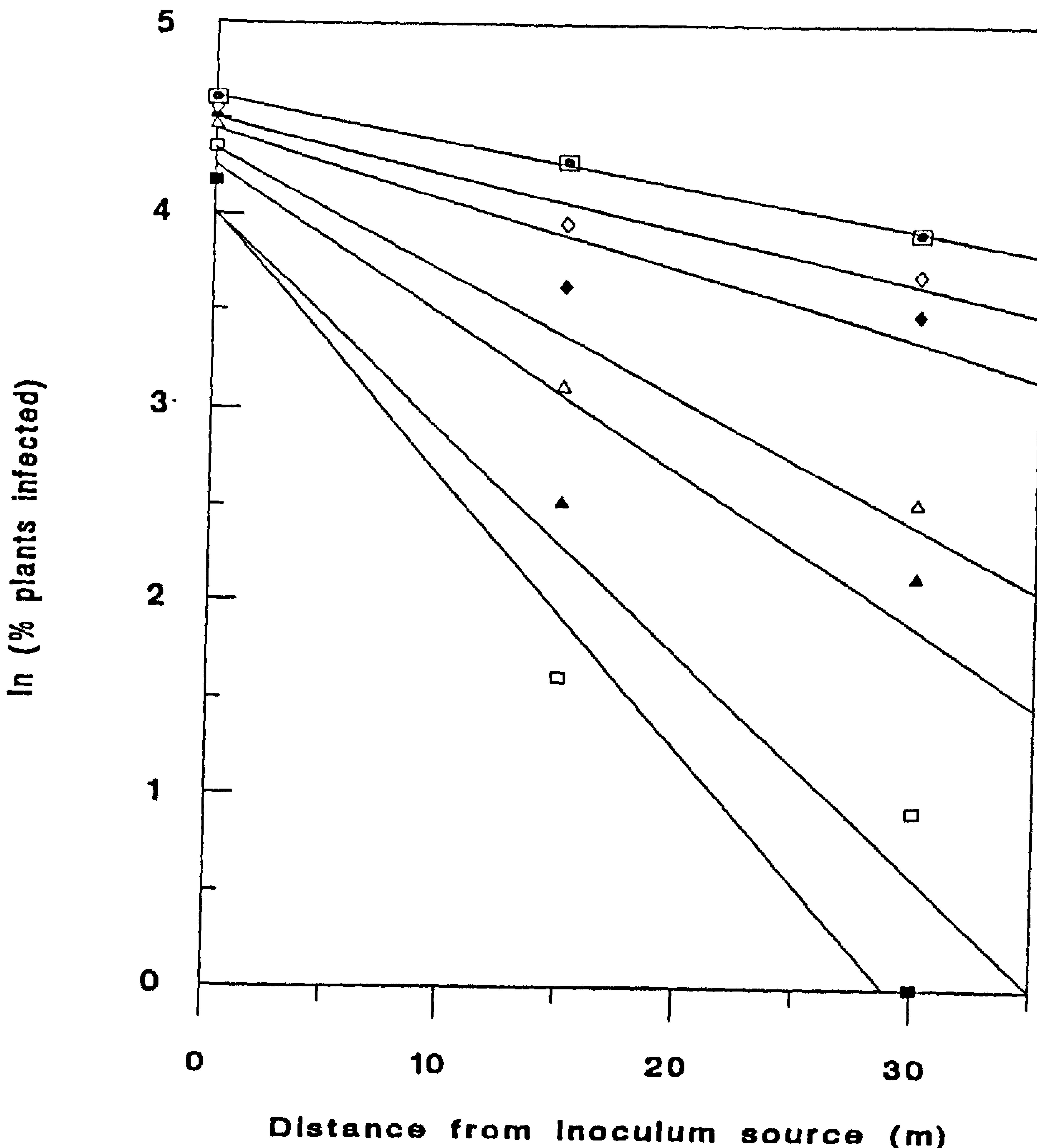



**Figure 7.16.** *A. linicola* disease gradients upwind from a line inoculum source on 24 June (■), 8 July (□), 22 July (▲), 5 August (△), 19 August (◆), 2 September (◇) and 17 September (⊡) 1993, fitted by the negative exponential model [ln (y) = ln (c) - dx]. The slopes of the regression lines (d) and the % variance accounted for ($r^2$) are given in Table 7.5.

### 7.4.5. Horizontal spore dispersal gradients

The negative exponential model (Kiyosawa & Shiyomi, 1972) gave a better fit to all sets of data than did the inverse power law model (Gregory, 1968). On average, the linear regressions of ln (y) on x (negative exponential model) accounted for 73% of the variance ($r^2$), whereas the regressions of ln (y) on ln (x) (inverse power law model) accounted for 53% of the variance (Table 7.6). Steep spore dispersal gradients were observed on 24 June ($b = -0.44$) and on 22 July ($b = -0.30$) up to 2 m and 10 m from the source, respectively (Fig. 7.17). On 22 July, 467 conidia $m^{-3}$ $h^{-1}$ were collected at the inoculum source ($x = 0$ m), but only 23 conidia $m^{-3}$ $h^{-1}$ were collected 10 m from the source (Fig. 7.17). Spore dispersal gradients became flatter with time and values of the slopes increased (became less negative), due to the production of secondary inoculum (Fig. 7.17). Although the numbers of *A. linicola* conidia collected at various distances from the inoculum source on 14 August were very small compared with those collected on previous dates, conidia were detected for the first time up to 40 m from the source (Fig. 7.17). On 18 August, 385 and 58 conidia $m^{-3}$ $h^{-1}$ were collected at the inoculum source ($x = 0$ m) and 40 m from the source, respectively (Fig. 7.17).

### 7.4.6. Vertical dispersal of *A. linicola* conidia

The linear regressions fitted all six sets of data quite well. On average, linear regressions of y (number of conidia $m^{-3}$ $h^{-1}$) on x (height in cm) accounted for 82% of the variance (Table 7.7). The concentration of conidia at heights within and above the linseed crop decreased with increasing height (Fig. 7.18). Generally, the mean number of *A. linicola* conidia collected during the sampling period was greater at

**Table 7.6.** Parameters estimated for the negative exponential model [ln (y)* = ln (c) - dx‡] and the inverse power law model [ln (y) = ln (a) - b ln (x)] used to describe the *A. linicola* spore dispersal gradients downwind from a line inoculum source in 1993.

| Sampling date | Negative exponential model | | | Inverse power law model | | |
|---|---|---|---|---|---|---|
| | ln(c)† | d¶ | $r^2$††† | ln(a)†† | b¶ | $r^2$††† |
| 24 June | 4.86 | -0.44 | 63.9 | 135.4 | -45.15 | 63.9 |
| 22 July | 5.83 | -0.30 | 90.4 | 83.5 | -6.41 | 23.3 |
| 2 August | 5.72 | -0.11 | 81.9 | 347.5 | -40.12 | 67.8 |
| 7 August | 5.07 | -0.02 | 56.6 | 285.5 | -13.64 | 72.3 |
| 14 August | 3.67 | -0.02 | 53.9 | 159.7 | -2.25 | 53.9 |
| 18 August | 5.83 | -0.05 | 91.5 | 327.5 | -7.84 | 83.2 |

* y : number of conidia $m^{-3}$ $h^{-1}$ collected by the rotorod spore samplers
‡ x : distance from the inoculum source (m).
† c : constant equal to the value of y at x = 0 m.
¶ b & d : slopes of the linear regressions.
†† a : constant equal to the value of y at x = 1 m.
††† $r^2$ : % variance accounted for.

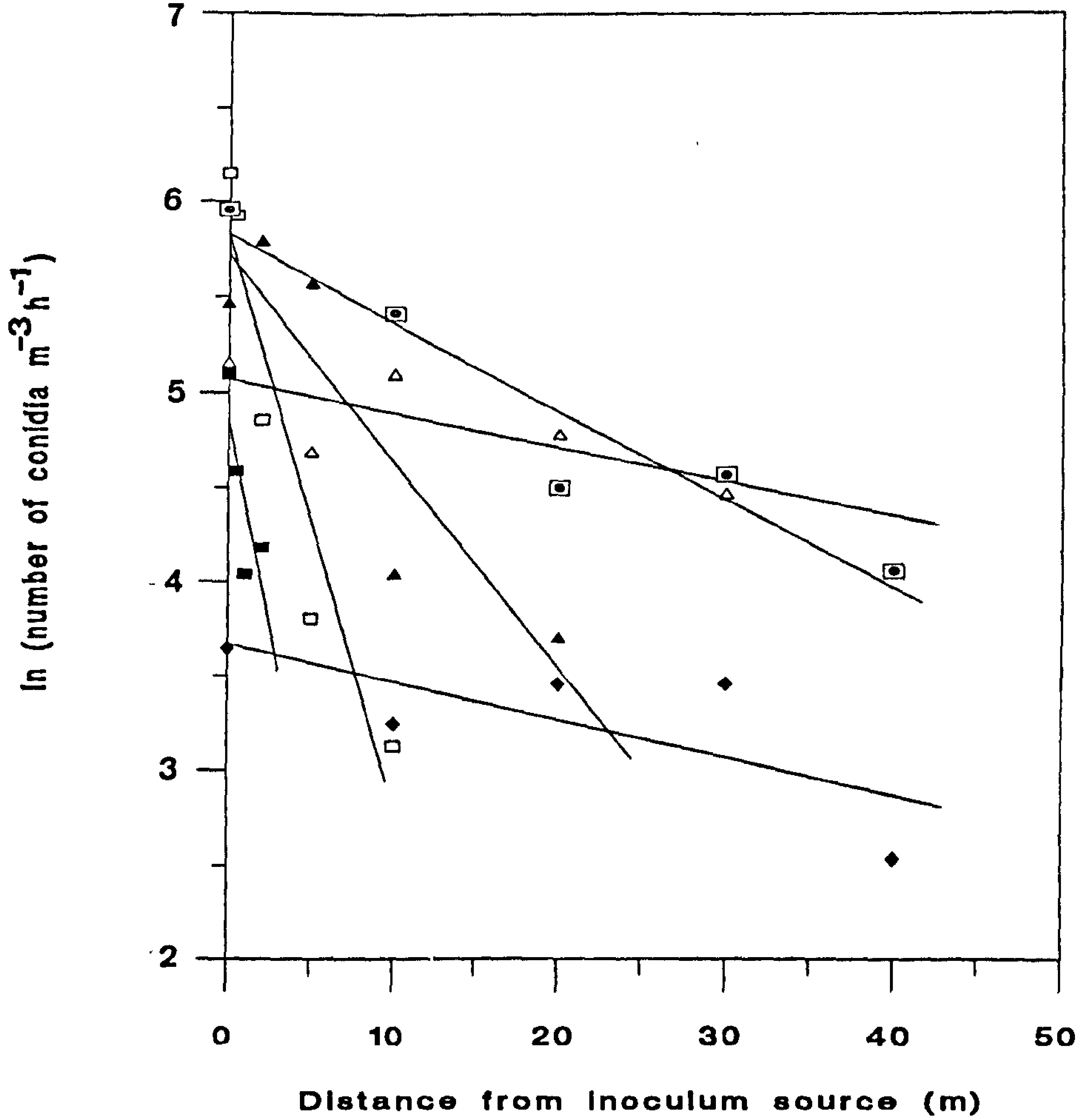



**Figure 7.17.** *A. linicola* spore dispersal gradients downwind from a line inoculum source as measured by the rotorod samplers at 30 cm above ground level on 24 June (■), 22 July (□), 2 August (▲), 7 August (△), 14 August (◆) and 18 August (▣) 1993. The gradients were fitted by the negative exponential model [ln (y) = ln (c) - dx]; the slopes of the regression lines (d) and the % variance accounted for ($r^2$) are given in Table 7.6.

**Table 7.7.** Parameters of the linear regressions ($y^{*} = a - bx^{\ddagger}$) used to describe the number of *A. linicola* conidia dispersed at different heights above the ground within or above a linseed crop in 1993.

| Sampling date | $a^{\dagger}$ | $b^{\P}$ | $r^{2\dagger\dagger\dagger}$ |
|---|---|---|---|
| 25 August | 89.4 | -0.55 | 76.9 |
| 31 August | 249.8 | -1.74 | 94.2 |
| 1 September | 472.0 | -2.47 | 90.8 |
| 2 September | 771.9 | -3.59 | 72.7 |
| 21 September | 397.3 | -2.08 | 90.4 |
| 22 September | 1374.0 | -6.42 | 66.9 |

* y : number of conidia $m^{-3}$ $h^{-1}$ collected by the rotorod spore samplers.
‡ x : height (cm) above ground level.
† a : constant equal to the value of y at x = 0 cm.
¶ b : slope of the linear regression.
††† $r^2$ : % variance accounted for.

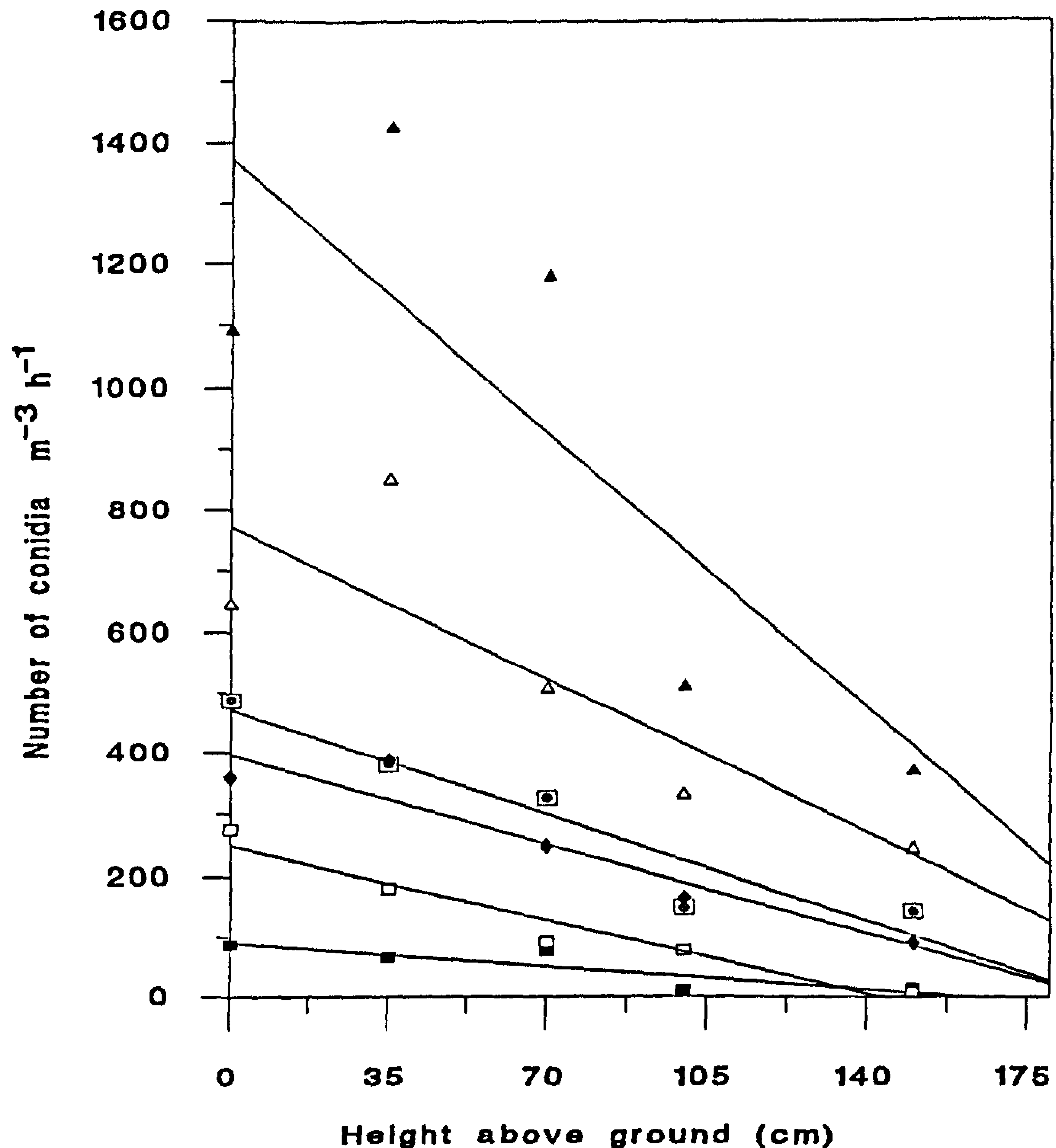



**Figure 7.18.** Vertical dispersal of *A. linicola* conidia as measured by the rotorod samplers on 25 August ( ■ ), 31 August ( □ ), 1 September (◆), 2 September ( ▣ ), 21 September ( △ ) and 22 September ( ▲ ) 1993. The samplers were operated within the linseed crop from 10:00 h until 16:00 h at 0, 35, 70, 100 and 150 cm above the ground level. The slopes of the regression lines (b) and the % variance accounted for ($r^2$) are given in Table 7.7.

ground level than at 150 cm above ground (492 and 144 conidia $m^{-3} h^{-1}$, respectively). There was also an increase in the number of conidia collected with time, with the greatest number being collected on 22 September, approximately 2 weeks before harvest (Fig. 7.18). During all sampling periods greater numbers of conidia were generally collected near ground level than at the top of the canopy. However, on 22 September a slightly greater number of conidia was collected at 35 cm above ground than at ground level (1430 and 1094 conidia $m^{-3} h^{-1}$, respectively) (Fig. 7.18).

## 7.5. Discussion

The results of this study confirm that conidia of *A. linicola*, like those of other *Alternaria* species, are mainly dispersed by wind. Air-borne *A. linicola* conidia were present above linseed crops during the entire growing season (April - September) in 1991, 1992 and 1993. Large numbers of conidia were collected by the Burkard spore sampler on the first dry day following a period of rain. However, few or no *A. linicola* conidia were collected during rainy days or extended dry periods.

The dispersal of *A. linicola* conidia followed a seasonal periodicity which was influenced by the prevailing weather conditions. Generally, the numbers of conidia dispersed on a particular day were correlated with the occurrence of conditions that favoured the sporulation of the fungus (rain, dew, high relative humidity, high temperature) during the previous day or night (see section 3.4.2.1). In 1991, 1992 and 1993, the greatest numbers of *A. linicola* conidia in the air above linseed crops were collected in July and August and were associated with high average temperatures during that period.

Conidia of many *Alternaria* species are air-borne and their dispersal follows a seasonal periodicity which is influenced by the prevailing weather conditions. Strandberg (1977) reported that the maximum concentrations of air-borne *A. dauci* conidia were observed in mid-season and were associated with the high temperatures that occurred in April and May. Dispersal of *A. cucumerina* and *A. alternata* above watermelon fields was also greater in mid-season, when the summer periods of rain had begun (Schenk, 1968). Moreover, the greatest numbers of air-borne *A.brassicicola* conidia were observed above an oilseed rape crop after a period of rain or prolonged leaf wetness (more than 13 h) with a mean temperature above 13°C. Dispersal of *A. helianthi* on sunflower (Allen *et al.*, 1983), *A. porri* on onions (Meredith, 1966) and *A. carthami* and *A. alternata* on safflower (Mortensen *et al.*, 1983) was also associated with prolonged rain. Datar & Mayee (1982) also reported that fluctuations in rainfall or relative humidity influenced the daily variations in the dispersal of *A. solani* conidia above tomato fields. Low temperatures at the end of the growing season suppressed sporulation of *A. alternata* on tomato plants and therefore the numbers of conidia dispersed during that period were less than those dispersed earlier in the growing season (Pearson & Hall, 1975).

However, the measurement of seasonal patterns of dispersal also depends on the efficiency of the spore sampler used. As the results of this study showed, the Burkard spore sampler was less efficient than the bait plants in detecting *A. linicola* conidia early or late in the growing season, when their concentrations in the air were very low. In both years the Burkard spore sampler detected air-borne *A. linicola* conidia at least one month before the appearance of symptoms on the linseed crop. However, by exposing bait plants, air-borne *A. linicola* conidia could be detected

up to three months before the symptoms were observed on the linseed crop. Furthermore, in both years, air-borne *A. linicola* conidia present in the linseed crops for 2 or 3 weeks after harvest were deposited on the bait plants, although they were not detected by the Burkard spore sampler. Harrison *et al.* (1965) also reported that air-borne conidia of *A. solani* could not be detected before late July, although symptoms were observed on potato plants early in the growing season, because the efficiency of the spore sampler used (weather vane spore sampler) was low.

A higher incidence of *A. linicola* infection was observed early in the growing season on the bait plants placed at ground level than on those placed 1 m above ground. Moreover, as the results of this study showed, the numbers of *A. linicola* conidia dispersed were greater near ground level than at some height above ground. However, there was no difference in the disease incidence between the bait plants exposed at ground level and those exposed 1 m above ground when the highest concentrations of air-borne conidia were collected by the Burkard spore sampler (July, August and early September).

According to the results of this study, the variations between years in the numbers of air-borne *A. linicola* conidia dispersed above linseed crops were also influenced by the prevailing weather conditions. Numbers of air-borne *A. linicola* conidia collected above the linseed crop may have been greater in 1992 than in 1991 because June was drier and hotter in 1992 than in 1991 and August was wetter in 1992 than in 1991, although weather conditions in July were similar in both years. The numbers of air-borne *A. linicola* conidia collected above the linseed crop in 1993 were also greater than in 1991. Approximately the same amount of rain fell during June, July and August in 1991 and 1993. However, in June 1993 most of

the rain fell in mid-June with the rest of the month being dry, whereas in 1991 the rainy days were better distributed during June. Moreover, the low mean temperature in June 1991 compared with that in June 1993 might have decreased the sporulation of *A. linicola* on the plant tissues during that period.

Differences in the numbers of *A. linicola* conidia dispersed above linseed crops between different years might have also been due to differences in the amounts of primary inoculum or to different cultural practices. In 1991, the field used had been sown with linseed for the first time and although no *A. linicola* infection was detected on the seed used for sowing, it is possible that a very low, but undetectable, level of infection was present on the seed. In 1992 and 1993, the fields had been sown with linseed for the second and third year, respectively. Although seed used in 1992 and 1993 was from the same origin as in 1991 the primary inoculum present in the field at the beginning of the growing season was probably greater in 1992 and 1993 than in 1991, due to the presence of the infected debris. In both years linseed stem debris, naturally infected by *A. linicola* the previous year, were left on the ground after harvest and the field was cultivated just before the sowing of the subsequent linseed crop. Moreover, the irrigation applied to the crop in 1992 and 1993 during the dry periods in June and July might have favoured the production of greater numbers of *A. linicola* conidia on the plant tissues. Humpherson-Jones & Maude (1982a) reported that the number of air-borne *A. brassicicola* conidia above oilseed rape crops increased with the duration of cropping and the highest concentration was observed during the harvest of the crop.

The results of this study also showed that, although wind is the main agent in the dispersal of *A. linicola* conidia, some conidia may be dispersed by

the splash dispersal mechanism. In 1992 and 1993, bait plants placed at ground level immediately after harvest of the linseed crop showed a greater incidence of *A. linicola* infection than did the bait plants placed 1 m above ground. This suggests that conidia of *A. linicola* produced on the infected stem debris left on the ground after harvest were splash-dispersed onto the bait plants placed at ground level and accounted for the greater disease incidence on these plants. Although there are no reports on the maximum height above an inoculum source to which *Alternaria* conidia can be splash-dispersed, it is unlikely that conidia deposited on the bait plants exposed 1 m above the ground were splash-dispersed. As field experiments with splash-dispersed conidia of *Pseudocercosporella herpotrichoides* (Fitt & Bainbridge, 1983) and pycnidiospores of *Septoria nodorum* (Griffiths & Ao, 1975) have shown, few spores are dispersed more than 50 cm above the inoculum source by splash. Moreover, photographic studies have demonstrated that 90% of the splash droplets produced on bean leaves reached a height of less than 5 cm above the leaves (Macdonald & McCartney, 1988).

The dispersal of *A. linicola* conidia, as monitored by the Burkard spore sampler during a 24-h period, showed a diurnal periodicity which was influenced by the wind speed. The numbers of conidia dispersed increased with increasing wind speed, reaching a maximum at midday (between 12:00 h and 13:00 h) when the wind speed was 2 - 3 m $sec^{-1}$. The highest concentration of conidia in the air was observed 3 h before the highest wind speed, suggesting that although wind is required for the dispersal of *A. linicola* conidia, strong winds may remove the greatest part of the conidial reserves within a short time. Therefore, the number

of conidia available for dispersal during a particular day depends not only on the number of conidia produced on the plant tissues the previous night but also on how strong the wind is on that day. Generally, as conidia of *Alternaria* species are considered to be firmly attached to conidiophores, their removal requires relatively high wind speeds and therefore they are mainly dispersed during the middle of the day. It has been reported that a wind speed of 2 - 3 m $sec^{-1}$ was required for releasing a great number of *A. dauci* conidia (Strandberg, 1977). Moreover, the pattern of diurnal dispersal of *A. linicola* conidia is similar to that of other *Alternaria* species from different geographical locations. In California, the highest concentration of *A. alternata* conidia in the air coincided with the highest wind speed (Pearson & Hall, 1975). In England, the numbers of air-borne *Alternaria* (Hirst, 1953) and *A. brassicicola* (Humpherson-Jones & Maude, 1982a) conidia dispersed during a 24-h period increased with increasing wind speed. In Canada, the highest concentrations of air-borne *A. dauci* conidia occurred at 13:00 h, 3 h before the highest wind speed (Langenberg *et al.*, 1977). In Israel, although the numbers of *A. porri* f. sp. *solani* conidia dispersed during a 24-h period increased with increasing wind speed, the maximum numbers of conidia were collected at 11:00 h, 4 h before the highest wind speed (Rotem, 1964).

However, no *A. linicola* conidia were dispersed between 21:00 h and 7:00 h, although there was an increase in the wind speed between 24:00 h and 5:00 h. It seems, that in addition to the wind speed other environmental factors can influence the release and dispersal of *A. linicola* conidia, although they were not investigated in this study. Rain, dew or high relative humidity have been reported to inhibit the release and subsequent dispersal of conidia of *A. dauci* (Langenberg

*et al.*, 1977) and *A. porri* f. sp. *solani* (Rotem, 1964; Meredith, 1966). However, temperature had no effect on the diurnal dispersal of *A. porri* f. sp. *solani* (Rotem, 1964).

Field experiments also showed that in a senescent linseed crop, air-borne *A. linicola* conidia were collected by the spore samplers not only within the canopy but also 80 cm above the canopy. However, the numbers of conidia dispersed decreased with increasing height above ground level. As the disease had already spread to the capsules at the time the vertical dispersal of *A. linicola* conidia was measured, these results suggest that more conidia were produced on the old, senescent leaves at the base of the plants than on the younger leaves at the top of the plants. Moreover, as the wind speed is greater above the crop canopy than within the canopy (Gregory, 1973), it is possible that the concentration of *A. linicola* conidia above the canopy was diluted so that a smaller number of conidia was collected above the canopy than within the canopy. These results also suggest that only a small number of *A. linicola* conidia which escape from the canopy may be involved in long-distance dispersal of the pathogen. The number of *A. linicola* conidia collected with sticky-slide spore samplers 20 cm above ground was greater than that collected above the linseed crop (Mercer *et al.*, 1992b). Humpherson-Jones (1992) also reported that the numbers of air-borne *A. brassicae* and *A. brassicicola* conidia collected decreased with increasing height within an oilseed rape crop.

In 1993, when the *A. linicola* spore dispersal gradients were studied downwind from a line inoculum source and at a height of 30 cm above ground level, the number of conidia dispersed decreased with increasing distance from the inoculum source. Furthermore, *A. linicola* conidia were collected by the spore

samplers even at 40 m from the foci of their production. Initially the gradients were steep near the inoculum source, gradually decreasing with increasing distance from the source and finally becoming flatter due to the production of secondary inoculum. These results suggest that wind was the main agent in the dispersal of *A. linicola* conidia within the linseed crop. Field experiments on the splash dispersal of *P. herpotrichoides* conidia (Fitt & Bainbridge, 1983) and *S. nodorum* (Griffiths & Ao, 1975) pycnidiospores have shown that few spores were dispersed by splash to distances more than 1 m from the inoculum source. Moreover, rain tower/wind tunnel studies have demonstrated that with a wind speed of 2.5 m $sec^{-1}$ droplets carrying conidia of *P. herpotrichoides* (Fitt & Nijman, 1983) or pycnidiospores of *S. nodorum* (Brennan *et al.*, 1985) were collected by slides at ground level at distances up to 4 m from the inoculum source.

The results of this study suggest that the disease caused by *A. linicola* on linseed crops is mainly spread by air-borne conidia. In 1991, 1992 and 1993 the increase in the disease incidence on the linseed crops coincided with the greatest concentrations of air-borne *A. linicola* conidia (July, August and early September). In 1991, when few *A. linicola* conidia were collected by the Burkard spore sampler above the linseed crop, the disease incidence on the plants was very low (4% by mid-August) (see section 9.4.3). Moreover, in 1992 when a greater number of conidia were dispersed above the linseed crop than in 1991 the disease incidence was also greater (60% - 80% by mid-August) (see section 9.4.3). In 1993, conditions unfavourable for sporulation of *A. linicola* on the plant tissues (extended dry period between mid-June and early July) influenced the numbers of conidia dispersed. Therefore, not only were few conidia dispersed in 1993 compared with

1992, but also the highest concentrations of these conidia occurred later in the season (August and early September). However, the disease incidence on the plants was high (see section 9.4.3) despite the small numbers of conidia collected.

In all years there was an unexpected decrease in the numbers of air-borne *A. linicola* conidia at the end of the growing season just before harvest despite the increase in the disease incidence on the senescent crop. It is possible that the low temperatures during that period suppressed the sporulation of the fungus on plant tissues.

Correlation between conidial dispersal and disease incidence has also been reported for *A. dauci* on onions (Langenberg *et al.*, 1977), *A. alternata* and *A. solani* on tomatoes (Rotem, 1964; Pearson & Hall, 1975), *A. brassicicola* (Humpherson-Jones & Maude, 1982a) and *A. brassicae* (Machegay *et al.*, 1990) on oilseed rape and *A. macrospora* on cotton (Rotem, 1991).

When the *A. linicola* disease gradients were studied from point (in 1992) or line (in 1993) inoculum sources, the gradients followed patterns similar to those of the spore dispersal gradients. In both years the disease incidence decreased with increasing distance from the inoculum source. Initially the gradients were very steep near the inoculum source but they became flatter with time due to the secondary spread of the disease. In both years the disease was first detected downwind from the inoculum source, suggesting that wind was the main agent in the dispersal of the inoculum (conidia). However, as the direction of the local wind varied constantly not only between days, but also within the day, the disease spread to all directions later in the season. These results suggest that, although wind is required for the dispersal of *A. linicola* conidia and subsequently for the spread of the disease in

a linseed crop, the direction of the prevailing wind is not very important.

The empirical models which fitted the *A. linicola* disease gradients data best were different in 1992 and 1993. In 1992, when the disease gradients were studied in four directions around a point inoculum source, the inverse power model suggested by Gregory (1968) gave a better fit than did the negative exponential model (Kiyosawa & Shiyomi, 1972). However, in 1993 when the disease gradients were studied downwind from a line inoculum source, the negative exponential model fitted the data better than did the inverse power law model. In the same year the disease gradients upwind from the line inoculum source were described equally well by both models.

Several different factors (geometry of the source, distance over which the gradients are studied, weather conditions, background infection, etc.) may affect the values of b and d (Gregory, 1968), and therefore disease gradients may be described best by different models on different occasions. Such factors were possibly responsible for the differences in the best models between 1992 and 1993. In 1992 the disease gradients were studied from a point inoculum source, whereas in 1993 they were studied from a line inoculum source. The distance over which the gradients were measured from the source was shorter in 1992 (20 m) than in 1993 (60 m). Moreover, in 1992 the inoculum source was introduced into the crop late in the growing season (GS 9), whereas in 1993 the inoculum source was introduced very early (GS 5). In 1993, the disease gradients were studied in two directions (downwind and upwind) and over different distances from the line inoculum source (60 m downwind and 30 m upwind). Moreover, although the downwind and upwind samples were collected on the same date, the sampling downwind from the source was more

intensive near the inoculum source than the sampling upwind from the source. This was probably a reason why the negative exponential model showed the background infection (5 %) in downwind disease gradients but not in the upwind disease gradients.

The results of this study suggest that wind is the main agent in the dispersal of *A. linicola* conidia. Conidia produced on inoculum sources (e.g. infected debris left on the ground after the harvest of the previous linseed crop) can be transported by the wind and subsequently spread the disease over short distances from the foci of their production. However, to understand the spread of the disease caused by *A. linicola* in linseed crops a number of factors should be considered. These include the size of the inoculum source, the crop density, the direction of the rows of the plants, the amount of the background infection and the wind speed. However, linseed crops that are free of *A. linicola* infection as a result of seed treatment, crop rotation or effective burial of debris are unlikely to be significantly affected by neighbouring infected linseed crops.

# CHAPTER VIII. SURVIVAL OF *ALTERNARIA LINICOLA*

## 8.1. Introduction

There are a variety of mechanisms by which *Alternaria* species survive between growing seasons, even under unfavourable environmental conditions, in order to provide inoculum for the infection of the succeeding crop. Survival of *Alternaria* species on seed seems to be the main mechanism for the introduction of *Alternaria* diseases into new areas (Maude & Humpherson-Jones, 1980b; Herr & Lipps, 1982; Bashan & Hernandez-Saavedra, 1992; Humpherson-Jones, 1992). However, once the *Alternaria* pathogens are established in a new area they may persist for long periods. Most *Alternaria* species can survive on infected debris that remains on the ground after crops are harvested, or on alternate hosts (Jackson, 1959; Von Ramm & Lucas, 1963; Pandotra, 1965; Netzer & Kenneth, 1969; Burns, 1974; Soteros, 1979; Humpherson-Jones & Maude, 1982a; Jeffrey *et al.*, 1984; Humpherson-Jones, 1989; Rotem, 1990). Conidia, hyphae and resting bodies like chlamydospores and microsclerotia, which are very resistant to unfavourable environmental conditions, have been reported to be involved in the survival of many *Alternaria* species (Atkinson, 1953; Rotem, 1968; Basu, 1971; Tsuneda & Skoropad, 1977a; Patterson, 1991).

In the case of *A. linicola,* it has been demonstrated that the pathogen survives as resting hyphae in the outer layer of the seed coat (Mercer & Hardwick, 1991). However, although infected seed is considered to be the main route of

disease transmission between growing seasons (Mercer *et al.*, 1991a), the possibility that infected debris, volunteer linseed plants or weeds are additional sources of primary inoculum has not been investigated.

## 8.2. Objectives

1. To investigate whether *A. linicola* can survive on infected debris, either left on the soil surface or buried beneath the soil surface.
2. To study the effects of infected debris on the onset of an epidemic.
3. To investigate whether *A. linicola* can overwinter on volunteer linseed plants or alternate hosts (weeds).
4. To study the survival structures of *A. linicola*.

## 8.3. Materials and Methods

### 8.3.1. Survival on debris

8.3.1.1. Glasshouse experiment

On 15 November 1992, approximately two months after harvest (harvest date : 17 September), linseed stem debris naturally infected by *A. linicola* was collected from a linseed field and cut into 10-cm long pieces. The debris was either placed on the soil surface or buried at a depth of 5 cm in plastic trays (35 x 26 x 7 cm) containing loam soil. For easy recovery the buried debris was placed in nylon net bags (20 x 30 cm, 2 mm mesh). Five trays with debris on the soil surface and five with debris buried beneath the soil surface were placed outdoors to be

exposed to the environmental conditions (alternating wet-dry, low temperatures) during winter (Group I) (Fig. 8.1). The remaining 10 trays were placed in a heated glasshouse (temperature range 15 - 25°C) and kept dry (Group II). Every month, starting from December 1992, two trays from each group, one with debris on the soil surface and the other with buried debris, were collected. The stem debris was examined under a stereo-microscope (x 25 magnification) for the presence of *A. linicola* conidia. If no *A. linicola* conidia were present, the debris was washed in running tap water to remove soil, air-dried overnight and incubated by the method described in section 2.2.1 to induce sporulation. After five days of incubation, the conidia produced were removed by shaking the debris in 50 ml of distilled water (0.01 ml of 0.01% Tween 80 was added to the water as a wetting agent) and the resulting conidial suspension was filtered through two layers of muslin. The concentration of conidia was determined with a haemocytometer (4 counts per conidial suspension) and was adjusted to $5 \times 10^5$ conidia $ml^{-1}$ by dilution with distilled water.

Conidial viability was tested by spreading 1 ml of each conidial suspension on the surface of V-8 juice agar plates prepared by the method described in section 2.1.7. One plate (20 ml per plate) was used for each conidial suspension (no replicate plates). The plates were sealed with parafilm, incubated in darkness at 20°C for 6 h and assessed for conidial germination.

The pathogenicity of the conidia produced was tested on linseed seedlings (10 days old) grown in a heated glasshouse (temperature range 15 -25°C) by the method described in section 4.3.4.3. The plants were inoculated with the conidial suspensions by the method described in section 2.1.6. Uninoculated control plants

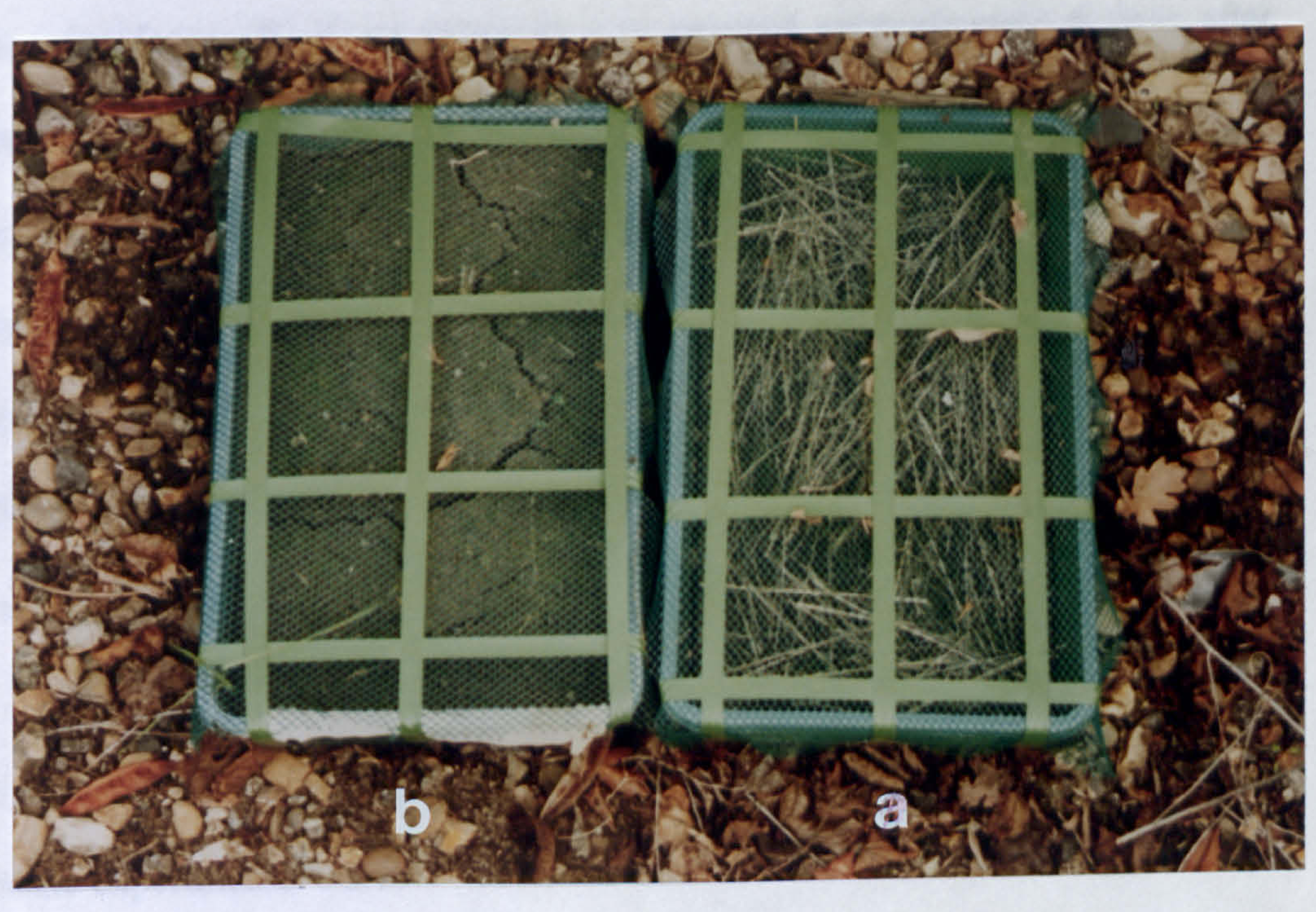



**Figure 8.1.** Trays with infected linseed stem debris used to study the survival of *A. linicola*. The debris placed either on the soil surface (a) or buried (in nylon net bags) 5 cm beneath the soil surface (b), was exposed to environmental conditions during the period 15 November 1992 to 15 April 1993.

were sprayed with distilled water containing 0.01% Tween 80. There were four replicate pots, with 10 plants each, for each treatment. After inoculation, the plants were covered for 72 h with polyethylene bags sprayed inside with water (100%) to provide a water-saturated atmosphere favourable for infection. The disease incidence (% plants infected) was assessed approximately 6 days after inoculation, after incubating the plant tissues (cotyledons or leaves) with symptoms by the method described in section 2.2.1 to induce sporulation. Assessments of conidial viability and pathogenicity were made on the conidia produced on the stem debris after incubation in dew chambers under diurnal NUV-light, and not on the conidia recovered from the debris immediately after the monthly sample.

Records of maximum and minimum temperatures and numbers of days with ground frost were obtained from a meteorological station situated 0.5 - 1 km from the experimental site.

8.3.1.2. Field experiment

To study if *A. linicola* can survive on infected debris under field conditions, linseed stem debris naturally infected by *A. linicola* was collected from a linseed field immediately after harvest in 1993 and stored at 5°C. A field cropped with linseed for three successive years was used for the experiment (Field A, Fig. 9.1). The field was deep-ploughed on 20 October. On 26 November the stem debris was cut into pieces approximately 10-cm long and placed in 10 nylon net bags (30 x 100 cm, 2 mm mesh) which were divided into two groups of five bags each. One group was placed on the soil surface and the other group was buried approximately 20 cm beneath the soil surface. The bags were placed 20

cm apart and were held in place with wooden poles (Fig. 8.2). Two bags with debris (one placed on the soil surface and one buried) were recovered at approximately monthly intervals, starting in December 1993 and the debris was treated by the method described in section 8.3.1.1.

The viability and pathogenicity of the conidia produced on the stem debris after its incubation in dew chambers under diurnal NUV-light was tested by the method described in section 8.3.1.1.

Records of maximum and minimum temperatures and numbers of days with ground frost were obtained from a meteorological station situated 0.5 - 1 km from the experimental site.

**8.3.2. Effects of infected debris on the onset of an epidemic**

8.3.2.1. Experiment I - 1992

In 1992, four wooden containers (A, B, C and D), each 90 x 90 x 40 cm, were filled with a 1 : 1 mixture of loam : soil-less compost with a slow release fertilizer [Croxden compost, produced by Nursery trades (Lea valley) Ltd] (Fig. 8.3). The containers were placed outdoors in groups of two (group I : A & B and group II : C & D). The distance between the groups was approximately 16 m. On 21 April 1992, stem debris from linseed plants, artificially inoculated with *A. linicola* in the previous season and left to overwinter outdoors, was cut into pieces 10-cm long and spread on the soil surface in containers A & B. No debris was placed on the soil surface in containers C & D (controls). Linseed seed (cv. Antares, untreated), with 17% incidence of *A. linicola* infection (on surface-sterilized seed), when tested by the method described in section 6.3.1, was sown in the containers A & C. Seed

**Figure 8.2.** Nylon net bags with linseed stem debris naturally infected by *A. linicola* used to study the survival of the pathogen under field conditions. The bags with the debris were either placed on the soil surface (a) or buried 20 cm beneath the soil surface (b) and exposed to the environmental conditions during the period 25 November 1993 to 25 April 1994.

**Figure 8.3**. Wooden containers for studying the effects of infected linseed stem debris on the onset of an *A. linicola* epidemic in 1992, 1993 and 1994.

from another source (cv. Antares, treated with prochloraz; 4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem) with 1% incidence of *A. linicola* infection (on surface-sterilized seed) was sown in containers B & D. Because of the small number of containers available, there were no replicates of treatments. When the first symptoms appeared on the cotyledons, 100 seedlings per container (c. 15 seedlings per row) were collected at random and incubated according to the method described in section 2.2.1 to induce sporulation. A total of three samples were taken at weekly intervals.

8.3.2.2. Experiment II - 1993

In 1993, six wooden containers (A, B, C, D, E and F), each 90 x 90 x 40 cm, were filled with the mixture of soil described in section 8.3.2.1 (Fig. 8.3). The containers were placed outdoors in groups of three (group I : A, B & F and group II : C, D and E). The distance between the two groups was approximately 16 m. On 13 May 1993, stem debris from linseed plants naturally infected by *A. linicola*, was cut into pieces 10-cm long and spread on the soil surface in containers A, B and F. No debris was placed on the soil surface in containers C, D and E (controls). Linseed seed (cv. Antares, untreated) with 14% incidence of *A. linicola* infection (on surface-sterilized seed), tested by the method described in section 6.3.1, was sown in containers A & E. The same seed treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem), with 4% incidence of *A. linicola* infection (on surface-sterilized seed) was sown in containers B & D. A third seed sample (cv. Antares, untreated) from another source with 1% incidence of *A. linicola* infection (on surface-sterilized seed) was sown in containers C & F. The seed was sown on 13 May with seven rows in each container. Because of the small number of containers available, there were no replicates of treatments. The incidence of *A. linicola* symptoms on

the young seedlings was assessed by the method described in section 8.3.2.1. A total of four samples were collected during the experimental period. The first three samples were collected at weekly intervals and the last sample was collected 2 weeks after the third sample.

8.3.2.3. Experiment III - 1994

In 1994, the six wooden containers (A, B, C, D, E and F) used in 1993 were refilled with the mixture of soil described in section 8.3.2.1 (Fig. 8.3). In October 1993, immediately after the harvest of the linseed crop, linseed stem debris, naturally infected by *A. linicola*, was collected from the field and placed in 12 nylon net bags (20 x 100 cm, 2 mm mesh). The bags with the debris were left to overwinter in the field : six of the bags were placed on the soil surface, whereas the other six were buried 10 cm beneath the soil surface. On 26 April the debris was recovered from the field and cut into pieces approximately 10-cm long. The debris that was left to overwinter on the soil surface was spread on the soil surface in containers E & F. The debris left to overwinter under the soil surface was buried 5 cm under the soil surface in the containers B & D. No debris was placed in containers A & C (controls). On 26 April linseed seeds (cv. Antares, untreated) with 4% (sample I) and 28% (sample II) incidence of *A. linicola* infection (on surface-sterilized seed), when tested by the method described in section 6.3.1, were sown in the containers. The seeds were sown at a depth of 5 cm and a rate of 600 seeds per container. The containers C, D and E were sown with seed from sample I, whereas the containers A, B and F were sown with seed from sample II.

### 8.3.3. Survival structures

In November 1992, linseed stem debris artificially inoculated with *A. linicola* was cut into pieces 10-cm long. Two plastic trays (35 x 26 x 7 cm), one containing sterilized loam soil and the other containing unsterilized loam soil were used for the experiment. The debris was either spread on the surface of the sterilized and unsterilized soil or placed in a nylon net bag (30 x 30 cm, 2 mm mesh) and suspended in the air (above the soil) (Fig. 8.4). The trays and the bag with the debris were placed outdoors to be exposed to the environmental conditions during winter. After two (January 1993) and four (March 1993) months, debris from the trays and the bag was recovered, stained with cotton blue in lactophenol (see section 2.1.8) and examined under a light microscope (x 250 magnification) for the presence of survival structures.

### 8.3.4. Survival on volunteers

In 1992, in order to study if volunteers can be a source of primary inoculum of *A. linicola*, a linseed field naturally infected by *A. linicola* was left unploughed after harvest (Field A, Fig. 9.1). On 6 October, approximately 18 days after harvest, the first volunteers with symptoms on their cotyledons were observed in the field (Fig. 8.5). Samples, of 200 plants each, were collected at random throughout the field and the cotyledons, stems, hypocotyledons or leaves with symptoms were examined under a stereo-microscope (x 40 magnification) for the presence of *A. linicola* conidia. If there were no conidia present, then the plant tissues were incubated by the method described in section 2.2.1 in order to induce sporulation. A total of six samples were collected at monthly intervals

**Figure 8.4**. Linseed stem debris, artificially inoculated with *A. linicola,* used to study the survival structures of the pathogen. The debris was placed either on the soil surface of sterilized (a) and unsterilized (b) soil or suspended in the air (c) and exposed to the environmental conditions during the period November 1992 to March 1993.

**Figure 8.5**. Symptoms of *A. linicola* on the cotyledons of volunteer linseed plants which had emerged in an unploughed linseed field on 6 October 1992.

until March and the percentage of plants with *A. linicola* infection on the cotyledons, stems, hypocotyledons and leaves was estimated.

### 8.3.5. Survival on alternate hosts

To study if *A. linicola* can overwinter on weeds, a linseed field naturally infected by *A. linicola* was left unploughed after harvest in 1992 (Field A, Fig. 9.1). The field was inspected at approximately two-weekly intervals for the presence of symptoms on weeds that grew in the field. In November 1992, necrotic lesions were observed on the leaves of a few plants of the species *Veronica agrestis* (common name : field speedwell). When leaves with lesions were collected and examined under a stereo-microscope (x 25 magnification) *Alternaria* conidia similar to those of *A. linicola* were observed on the lesions. Lesions were excised, surface sterilized with 1% sodium hypochlorite (NaOCl) for 1 min, washed with sterile distilled water and placed on V-8 juice agar plates (see section 2.1.7). The plates were sealed with parafilm and incubated under diurnal NUV-light [12h NUV-light (365 nm)/12 h darkness] at 15°C. After 7 days of incubation the fungal colonies that grew readily from the tissues were examined under a stereo-microscope (x 25 magnification) for the presence of conidia similar to those of *A. linicola* conidia. Two single-spore cultures were prepared from the colonies on which *Alternaria* conidia similar to those of *A. linicola* were present. Fifty conidia of a single-spore isolate and 50 *Alternaria*-type conidia found on the leaves of the *V. agrestis* plants in the field, were examined under a light microscope (x 400 magnification). The total length (including the beak) of each conidium, the maximum width of the cells and the length of the beak were

measured. The dimensions of these conidia were compared with those of conidia of an *A. linicola* isolate (Al 24, belonging to the Rothamsted Experimental Station collection) and with the dimensions of conidia of *A. linicola* given by the International Mycological Institute (David, 1991).

The pathogenicity of the mixture of the two single-spore cultures isolated from the *V. agrestis* plants was tested on linseed seedlings and on *V. agrestis* plants. Moreover, the pathogenicity of these cultures was compared with that of two single-spore isolates of *A. linicola* (Al 24 & Al 27) on *V. agrestis* plants and on linseed seedlings. *V. agrestis* plants and linseed seedlings were grown from commercial weed and linseed seed (cv. Antares, treated with prochloraz and free of *A. linicola* infection), respectively, in six trays (35 x 26 x 7 cm). The trays containing the mixture of soil described in section 2.1.5 were placed in an unheated glasshouse (temperature range 10 - 25$^{0}$C). For the preparation of the inoculum for artificial inoculation, the method described in section 2.1.3 was used. When the *V. agrestis* plants and the linseed seedlings were 38 and 14 days old, respectively, they were inoculated with spore suspensions of 4 x $10^{4}$ conidia $ml^{-1}$ by the method described in section 2.1.6. Four trays (two with *V. agrestis* plants and two with linseed seedlings) were inoculated with the conidial suspension of the fungus that had been isolated from the *V. agrestis* plants. Four more trays (two with *V. agrestis* plants and two with linseed seedlings) were inoculated with the mixture of two *A. linicola* isolates (Al 24 & Al 27). The other four trays were sprayed with water (controls). All the trays were covered with polyethylene bags sprayed inside with water (100% r.h.) to provide a water-saturated atmosphere favourable for infection and incubated in a heated

glasshouse (temperature range 15 - 25$^0$C) until the symptoms appeared.

## 8.4. Results

### 8.4.1. Survival on debris

#### 8.4.1.1. Glasshouse experiment

*A. linicola* survived on the infected stem debris during the experimental period (15 November 1992 to 15 April 1993) irrespective of the treatment of the debris. The fungus remained viable, even after 5 months of exposure of the stem debris to unfavourable environmental conditions both indoors (dryness) and outdoors (alternating wet-dry conditions, low temperatures, ground frost) (Table 8.1).

No sporulation of *A. linicola* was observed on the debris (either on those kept indoors or on those left to overwinter outdoors) immediately after the monthly samples were taken during the period 15 November 1992 to 15 March 1993. Some conidia were present on 15 April 1993 but only on the stem debris left to overwinter outdoors on the soil surface. However, conidiophores and conidia of *A. linicola* were produced on all the stem debris, even on those kept under dry conditions (indoors), after incubation in dew chambers under diurnal NUV-light.

The percentage of conidial germination was high and by mid-April more than 95% of the conidia produced on the stem debris kept indoors under dry conditions (either on the soil surface or buried 5 cm underneath the soil surface), were viable (Fig. 8.6). Similarly, the viability of the conidia produced on the debris left to overwinter (for 5 months) outdoors on the soil surface was high

**Table 8.1.** Mean maximum and minimum temperatures and numbers of days with ground frost during the periods 15 November 1992 to 15 April 1993 and 25 November 1993 to 25 April 1994.

| Period of the year | Mean air temperature | | Number of days with ground frost |
|---|---|---|---|
| | Maximum | Minimum | |
| **1992 - 1993** | | | |
| 15 Nov. 1992 - 15 Dec.1992 | 8.9 | 2.7 | 18 |
| 15 Dec. 1992 - 15 Jan. 1993 | 6.2 | 0.4 | 20 |
| 15 Jan. 1993 - 15 Feb. 1993 | 7.7 | 3.1 | 31 |
| 15 Feb. 1993 - 15 Mar. 1993 | 7.9 | 1.7 | 20 |
| 15 Mar. 1993 - 15 Apr. 1993 | 11.4 | 3.8 | 17 |
| **1993 - 1994** | | | |
| 25 Nov. 1993 - 25 Dec. 1993 | 7.9 | 2.5 | 13 |
| 25 Dec. 1993 - 25 Jan. 1994 | 7.2 | 1.3 | 23 |
| 25 Jan. 1994 - 25 Feb. 1994 | 6.1 | 0.4 | 22 |
| 25 Feb. 1994 - 25 Mar. 1994 | 10.5 | 3.4 | 16 |
| 25 Mar. 1994 - 25 Apr. 1994 | 10.4 | 3.5 | 13 |

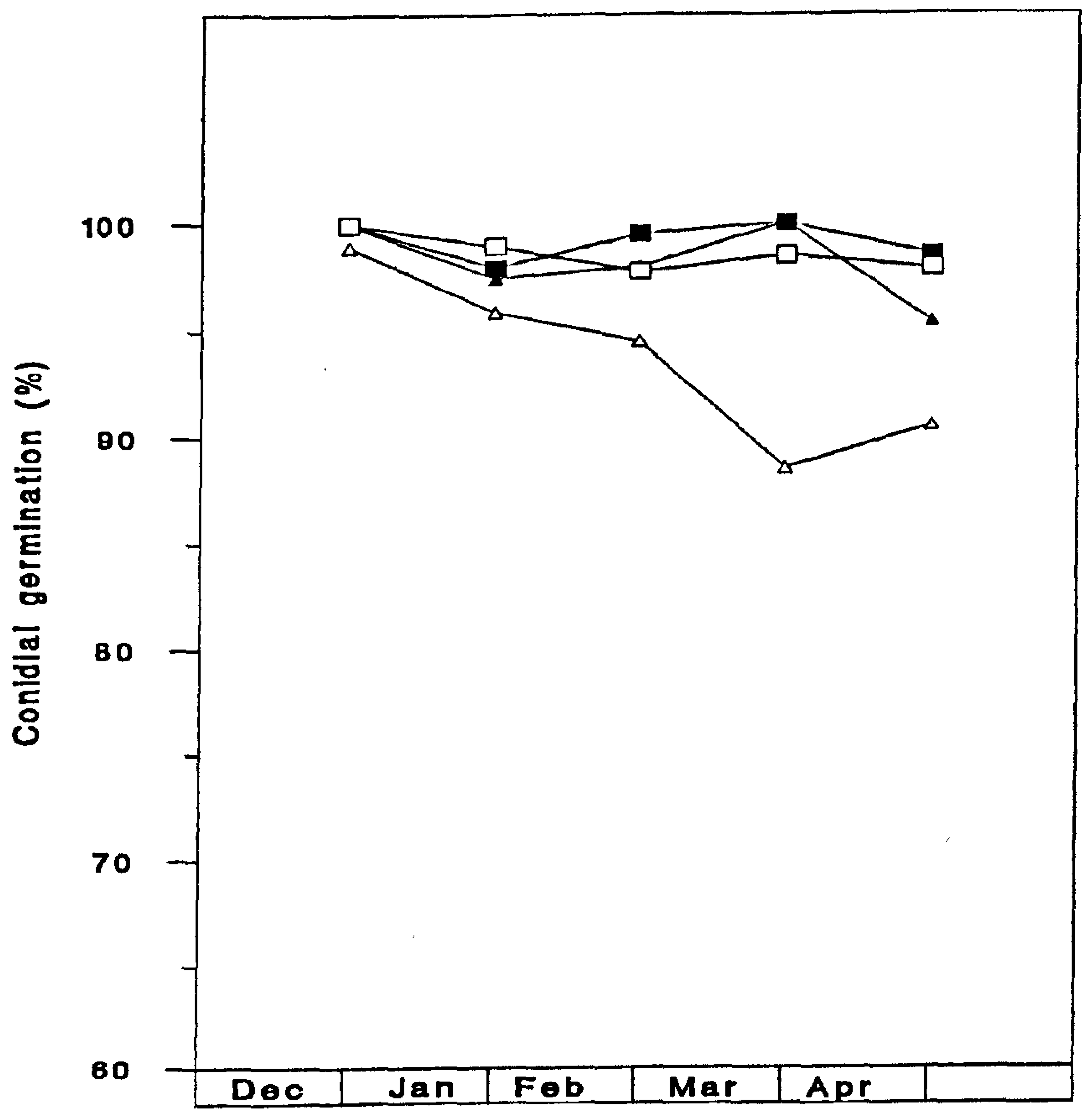



**Figure 8.6.** Viability (% germination) of *A. linicola* conidia produced on infected linseed stem debris left to overwinter indoors (dry conditions, temperature range 15 - 25°C) ( ■ , ▲ ) or outdoors exposed to the environmental conditions ( □ , △ ), either on the soil surface ( ■ , □ ) or buried 5 cm underneath the soil surface ( ▲ , △ ), during the period 15 November 1992 to 15 April 1993. The mean maximum - minimum temperatures and the numbers of days with ground frost are given in Table 8.1. No statistical analyses were done on the data as they were no replicates.

(95%) (Fig. 8.6). Although there was a slight decrease during the experimental period (15 November 1992 to 15 April 1993) in the percent germination of the conidia produced on the debris kept outdoors 5 cm beneath the soil surface, 91% of these conidia were viable at the end of the fifth month (Fig. 8.6).

The pathogenicity to young linseed seedlings of the conidia produced as the percent of plants showing symptoms varied during the 5 months experimental period (Fig. 8.7). However, conidia of *A. linicola* produced on stem debris kept either indoors or outdoors for 5 months were still pathogenic to linseed (Fig. 8.7).

8.4.1.2. Field experiment

In 1993, when the linseed stem debris, naturally infected by *A. linicola,* was left to overwinter in the field either on the soil surface or 20 cm beneath the soil surface, the fungus remained viable during the experimental period (25 November 1993 to 25 April 1994). The pathogen survived on the debris despite the low temperatures and ground frosts that occurred during that period (Table 8.1). Conidiophores and conidia of *A. linicola* were produced on these debris after its incubation in dew chambers under diurnal NUV-light. All the conidia produced on the debris left for one month, either on the soil surface or buried 20 cm beneath the surface, were viable (100% germination) (Fig. 8.8). However, after five months of exposure (25 April 1994) only 88% and 70% of the conidia produced on the debris left on the soil surface or buried beneath the surface, respectively, were viable and pathogenic to linseed plants (Fig. 8.8 & Fig. 8.9).

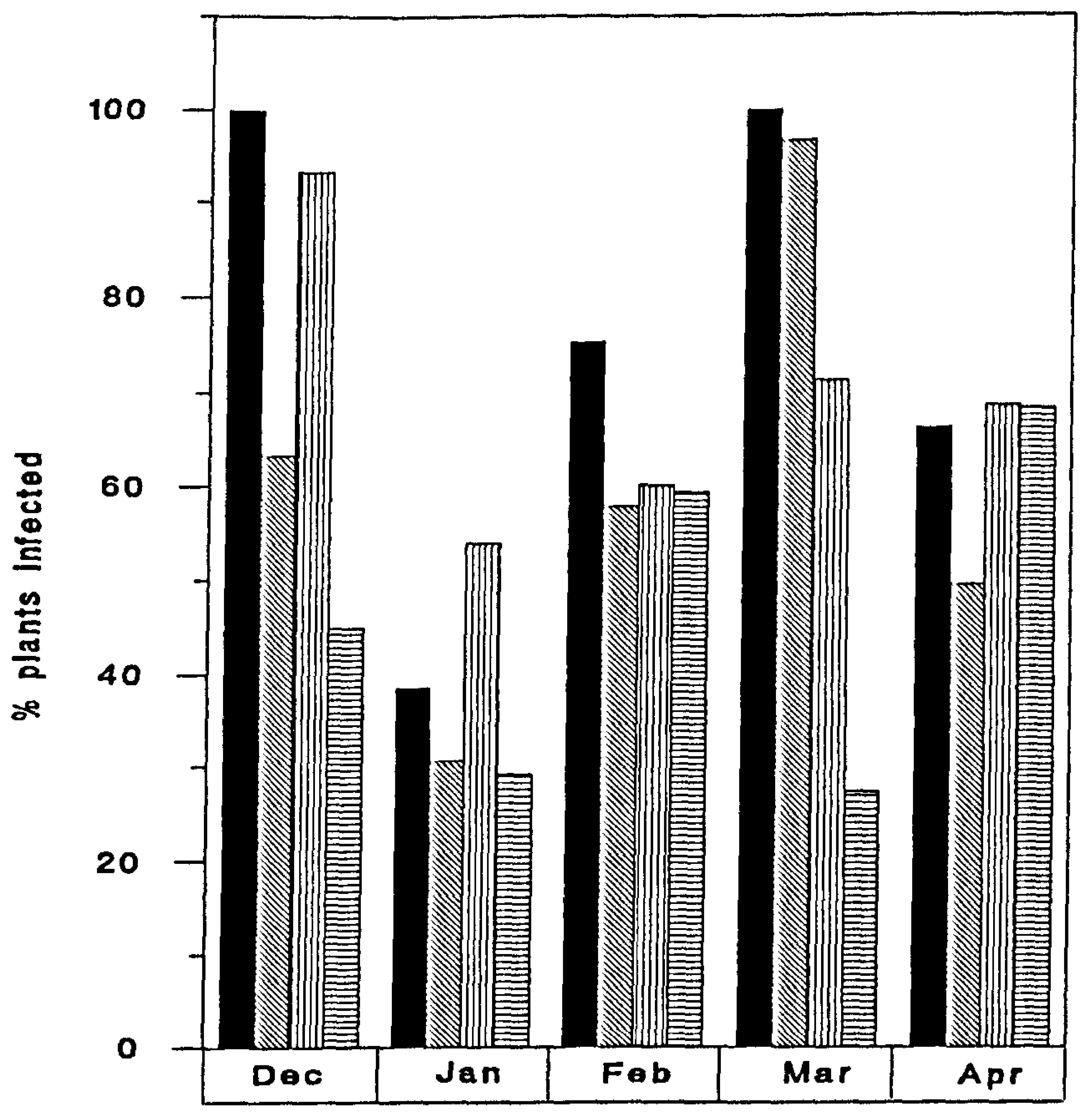



**Figure 8.7.** Pathogenicity (% seedlings infected) of *A. linicola* conidia produced on infected linseed stem debris left to overwinter indoors (dry conditions, temperature range : 15 - 25°C) ( ■ , ▧ ) or outdoors exposed to the environmental conditions ( ▥ , ▤ ) either on the soil surface (■ , ▥) or buried 5 cm beneath the soil surface ( ▧ , ▤ ), during the period 15 November 1992 to 15 April 1993. SED (32 d.f.) = 10.88. The mean maximum - minimum temperatures and the numbers of days with ground frost are given in Table 8.1.

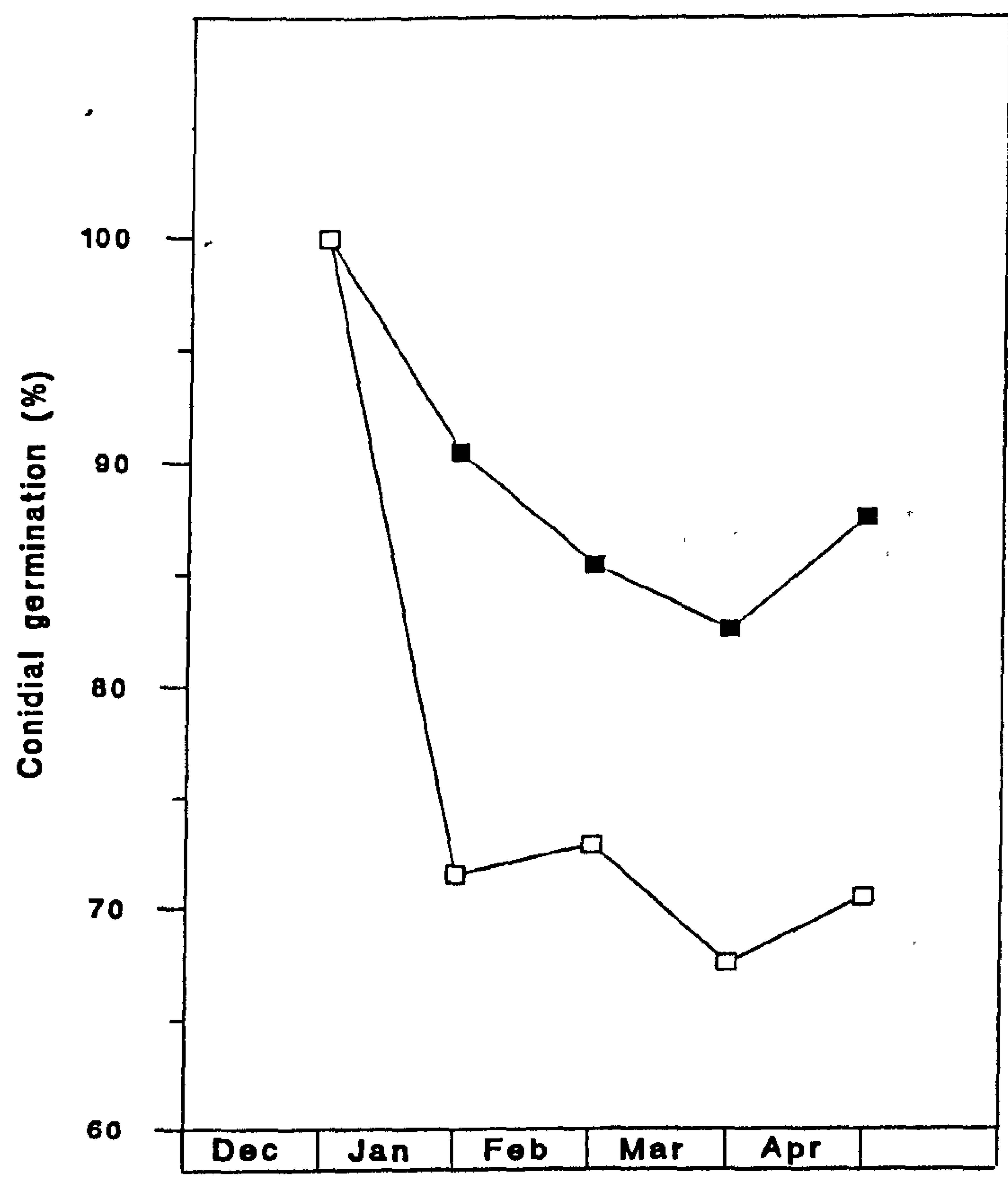



**Figure 8.8.** Viability (% germination) of *A.linicola* conidia produced on infected linseed stem debris left to overwinter in the field, either on the soil surface (■) or buried 20 cm beneath the soil surface (□) during the period 25 November 1993 to 25April 1994. No statistical analyses were done on the data as they were no replicates.

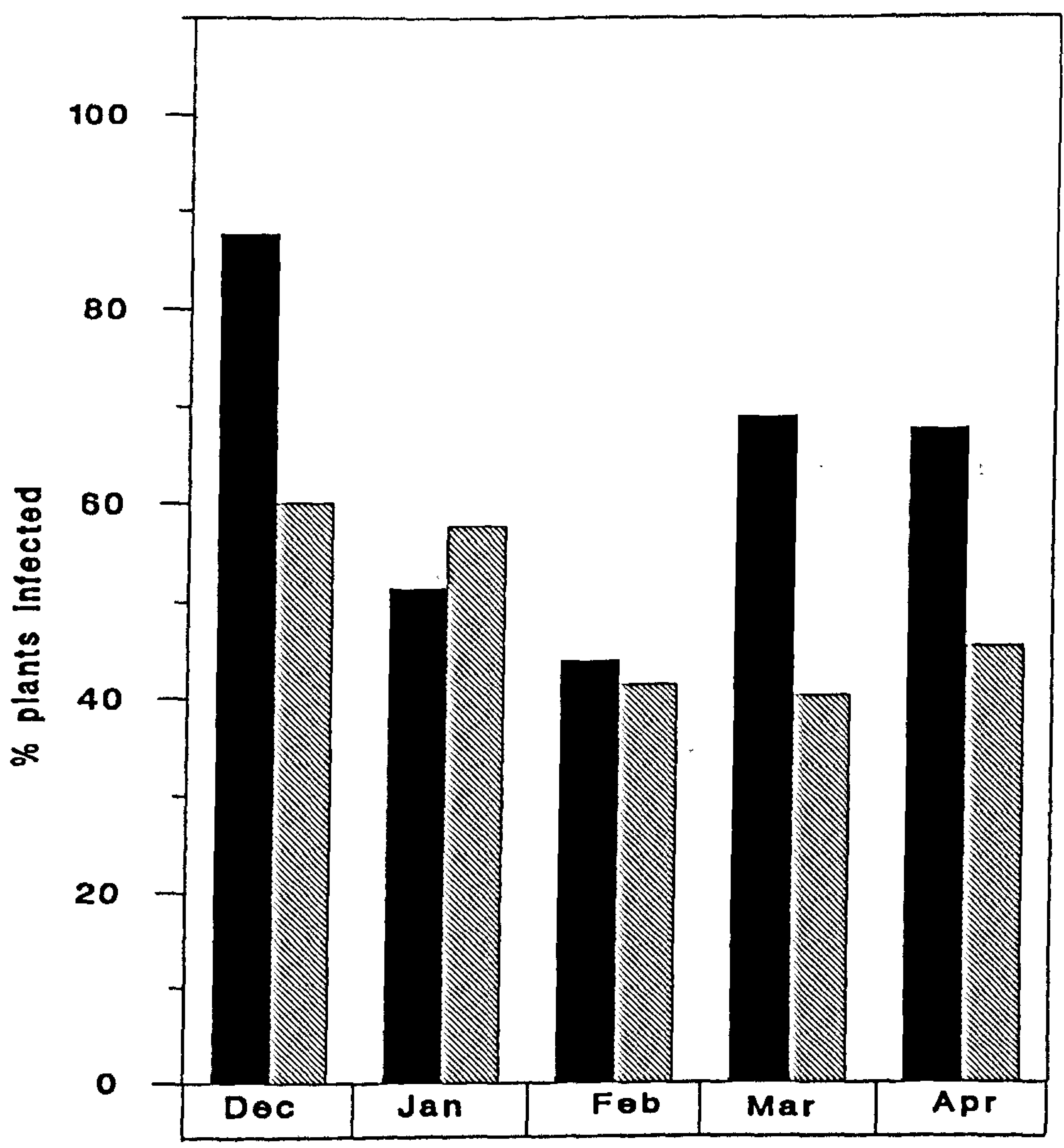



**Figure 8.9.** Pathogenicity (% seedlings infected) of *A. linicola* conidia produced on infected linseed stem debris left to overwinter in the field, either on the soil surface ( ■ ) or buried 20 cm beneath the soil surface ( ▧ ) during the period 25 November 1993 to 25 April 1994. SED (24 d.f.) = 8.94. The mean maximum - minimum temperatures and the numbers of days with ground frost are given in Table 8.1.

### 8.4.2. Effects of infected debris on the onset of an epidemic

#### 8.4.2.1. Experiment I - 1992

Ten days after sowing, when the linseed plants were at growth stage 2 (Fig. 1.2), the first symptoms of *A. linicola* infection appeared on the cotyledons (Fig. 8.10). When seed with 1% incidence of *A. linicola* infection and treated with prochloraz was sown in the container with infected debris on the soil surface, 1% and 4% of the emerged seedlings showed symptoms by 10 May and 25 May, respectively (Fig. 8.11). However, when the same seed was sown in the container without debris (control) no symptoms of *A. linicola* were observed on the cotyledons of the emerged plants (Fig. 8.11). When untreated seed with 17% incidence of *A. linicola* infection was sown in soil with infected debris, the disease incidence on the emerged seedlings was 19% and 37% on 10 May and 25 May, respectively (Fig. 8.11). In soil without debris (control) 10% and 16% of the plants emerged from treated seed had *A. linicola* infection on their cotyledons by 10 May and 25 May, respectively (Fig. 8.11).

#### 8.4.2.2. Experiment II - 1993

The first symptoms of *A. linicola* infection appeared on the cotyledons of the emerged seedlings approximately 3 weeks after sowing (1 June). Generally the disease incidence was greater on the plants which emerged in containers with infected debris on the soil surface than on those which emerged in containers without debris (Fig. 8.12).

When untreated seed with 14% incidence of *A. linicola* infection was sown in the containers with or without debris on the soil surface, 49% and 5% of the emerged plants, respectively, showed symptoms on their cotyledons 19 days after sowing (1 June) (Fig. 8.12A). The disease incidence on these plants increased with time and one month later it was 95% and 58%, respectively (Fig. 8.12A). Seed

**Figure 8.10**. Symptoms of *A. linicola* infection on cotyledons of linseed seedlings (cv. Antares, untreated with 17% incidence of infection on surface sterilized seed) which emerged in soil with infected linseed stem debris on its surface on 10 May 1992.

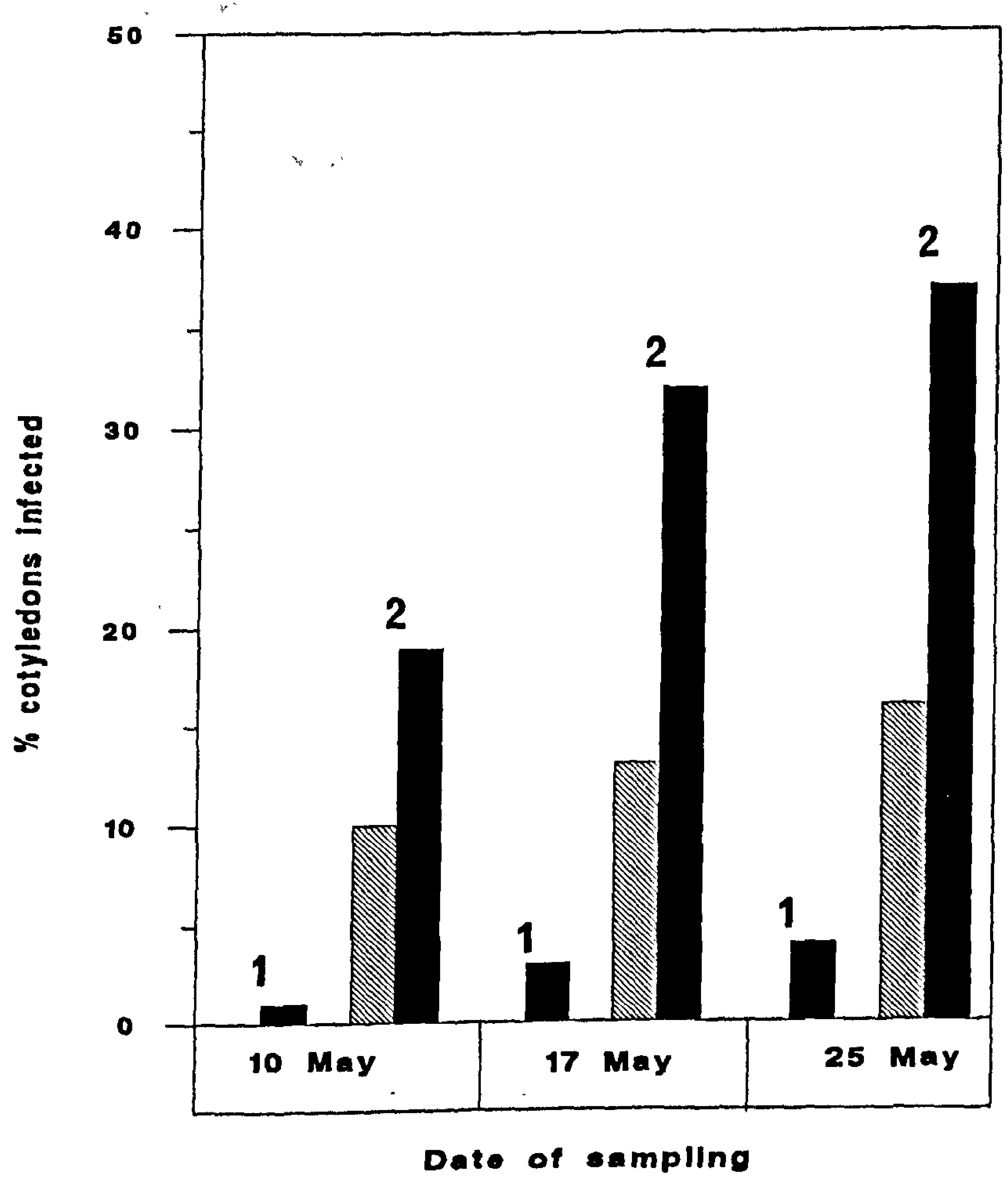



**Figure 8.11.** Effects of infected linseed stem debris on the incidence of *A. linicola* on the cotyledons of linseed seedlings derived from seed (cv. Antares) with 1% (1) and 17% (2) incidence of *A. linicola* infection and sown in soil with ( ■ ) or without ( ▨ ) debris on the surface in 1992.

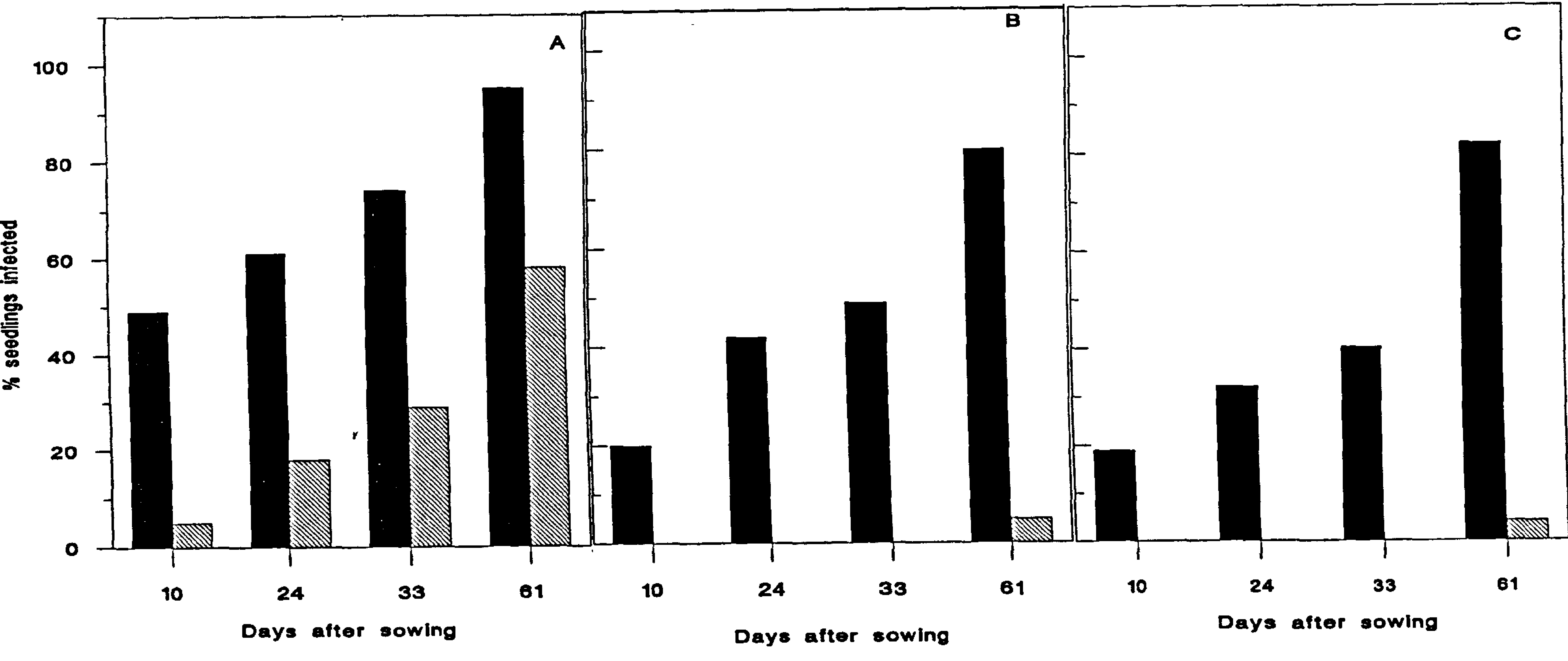



**Figure 8.12.** Effects of infected linseed stem debris on the incidence of *A. linicola* on the cotyledons of linseed seedlings derived from seed (cv. Antares) : (A) untreated, with 14% incidence of *A. linicola* infection; (B) treated with prochloraz (4 g a.i. $kg^{-1}$ seed, Prelude 20LF, Agrichem), with 4% incidence of *A. linicola* infection, or (C) untreated with 1% incidence of *A. linicola* infection and sown in soil with ( ■ ) or without ( ▧ ) debris on the surface in 1993.

from the same origin, treated with prochloraz with 4% incidence of *A. linicola* infection produced 20% and 80% of emerged plants with symptoms of *A. linicola* infection 3 (1 June) and 7 (4 July) weeks after sowing, respectively, in the container with debris on the soil surface (Fig. 8.12B). Similarly, when untreated seed with a low incidence of *A. linicola* infection (1%) was sown in soil with infected debris on the surface, 19% and 82% of the emerged plants showed symptoms 3 and 7 weeks after sowing, respectively (Fig. 8.12C). However, the disease incidence on the emerged plants was 0% and 5% on 1 June and 4 July, respectively, when the same seed was sown in soil without debris on the surface (Fig.8.12C).

8.4.2.3. Experiment III - 1994

On 19 May, approximately 3 weeks after sowing, the first symptoms of *A. linicola* infection were observed on the cotyledons of the emerged seedlings (Fig. 8.13). When untreated seeds with 28% and 4% incidence of *A. linicola* infection were sown in soil with debris on the surface, 14% and 8% of the emerged seedlings showed symptoms by 19 May (Fig 8.13). However, when seeds from the same origin were sown in soil either with buried debris or without debris, the disease incidence on the emerged seedlings was much lower (Fig. 8.13). Generally, the disease incidence on the emerged seedlings increased with time, with the rate of increase being greater on the seedlings that emerged in soil with debris on the surface than on those that emerged in soil with buried debris or without debris.

By 7 June, approximately 42 days after sowing, 96%, 10% and 7% of the emerged plants in soil with debris on the surface, with buried debris or without debris, respectively, had become infected by *A. linicola* when untreated seed with 28% incidence of *A. linicola* infection was used (Fig. 8.13). However, when untreated

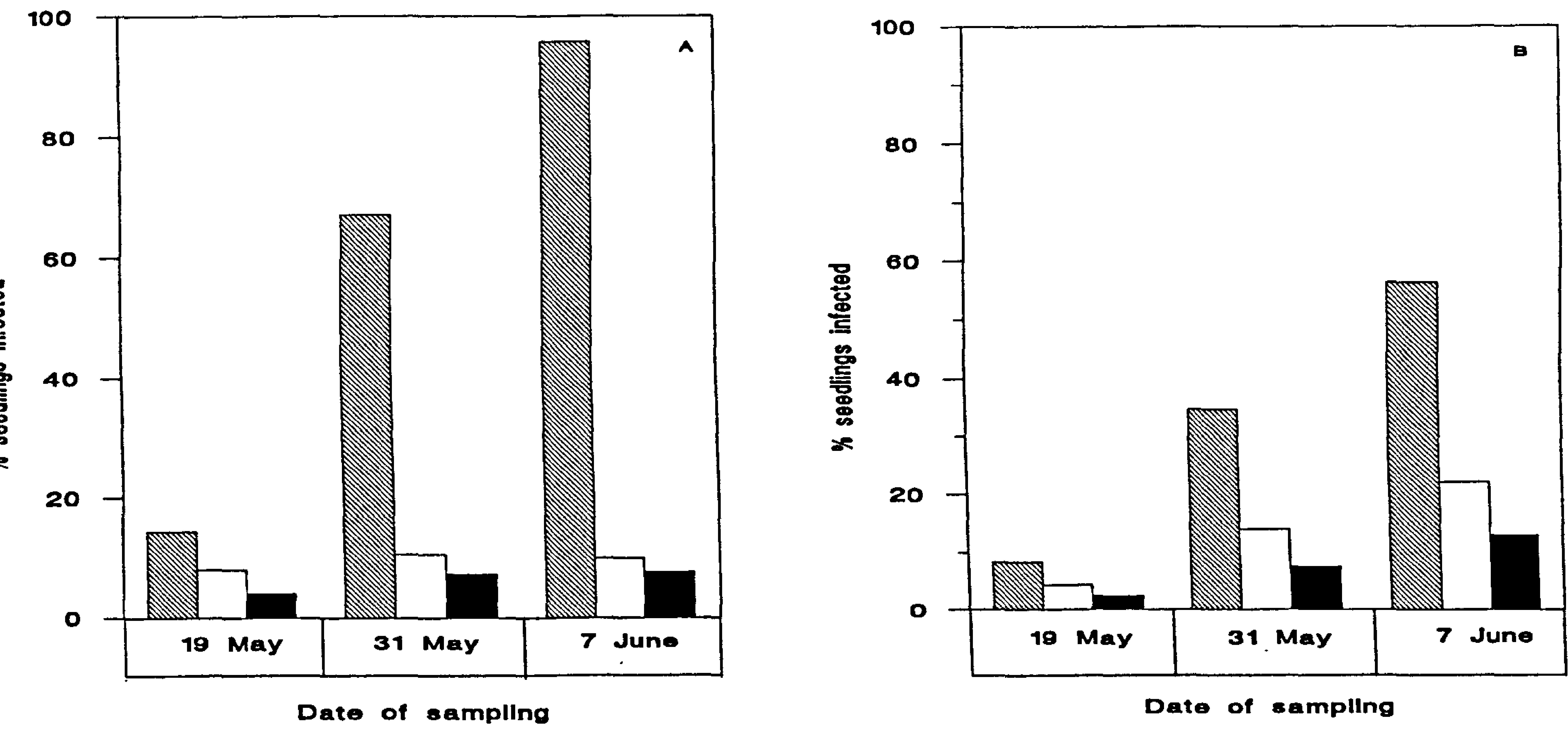



**Figure 8.13.** Effects of infected linseed stem debris on the incidence of *A. linicola* on the cotyledons of linseed seedlings derived from seed (cv. Antares, untreated) with 28% (A) and 4% (B) incidence of *A. linicola* infection and sown in soil with debris on the soil surface ( ▧ ), debris buried 5 cm beneath the soil surface ( □ ) or without debris ( ■ ) in 1994.

seed with 4% incidence of *A. linicola* infection was used, 56%, 22% and 13% of the emerged plants in soil with debris on the surface, with buried debris or without debris, respectively, showed symptoms of *A. linicola* infection on their cotyledons (Fig. 8.13).

### 8.4.3. Survival structures

Structures resembling chlamydospores were observed on the stem debris in contact with the soil (sterilized or unsterilized) at the end of the second month (January 1993) of its exposure outside. These structures were produced from differential swellings in individual hyphal cells of the "curly type" mycelium (Neergaard, 1945) and they were 8 - 15 $\mu$m in diameter, dark brown, single-celled and thick-walled (Fig. 8.14). Similar structures were also present inside *A. linicola* conidia formed on stem debris which was in contact with soil. In this case the chlamydospores were formed either by contraction of the protoplasm of individual cells of the conidium followed by rounding up of the cell contents or by movement of the protoplasm of some cells into neighbouring cells which became denser and darker (Fig. 8.14).

Two months later (March 1993), when the stem debris that was in contact with the soil was examined again under the light microscope, the same structures were present in both mycelium and conidia of *A. linicola*. Moreover, conidiophores arising from the mycelial chlamydospores produced abundant conidia (Fig. 8.14). However, none of these structures was observed on the stem debris that was not in contact with soil.

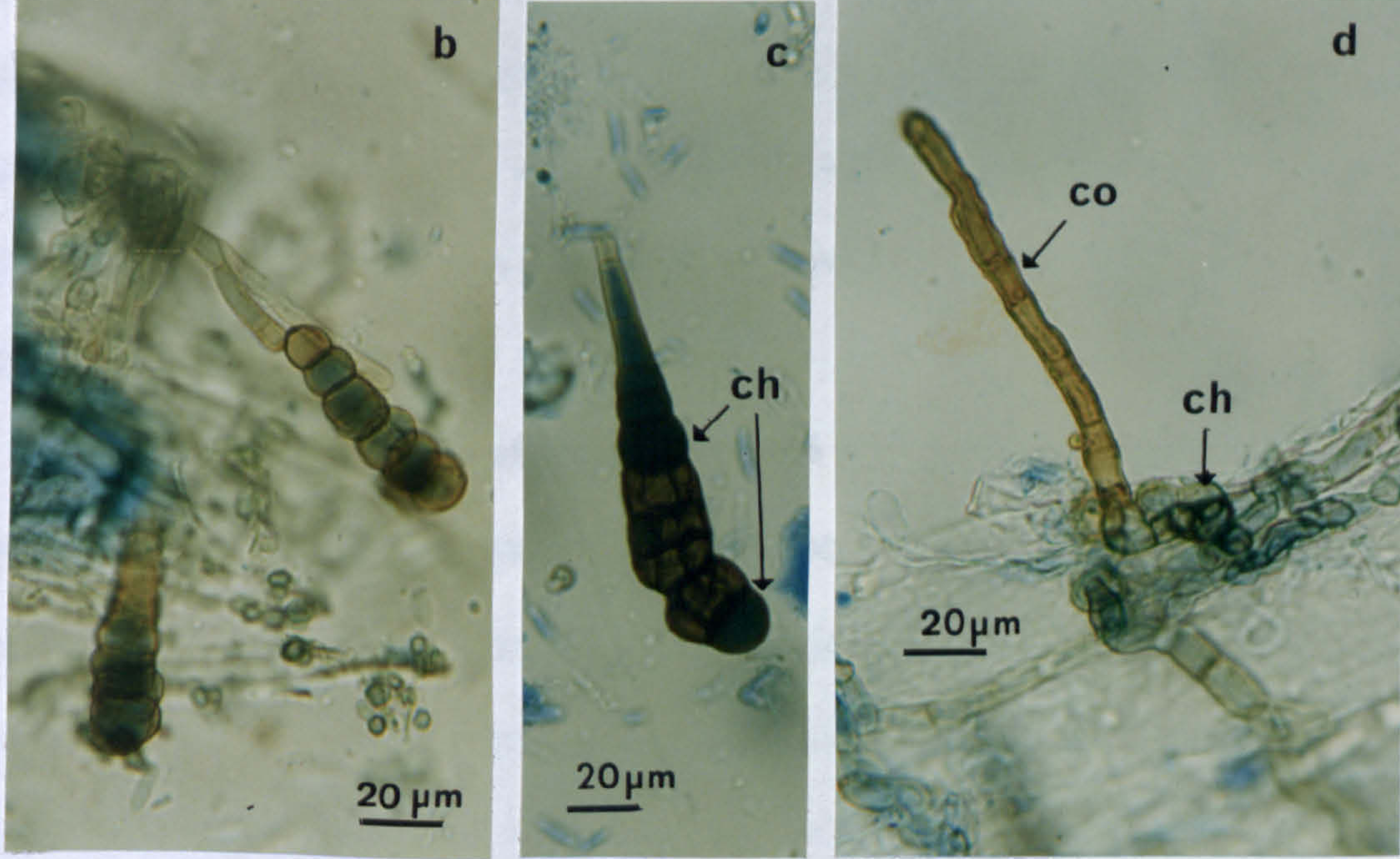



**Figure 8.14.** Structures resembling chlamydospores in mycelium and conidia of *A. linicola* observed in linseed stem debris placed on the soil surface and exposed to the environmental conditions during the period : November 1992 - March 1993. (a) mycelial chlamydospores (January 1993); (b) chlamydospores inside a conidium formed by contraction of the protoplasm of individual cells (January 1993); (c) protoplasm movement in two cells of a conidium forming chlamydospores (January 1993); (d) conidiophore arising from mycelial chlamydospores (March 1993); ch : structures resembling chlamydospores; co : conidiophore. Preparations were stained with cotton blue in lactophenol.

### 8.4.4. Survival on volunteers

In 1992, approximately 18 days after harvest, the first symptoms of *A. linicola* infection appeared on the cotyledons and hypocotyledons of volunteers in the field. Initially, the symptoms on the cotyledons were tiny specks (0.5 mm in diameter) which had a Prout's brown colour (Ridgway, 1912) (Fig. 9.9). Gradually, the specks enlarged to become lesions (3 - 4 mm in diameter) with a definite margin (Fig. 8.15). The colour of these lesions varied from Prout's brown to Dresden brown with a light-coloured centre (Dresden brown) (Fig. 8.15). Concentric zonation was sometimes observed on some of these lesions and several lesions coalesced to form large necrotic areas, particularly near the leaf margin. Under humid conditions the lesions had a water-soaked appearance. Symptoms were visible on both the surfaces of the cotyledons, although their colour was lighter on the lower surface. The lesions on the hypocotyledons were 1 - 3 mm long and 0.2 mm wide, with a Buckthorn brown colour (Fig. 8.16).

On 6 October (18 days after harvest), 67% and 31% of the volunteers showed symptoms of *A. linicola* infection on their cotyledons and hypocotyledons, respectively (Fig. 8.17). One month later the incidence of the disease on the cotyledons was 85% and lesions similar to those on the hypocotyledons appeared for the first time on the stems (Fig 8.17). As the lesions on the cotyledons expanded, a yellow halo appeared and some of the cotyledons died. By mid-November, 25% of the infected plants had dead cotyledons which were covered with conidiophores and conidia of *A. linicola.* On 13 December, when all the volunteers had symptoms on their cotyledons, the first lesions appeared on the leaves. These lesions were 0.5 mm in diameter with a Prout's brown colour

**Figure 8.15**. Symptoms of *A. linicola* infection on the cotyledons of volunteer linseed plants (cv. Antares) emerging in an unploughed linseed field immediately after harvest (6 October 1992).

**Figure 8.16**. Symptoms of *A. linicola* infection on cotyledons and hypocotyledons of volunteer linseed plants (cv. Antares) emerging in an unploughed linseed field immediately after harvest (6 October 1992).

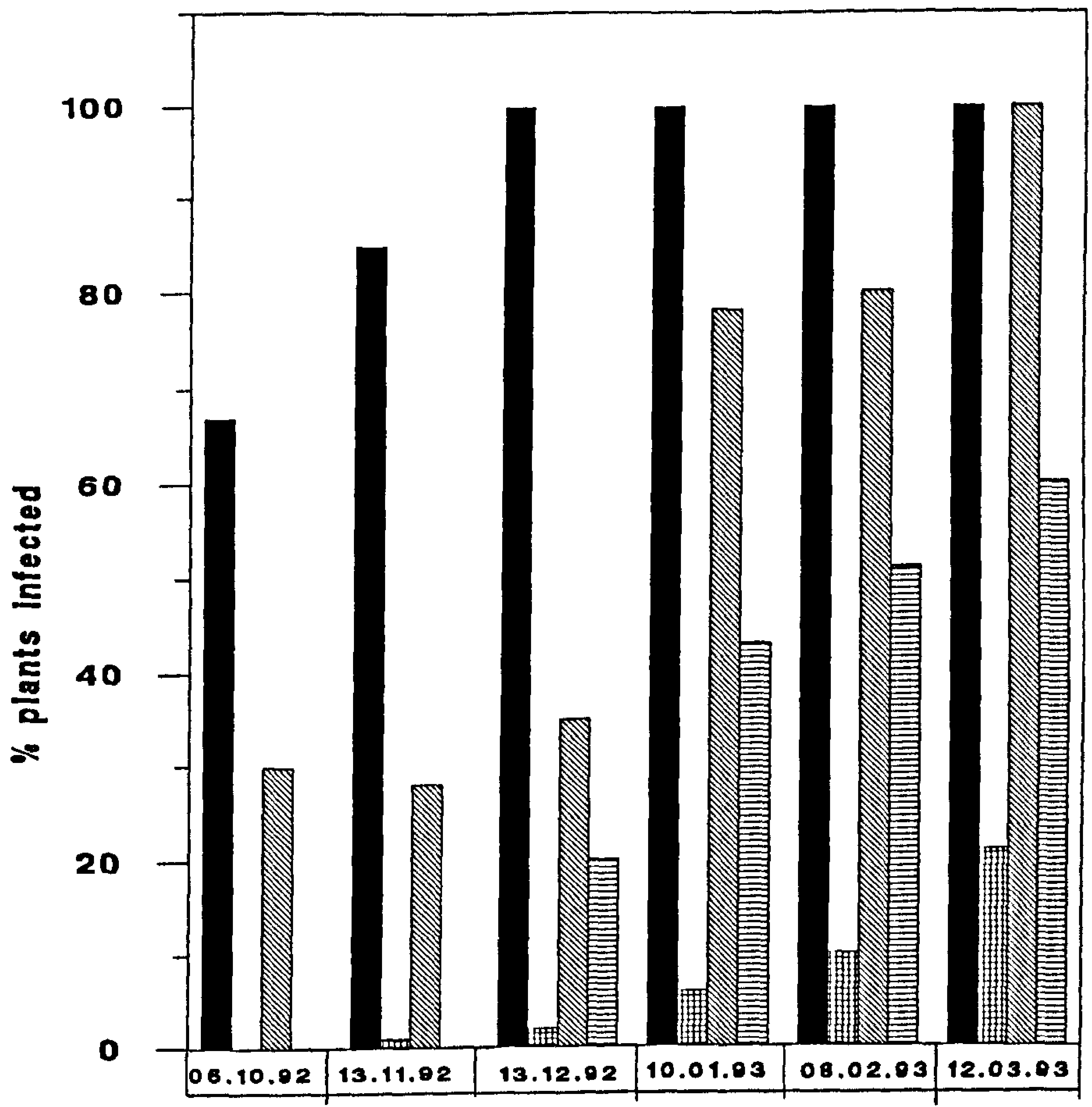



Figure 8.17. Development of *A. linicola* lesions on cotyledons ( ■ ), stems ( ▦ ), hypocotyledons ( ▧ ) and leaves ( ▤ ) of volunteer linseed plants (cv. Antares) which emerged in an unploughed linseed field during the period 6 October 1992 to 12 March 1993.

(Ridgway, 1912) (Fig. 9.9). Although there were 25 days with minimum temperature below $0^{0}C$ and 58 days with ground frost between December 1992 and March 1993, a great number of the volunteers survived. By 13 March 1993, 100% of the volunteers had dead cotyledons covered with *A. linicola* conidia and had symptoms on the hypocotyledons. However, the disease incidence on the leaves and stems was 60% and 21%, respectively (Fig. 8.17).

**8.4.5. Survival on alternate hosts**

The dimensions of the *Alternaria* conidia observed on leaves of the *V. agrestis* plants on November 1992 were similar to those of conidia produced in cultures of isolates from the same plants and to those of conidia of the *A. linicola* isolate (Al 24). These dimensions were also similar to those of conidia in the International Mycological Institute description of *A. linicola* (David, 1991) (Table 8.2). Moreover, the fungus that was isolated from *V. agrestis* plants was pathogenic to linseed and 5 days after inoculation, 100% of the linseed seedlings showed symptoms of *A. linicola* infection on their cotyledons. However, few symptoms (yellow lesions, 1 - 2 mm in diameter) were observed on some leaves of the *V. agrestis* plants inoculated either with the fungus isolated from the *V. agrestis* plants or with the *A. linicola* isolate from linseed plants (Al 24). *A. linicola* conidia were observed on the symptomless leaves of the artificially inoculated *V. agrestis* plants when they were examined under the light microscope. These conidia had not only germinated but also penetrated the leaf tissue (Fig. 8.18). The fungus was re-isolated from the artificially inoculated *V. agrestis* plants. Neither conidia nor symptoms of *A. linicola* were observed on *V. agrestis* plants or on linseed plants that had been sprayed with water (controls).

**Table 8.2.** Dimensions of *A. linicola* conidia derived from different sources.

| Host | Source | Total length (including beak) (μm) | Beak (μm) | Maximum cell width (μm) |
|---|---|---|---|---|
| *Veronica agrestis*[1] | Leaves | 104-259[5] | 52-170 | 14-19 |
| *Veronica agrestis*[2] | Culture | 104-297 | 57-198 | 14-19 |
| Linseed[3] | Al 24 | 106-299 | 57-179 | 14-19 |
| Linseed[4] | IMI | 36-360 | 16-230 | 17-24 |

[1] Conidia of *A. linicola* found on the leaves of *V. agrestis* plants in the field.
[2] Culture of *A. linicola* isolated from *V. agrestis* plants sampled in the field.
[3] Culture of *A. linicola* belonging to the Rothamsted Experimental Station collection and isolated from linseed plants in 1991.
[4] Dimensions of *A. linicola* conidia given by the International Mycological Institute (David, 1991).
[5] Ranges of dimensions.

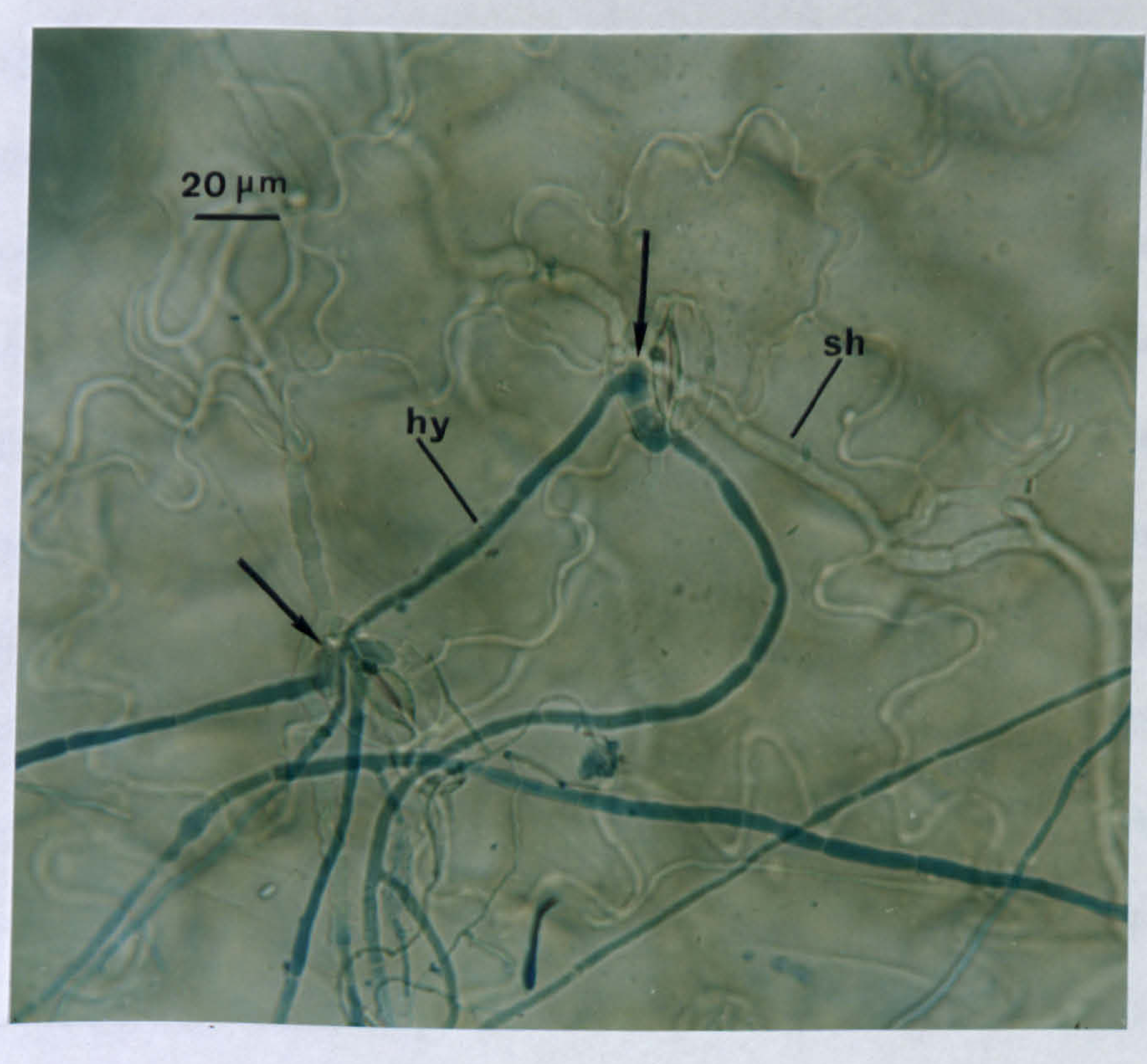



**Figure 8.18**. Penetration of *Veronica agrestis* leaf by *A. linicola*, 5 days after inoculation; (hy) : hyphae on the leaf surface; (sh) : subcuticular hyphae.

## 8.5. Discussion

The results of the present study showed that infected linseed stem debris, volunteer linseed plants and the weed *V. agrestis* can be sources of primary inoculum for the infection of linseed crops by *A. linicola* in addition to the seed. Glasshouse and field experiments showed that *A. linicola* can survive for 5 months during the period between two linseed growing seasons (October - April) on infected debris either on the soil surface or buried 20 cm beneath the soil surface. The survival of *A. linicola* on the debris was not affected by the low temperatures or the ground frost during winter. No sporulation occurred on the debris before until April because the conditions were unfavourable for sporulation (dryness in the glasshouse and low temperatures in the field). However, the fungus produced conidia on the debris when it was exposed to high relative humidity (dew chambers), high temperatures and diurnal NUV-light. These conidia were not only viable (high percent of germination), but also pathogenic to linseed seedlings, causing severe symptoms on the cotyledons. Survival on debris seems to be the main means of overseasoning for most of *Alternaria* species. *A. brassicae* and *A. brassicicola* can survive and produce conidia on infected oilseed rape stubble for up to 5 months (Humpherson-Jones, 1989). Survival on debris has also been reported for *A. macrospora* on cotton (Rotem, 1990), *A. porri* on onions (Pandotra, 1965), *A. dauci* on carrots (Soteros, 1979), *A. cucumerina* on cucurbits (Jackson, 1959), *A. alternata* on tobacco (Von Ramm & Lucas, 1963), *A. triticina* on wheat (Prabhu & Prasada, 1966) and *A. solani* on potatoes and tomatoes (Rotem, 1968).

The present study also showed that infected linseed stem debris left on the

soil surface after harvest can initiate an epidemic which starts early in the growing season. The progress of this epidemic depends not only on the environmental conditions but also on the level of infection on the seed used for sowing. Although the fungus can survive equally well on debris left on the soil surface and on buried debris, it seems that the disease progresses less rapidly when initiated from debris that is buried (even at the same depth as the seed). A possible explanation might be that as no sporulation occurs on the buried debris, the pathogen can colonize the seed only by means of hyphal growth. However, in this case the closer to the stem debris the seed is, the more rapidly the infection of the seed will take place. Moreover, seedlings which emerged in soil with infected debris on the surface were also exposed to infections by air-borne or splash-dispersed conidia that were produced on the debris after wet periods. Jeffrey *et al.* (1984) also reported that *A. helianthi* survived on sunflower stem debris and that infected debris placed on the soil surface initiated severe disease epidemics on emerging sunflower plants. According to Bashan & Hernandez-Saavedra (1992), cotton stem debris infected by *A. macrospora* and left on the soil surface after harvest caused a severe epidemic which spread rapidly in a subsequent crop.

For most *Alternaria* species, survival on debris depends on the survival of the debris itself. Generally debris buried in the soil is likely to be exposed to high soil moisture and microbial activity which decompose it. In the present study linseed stem debris was not decomposed, even when it was buried 20 cm beneath the soil surface. It is possible that either the microbial activity in the soil was low during winter (low temperatures) or the structure of the linseed stems did not allow their decomposition during the experimental period (5 months). This may explain why

*A. linicola* survived equally well on debris left on the soil surface and on debris buried beneath the soil surface. However, decomposition of buried cotton stem debris reduced the survival of *A. macrospora* (Bashan & Hernandez-Saavedra, 1992). Survival of *A. helianthi* on sunflower stem debris was also reduced if the debris was buried (Jeffrey *et al.*, 1984). Furthermore, survival of *A. solani* on potato and tomato stem debris was more successful (for up to 8 months) when the debris was left on the soil surface than when it was buried (Rotem, 1968).

These results suggest that structures resembling chlamydospores, which developed in both mycelium and conidia of *A. linicola* when stem debris were brought in contact with soil, may be involved in the long-term survival of the fungus. It is not known if the resting hyphae of *A. linicola* observed by Mercer & Hardwick (1991) in the outer layer of the linseed seed coat are similar to the structures resembling chlamydospores observed in the present study in the hyphae of *A. linicola.* Moreover, this is the first time that chlamydospores have been reported inside *A. linicola* conidia. Although conidiophores and conidia were produced by the mycelial chlamydospores early in spring (March), the role of the chlamydospores formed inside the conidial cells in the survival of *A. linicola* is not clear. Formation of chlamydospores in hyphal or conidial cells has also been reported for *A. solani* (Basu,1971; Patterson, 1991) and *A. raphani* (Atkinson, 1953). Tsuneda & Skoropad (1977a) reported that *A. brassicae* forms chlamydospores and microsclerotia, with the latter being very resistant to desiccation and low temperatures (- 40°C).

These results suggest that *A. linicola* can overwinter on volunteers or on weeds that grow in unploughed linseed fields after the harvest of the crop.

Volunteer linseed plants can become infected by *A. linicola* early in winter and if some of them survive the low temperatures during winter, they can transfer the inoculum to following linseed crops. Moreover, the common weed *V. agrestis,* which can be found in most fields during winter (Hanf, 1973), may serve as an overseason carrier of the pathogen, although this study suggests that most of the infections are symptomless. It is also possible that *A. linicola* can survive on other weed species that grow in linseed or neighbouring fields during winter without causing any symptoms, although this was not investigated. Many other *Alternaria* species have been reported to survive on volunteers or wild plants. *A. macrospora* can survive on volunteer cotton plants, although these plants are rare in intensive cotton growing areas (Bashan & Hernandez-Saavedra, 1992). *A. longipes*, a pathogen of tobacco plants, causes symptomless infections on the weeds *Datura stramonium* and *Nicandra physaloides* (Riley, 1949). *A. dauci* (Strandberg, 1992) survives on wild carrot plants and *A. radicina* on the weed *Fumaria muralis* (Soteros, 1979), whereas *A. helianthi* survives during winter on *Helianthus annuus* plants (Jeffrey *et al.,* 1984).

Nevertheless, the results of this study emphasize the need for effective burial of the linseed stem debris soon after harvest by deep-ploughing. Although ploughing may not reduce the primary inoculum of *A. linicola*, it may decrease the rate of the progress of the disease in the succeeding crop. However, as large numbers of capsules are deposited on the soil during harvest, it is necessary for the seeds to be allowed to germinate before ploughing to eliminate volunteers which can be another source of inoculum for the following linseed crop. Further investigation is needed on the potential survival of *A. linicola* on weeds, especially on those that grow in

the fields during the period between the harvest of the linseed crop and the sowing of the following linseed crop (September - April). Although crop rotation has been suggested as a means of reducing the primary inoculum of other *Alternaria* pathogens, the longevity of the chlamydospores of *A. linicola* over a number of years and the methods for their eradication need further study.

# CHAPTER IX. EFFECTS OF FUNGICIDE SPRAYS ON *ALTERNARIA LINICOLA* ON LINSEED CROPS

## 9.1. Introduction

*Alternaria linicola* Groves & Skolko is considered to be the most important seed-borne pathogen of linseed (*Linum usitatissimum* L.) in the UK (Mercer *et al.*, 1991a). The epidemiology of the disease caused by *A. linicola* on linseed is only partly understood. The pathogen can decrease emergence by up to 50%, yield by up to 35%, and it can also affect the oil quantity and quality (Mercer *et al.*, 1991a). *A. linicola* causes damping-off symptoms on young seedlings, but if the seedlings survive then symptoms appear on the cotyledons and the lower leaves. According to Mercer *et al.* (1991a) there are no symptoms on the upper leaves but by the end of the growing season, just before harvest, symptoms appear on the upper leaves and capsules, through which the seeds become infected.

Reports on *A. linicola* on linseed crops have discussed disease development during the growing season (Mercer *et al.*, 1991a; Fitt *et al.*, 1991b; 1991c; Fitt & Vloutoglou, 1992). According to those reports the severity of the disease fluctuates from year to year, depending on the weather conditions. Generally, disease outbreaks occur during the period between flowering and harvest, especially when this period is wet (Mercer *et al.*, 1991a). Field studies on the effects of the application of different fungicide sprays to the crop have given variable results depending on the weather conditions during the growing season. Therefore, those studies suggest that the only

effective method for controlling *A. linicola* is seed treatment. Until a few years ago iprodione was used as a seed treatment but because the pathogen developed resistance to iprodione (Mercer *et al.*, 1988), this fungicide has recently been replaced by prochloraz.

## 9.2. Objectives

To study the effects of different fungicides applied as foliar sprays on disease development and crop yield.

## 9.3. Materials and Methods

### 9.3.1. Husbandry and site details

In 1991, a field experiment was sown on the Rothamsted farm (Long Hoos, field A) (Fig. 9.1). The previous crops were spring barley in 1989 and winter wheat in 1990. The experimental area was approximately 0.16 ha and the linseed seed (cv. Antares, untreated) was sown on 10 April at a rate of c. 600 seeds $m^{-2}$. Before sowing the seed was tested *in vitro* by the method described in section 6.3.1 and was found to be free of *A. linicola* infection. Nitram (34.5% nitrogen, ICI Agrochemicals Ltd) was applied on 9 April at 220 kg $ha^{-1}$. The crop was desiccated on 2 September with diquat (120 g a.i. $ha^{-1}$, Reglone, ICI Agrochemicals Ltd). "Vassgro Spreader" (300 ml $ha^{-1}$, Vass) was added to the desiccant as a wetting agent. The crop was combine harvested on 10 October.

For the 1992 field experiment the same site (Long Hoos, field A) (Fig. 9.1),

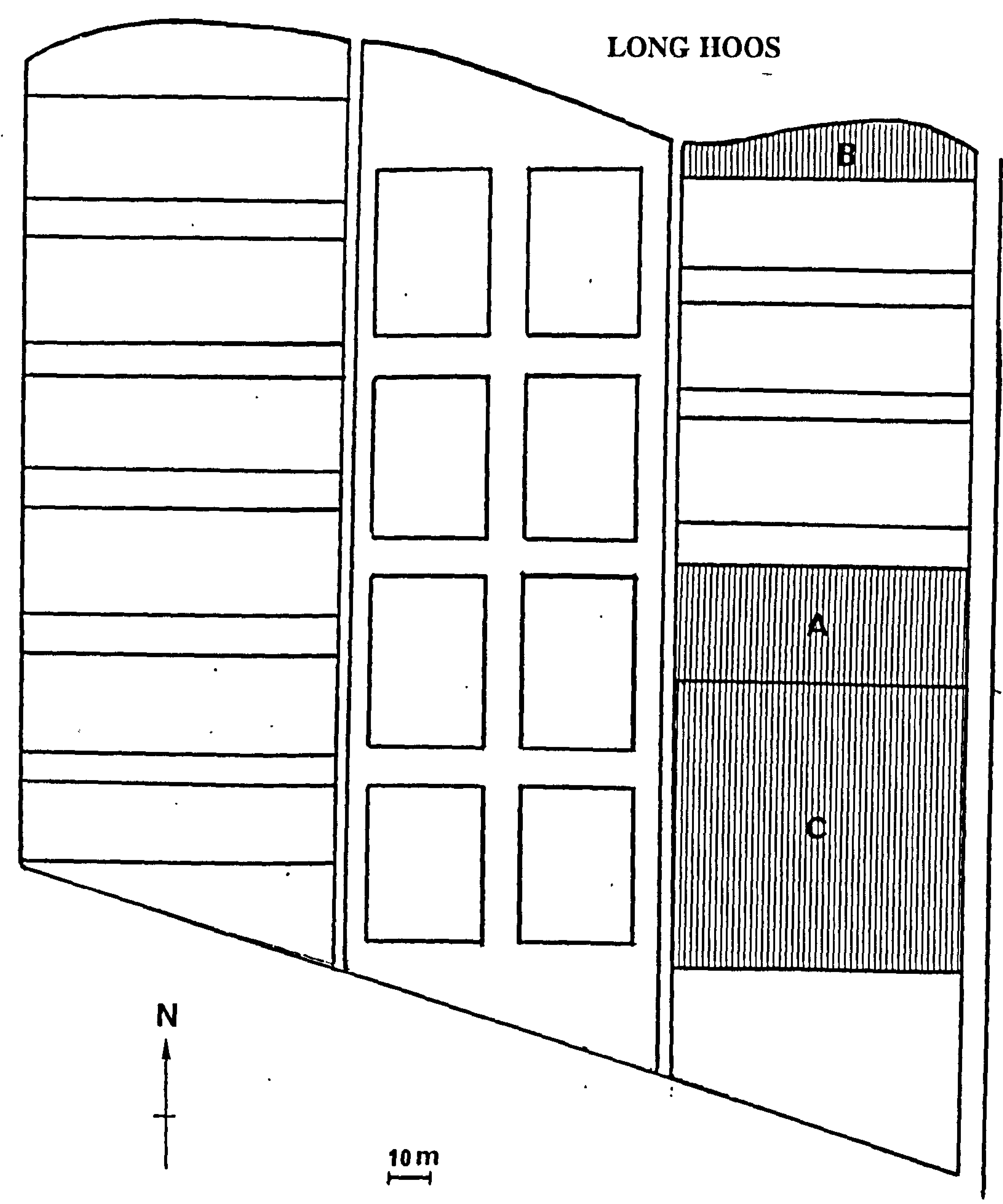



**Figure 9.1.** Siting of linseed crops in 1991, 1992 and 1993 field studies.
A = field where : a) the effects of fungicide sprays on disease development and yield were studied in 1991 and 1992, and b) the Burkard spore sampler was operated in 1992 and 1993. B = field where the Burkard spore sampler was operated in 1991. A & C = fields where the disease and spore dispersal gradients were studied in 1993.

and seed from the same origin (cv. Antares, untreated) as in 1991 were used. The seed was sown on 21 April at a rate of c. 600 seeds $m^{-2}$. Nitram (34.5% nitrogen, ICI Agrochemicals Ltd) was applied on 13 May at 220 kg $ha^{-1}$. The herbicides bentazone (960 g a.i. $ha^{-1}$, Basagran, BASF) and bromoxynil + clopyralid (240 : 50 g a.i. $ha^{-1}$, Vindex, DowElanco) were applied on 2 June. The experimental area was irrigated by using an overhead oscillating system on 18 June, 26 June, 29 June and 28 July with 12 mm of water each time. The crop was desiccated on 16 September with glyphosate (980 g a.i. $ha^{-1}$, Roundup, Monsanto) and combine harvested on 17 September.

9.3.2. Emergence

In both years the emergence of the plants was assessed approximately every 7 days, starting 20 days after sowing. Each time 10 assessments were made at random on row-lengths selected throughout the field. The number of plants which had emerged in a 0.5 m row-length and the number of plants with symptoms on their cotyledons were counted. The plants with symptoms on their cotyledons were incubated by the method described in section 2.2.1 to induce sporulation, and examined under a stereo-microscope (x 40 magnification) for the presence of *A. linicola* conidia. Totals of four and three emergence counts were done in 1991 and 1992, respectively. No statistical analyses were done on the data as there were no replicates of treatments.

9.3.3. Experimental design - Sampling method

In both years the experiments were in randomized blocks. There were five blocks, each of four plots. Each plot of 3 x 10 m was separated from the adjacent plots by a 3 x 10 m strip of linseed (Fig. 9.2 & Fig. 9.3 in 1991 and 1992, respectively).

| I | | - | | - | | B | | B | | - | | I | | B | | P | | I |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | | B | | I | | P | | I | | P | | - | | P | | - | | B |

Figure 9.2. Plan of the 1991 field experiment for testing the effects of fungicide sprays on *A. linicola* infection of linseed plants (cv. Antares). Treatments : I = iprodione (125 g a.i. $ha^{-1}$); P = prochloraz (72 g a.i. $ha^{-1}$); B = benomyl (140 g a.i. $ha^{-1}$); (-) = unsprayed.

| I | | P | | P | | - | | P | | - | | C | | P | | I | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| - | | C | | I | | C | | C | | I | | I | | - | | - | | C |

Figure 9.3. Plan of the 1992 field experiment for testing the effects of fungicide sprays on *A. linicola* infection of linseed plants (cv. Antares). Treatments : I = iprodione (250 g a.i. $ha^{-1}$); P = prochloraz (157.5 g a.i. $ha^{-1}$); C = chlorothalonil (250g a.i. $ha^{-1}$); ( - ) : unsprayed.

Samples of 10 plants each were collected at random on two "W" shaped transects across each plot (Fig. 9.4). In 1991, a total of 12 samples were collected during the growing season with the first six samples being collected at two-weekly intervals and the last six samples at weekly intervals. In 1992, a total of seven samples were collected during the growing season at two-weekly intervals. Growth stages were identified using a key (Turner, 1987; Fig. 1.2) and the dates on which the samples were taken and the growth stages of the plants are described in Tables 9.1 & 9.2 for 1991 and 1992, respectively.

9.3.4. Disease assessments

In both years, cotyledons, stems, leaves, buds, sepals, capsule cases and seeds were assessed for the presence of lesions or other symptoms indicating infection by *A. linicola*. As the symptoms produced by *A. linicola* on cotyledons, stems and leaves of linseed plants were similar to those caused by other fungi (e.g. *Botrytis cinerea*), the identification of the pathogen was only possible after placing the plant tissues in dew chambers and incubating them by the method described in section 2.2.1. In 1992, the number of leaves present at different heights on the stem, counting from the base upwards, the number of leaves with brown lesions or brown tips and the number of leaves infected by *A. linicola* were also assessed on two plants per plot on the sample taken on 17 July from unsprayed plots.

The incidence of *A. linicola* on sepals, capsule cases and seeds was also assessed : for each sample twenty capsules per plot were collected and one sepal per capsule, the underneath part of the capsule case and the seed were removed. All plant parts were treated by the method described in section 2.2.1 to induce

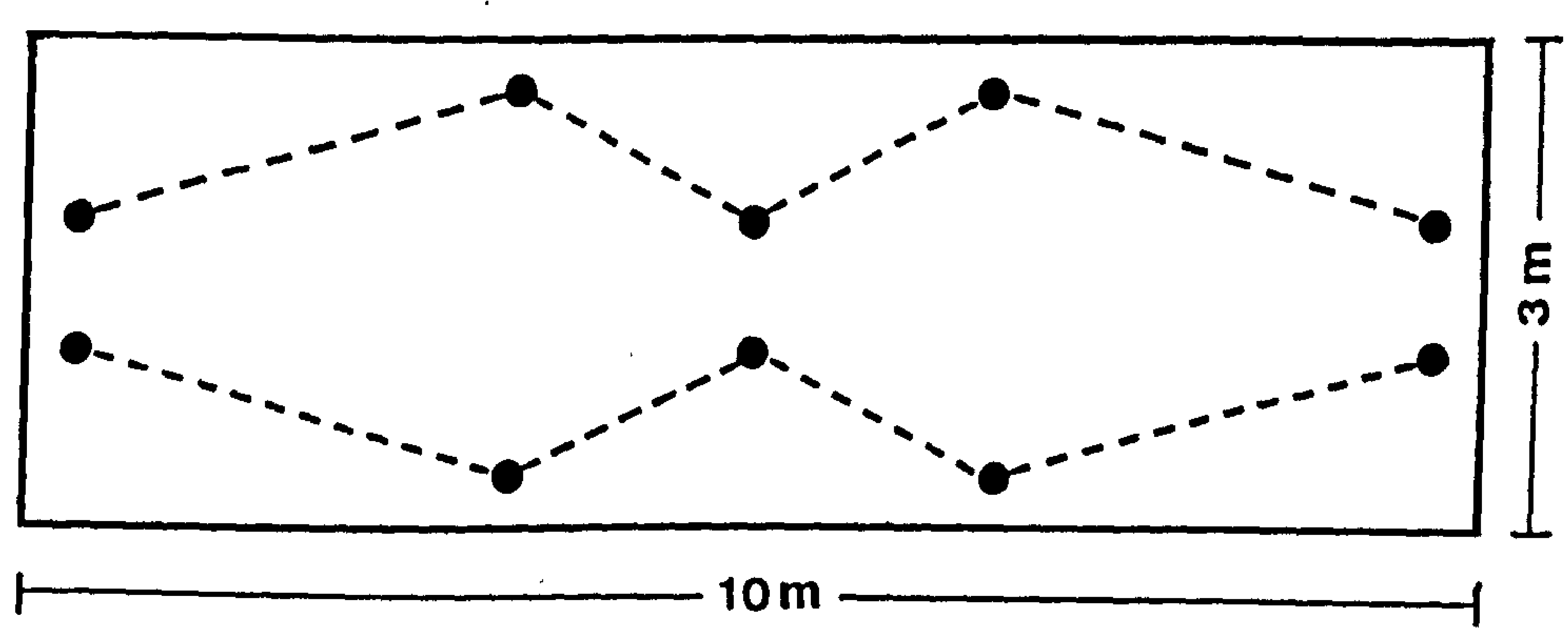



**Figure 9.4.** Method used for sampling within experimental plots in 1991 and 1992. ( ● ) : sampling positions.

**Table 9.1.** Growth stages of linseed (cv. Antares) at which samples were taken and fungicides were applied in 1991.

| Growth stage (GS)[1] | Date of sampling | Date of spray |
|---|---|---|
| 5 | 3 June | |
| 6 | 17 June | |
| 6 | | 20 June |
| 6 - 7 | 1 July | |
| 7 | | 10 July |
| 7 - 8 | 15 July | |
| 8 | | 23 July |
| 8 - 9 | 29 July | |
| 9 - 10 | | 7 August |
| 9 - 10 | 12 August | |
| 10 - 11 | 19 August | |
| 11 | 26 August | |
| 11 | | 27 August |
| 12 | 3 September | |
| 12 | 9 September | |
| 12 | 16 September | |
| 12 | 20 September | |

[1] Turner, 1987 (Fig. 1.2).

**Table 9.2.** Growth stages of linseed (cv. Antares) at which samples were taken and fungicides were applied in 1992.

| Growth stage (GS)[1] | Date of sampling | Date of spray |
|---|---|---|
| 6 | 20 June | |
| 6 | | 24 June |
| 6 | 2 July | |
| 6-7 | | 8 July |
| 7 | 17 July | |
| 7-8 | | 22 July |
| 8 | 30 July | |
| 9 | | 6 August |
| 9-10 | 13 August | |
| 10 | | 19 August |
| 11 | 29 August | |
| 12 | | 10 September |
| 12 | 12 September | |

[1] Turner, 1987 (Fig. 1.2).

sporulation and examined under a stereo-microscope (x 40 magnification) for the presence of *A. linicola* conidia. Each year one sample of fresh seed (post-harvest seed) per plot was also collected, stored at -15°C (1991) or 5°C (1992) and tested for *A. linicola* infection by the method described in section 6.3.1, 8 and 14 months after harvest in 1991 and 1992, respectively. After harvest, samples of seeds from each plot were collected, dried at 80°C, weighed and the seed yield per hectare (corrected to 90% dry weight) was calculated. In 1992, the mean crop height and the mean number of mature capsules per plant were also assessed on the sample taken on 29 August. In both years, records of daily rainfall and temperature (maximum and minimum) were obtained from a meteorological station situated 0.5 - 1 km from the experimental site.

9.3.5. Fungicides

In 1991, fungicide spray treatments were benomyl (140 g a.i. $ha^{-1}$), iprodione (125 g a.i. $ha^{-1}$) or prochloraz (72 g a.i. $ha^{-1}$) (Table 9.3), applied at half the recommended dose, at two-weekly intervals, starting 52 days after emergence. In 1992, fungicide spray treatments were chlorothalonil (250 g a.i. $ha^{-1}$), iprodione (250 g a.i. $ha^{-1}$) or prochloraz (157.5 g a.i. $ha^{-1}$) (Table 9.3), applied at two-weekly intervals, starting 44 days after emergence. In both years the fungicides were applied by a hand-operated sprayer; untreated plots served as controls. The dates on which the fungicides were applied and the growth stages of the plants in 1991 and 1992 are shown in Tables 9.1 & 9.2, respectively.

**Table 9.3.** Information about the fungicides tested in 1991 and 1992.

| Product name | Main supplier | Active ingredient | Active ingredient content | Formulation type[1] |
|---|---|---|---|---|
| **Field experiment in 1991** | | | | |
| Benlate | Du Pont | benomyl | 50% | WP |
| Rovral | RP Agric. | iprodione | 50 g/l | SC |
| Sportak 45 | Schering Agrochemicals Ltd. | prochloraz | 450 g/l | EC |
| **Field experiment in 1992** | | | | |
| Chiltern Chlorothalonil 500 | Chiltern | chlorothalonil | 500 g/l | SC |
| Rovral Flo | RP Agric. | iprodione | 250 g/l | SC |
| Sportak 45 | Schering Agrochemicals Ltd. | prochloraz | 450 g/l | EC |

[1] WP = wettable powder; SC = suspension concentrate ( = flowable); EC = emulsifiable concentrate.

## 9. 4. Results

### 9.4.1. Emergence

Although the crop was sown earlier in 1991, it emerged more slowly than in 1992. The maximum emergence was 100% by 7 May, with 70% of emerged plants with damaged cotyledons (Fig. 9.5). Two different types of symptoms were observed on these cotyledons : a) bites caused by flea beetles and b) small lesions (0.5 - 1 mm in diameter) which were light brown in color, surrounded by a yellow halo and observed mainly at the tips of the cotyledons. Fungi belonging to the genus *Pythium* were consistently isolated from such lesions. The lower percent of emergence at the end of May compared with that in the beginning of the month might have been due to damping-off of seedlings caused by *Pythium* spp.

In 1992, the emergence was only 56% by 25 May, with 100% of emerged plants showing flea beetle damage on their cotyledons (Fig. 9.6). In both years no infection by *A. linicola* was detected on the seedlings during the emergence counts.

### 9.4.2. Disease identification

In both years, it was difficult to distinguish visually between the symptoms caused by *A. linicola* and those caused by *B. cinerea* or natural senescence on leaves, buds and sepals (Fig. 9.7). The identification of *A. linicola* infection on those plant parts was only possible after incubating the plant tissues by the method described in section 2.2.1 in order to induce sporulation. Moreover, stem lesions caused by *A. linicola* could be confused with those caused by *Septoria linicola* (Pasmo), as both were

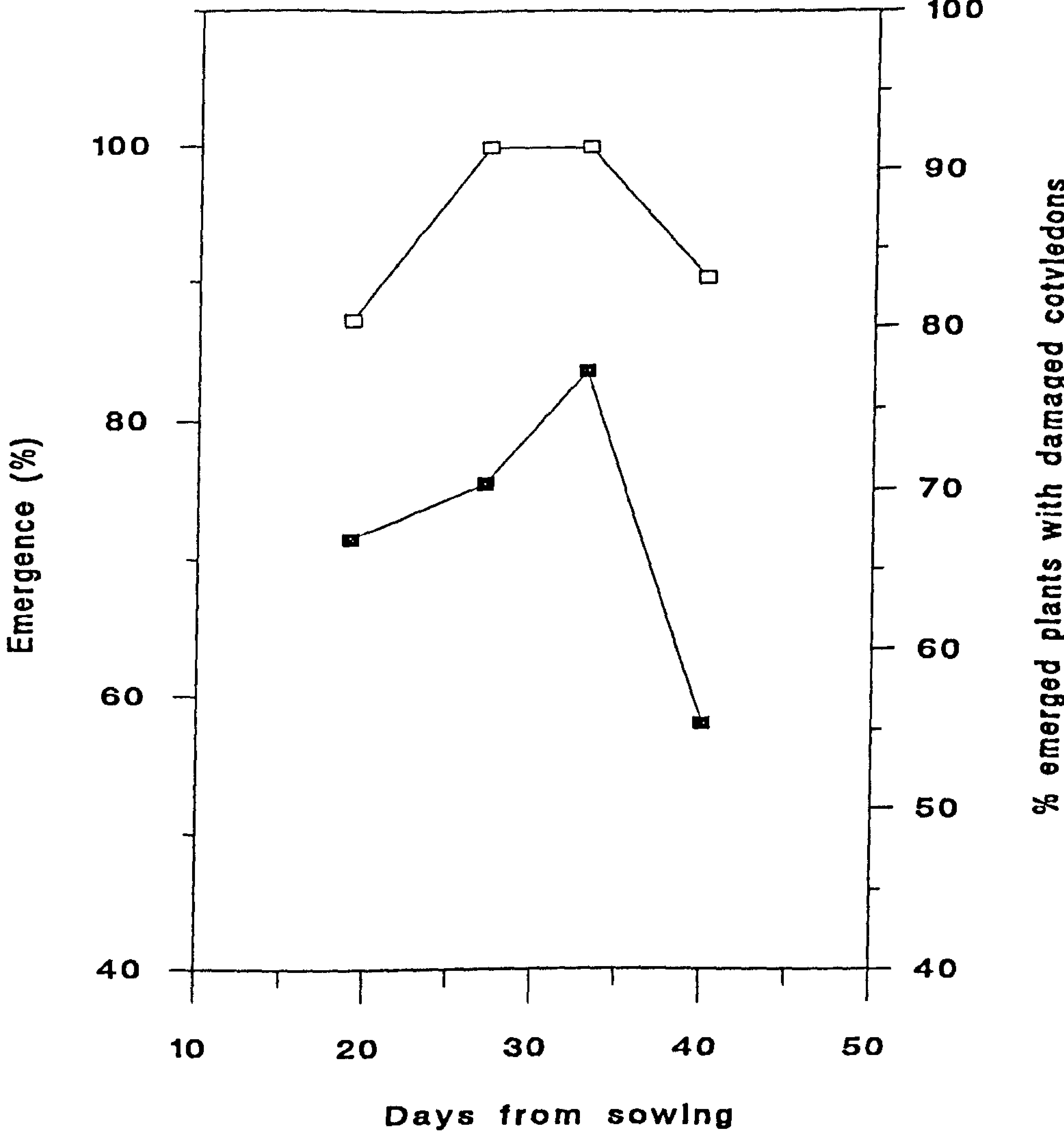



**Figure 9.5.** Emergence (%) of linseed ( □ ) and percentage of emerged plants with damaged cotyledons ( ■ ) in plots sown with untreated seed (cv. Antares) on 10 April 1991. No statistical analyses were done on the data as there were no replicates of treatments.

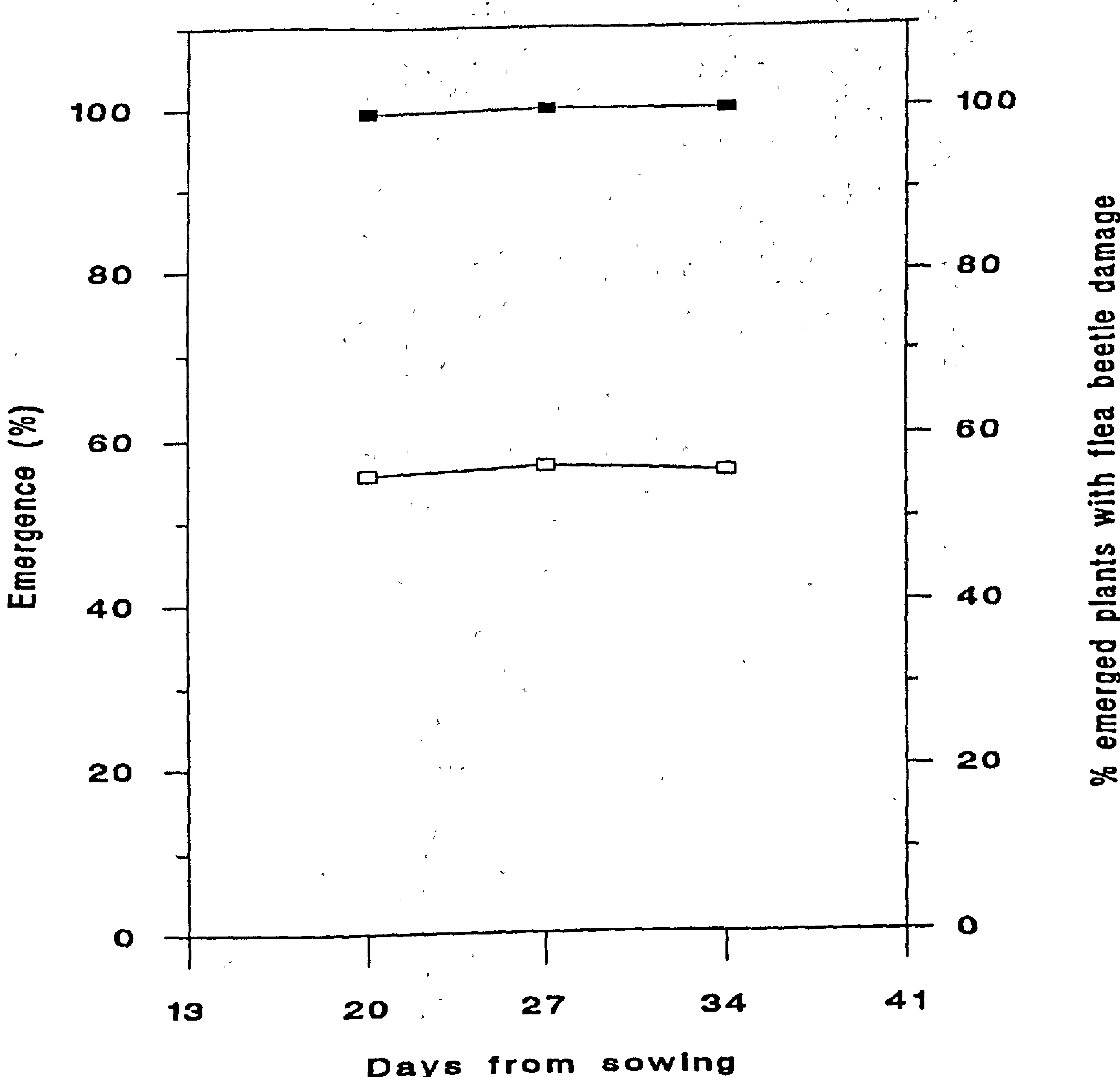



**Figure 9.6.** Emergence (%) of linseed plants (□) and percentage of emerged plants with damaged cotyledons (■) in plots sown with untreated seed (cv. Antares) on 21 April 1992. No statistical analyses were done on the data as there were no replicates of treatments.

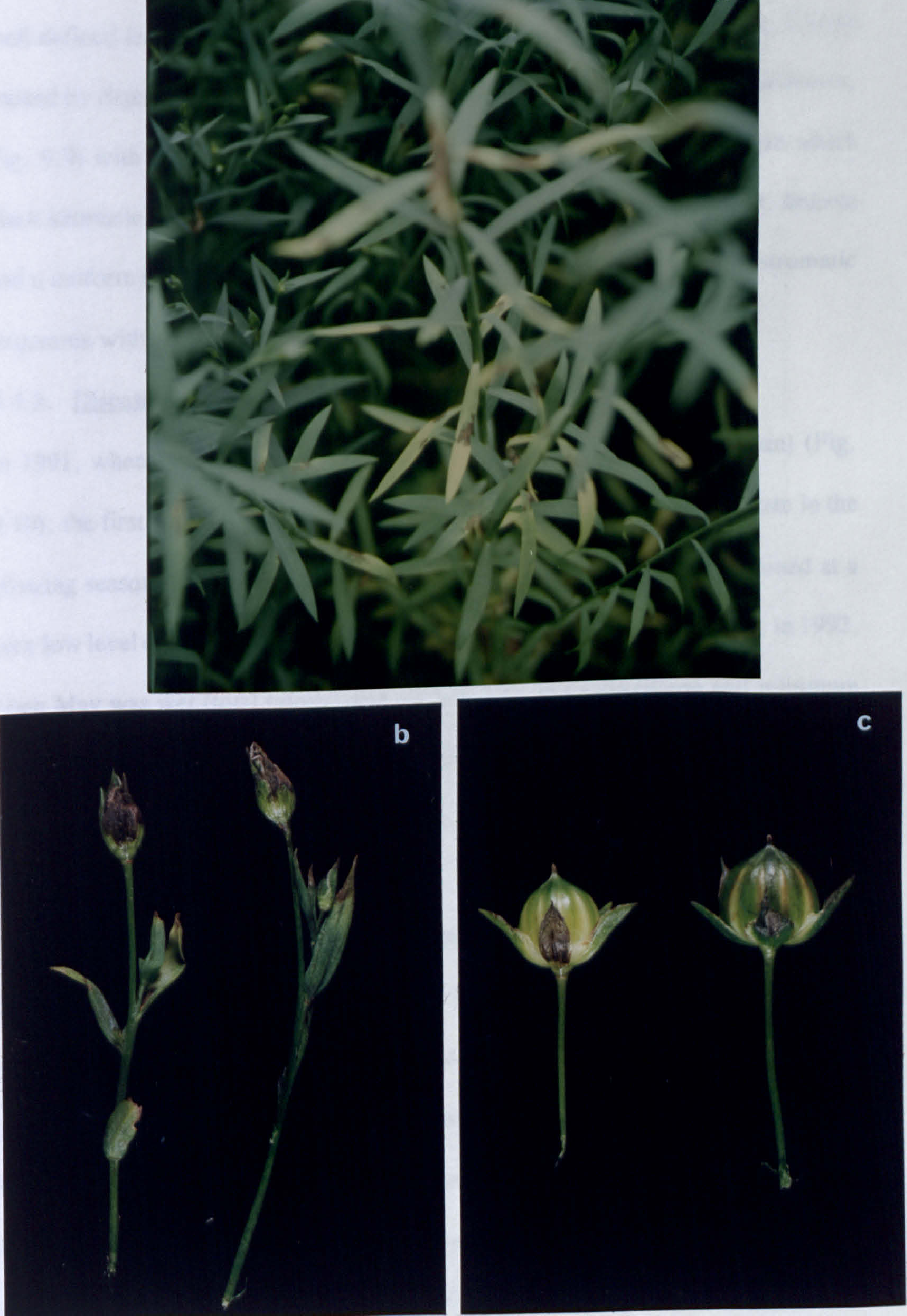



**Figure 9.7.** Symptoms on leaves (a), buds (b) and sepals (c) of linseed plants (cv. Antares) sampled from unsprayed plots on 19 August 1991 from which *A. linicola* was isolated. Similar symptoms were caused by *B. cinerea.*

well defined lesions which often surrounded the stem (Fig. 9.8). However, lesions caused by *Septoria linicola* on linseed stems had a brown colour (Dresden brown, Fig. 9.9) with a light coloured centre (Buckthorn brown, Fig. 9.9) within which black stromatic structures (pycnidia) could be observed. Lesions caused by *A. linicola* had a uniform chocolate-brown colour (Prout's brown, Fig. 9.9) with no stromatic structures within them.

9.4.3. Disease assessments

In 1991, when May was dry (total rainfall 15 mm, 14% of 30-year mean) (Fig. 9.10), the first symptoms of *A. linicola* infection on the plants appeared late in the growing season (mid-August) and the disease incidence in the crop remained at a very low level during the rest of the growing season (Table 9.4). However, in 1992, when May was wet (total rainfall 103 mm) and the mean maximum and minimum temperature was higher than in 1991 (Fig. 9.10), not only was the disease detected earlier in the growing season than in 1991, but also it spread rapidly throughout the rest of the season (Fig. 9.11).

In 1991, chocolate-brown lesions appeared for the first time on the leaves, buds and sepals on 19 August (GS 11) (Table 9.4) (Fig. 9.7). Subsequently the fungus was isolated from leaves, buds and sepals as long as these plant parts were present. On 26 August (GS 12) *A. linicola* lesions were first observed on the stems and on 20 September (GS 12) the fungus was detected for the first time on the capsule cases. *A. linicola* was isolated from the pre-harvest seed only on the samples collected on 19 August (GS 11) and on 20 September (GS 12) from plots sprayed with iprodione and benomyl, respectively (Table 9.4).

In 1992, symptoms of *A. linicola* infection were first observed on the

**Figure 9.8**. Symptoms of *A. linicola* (a) and *Septoria linicola* (b) infection on linseed stems (cv. Antares) sampled from unsprayed plots on 13 August 1992.

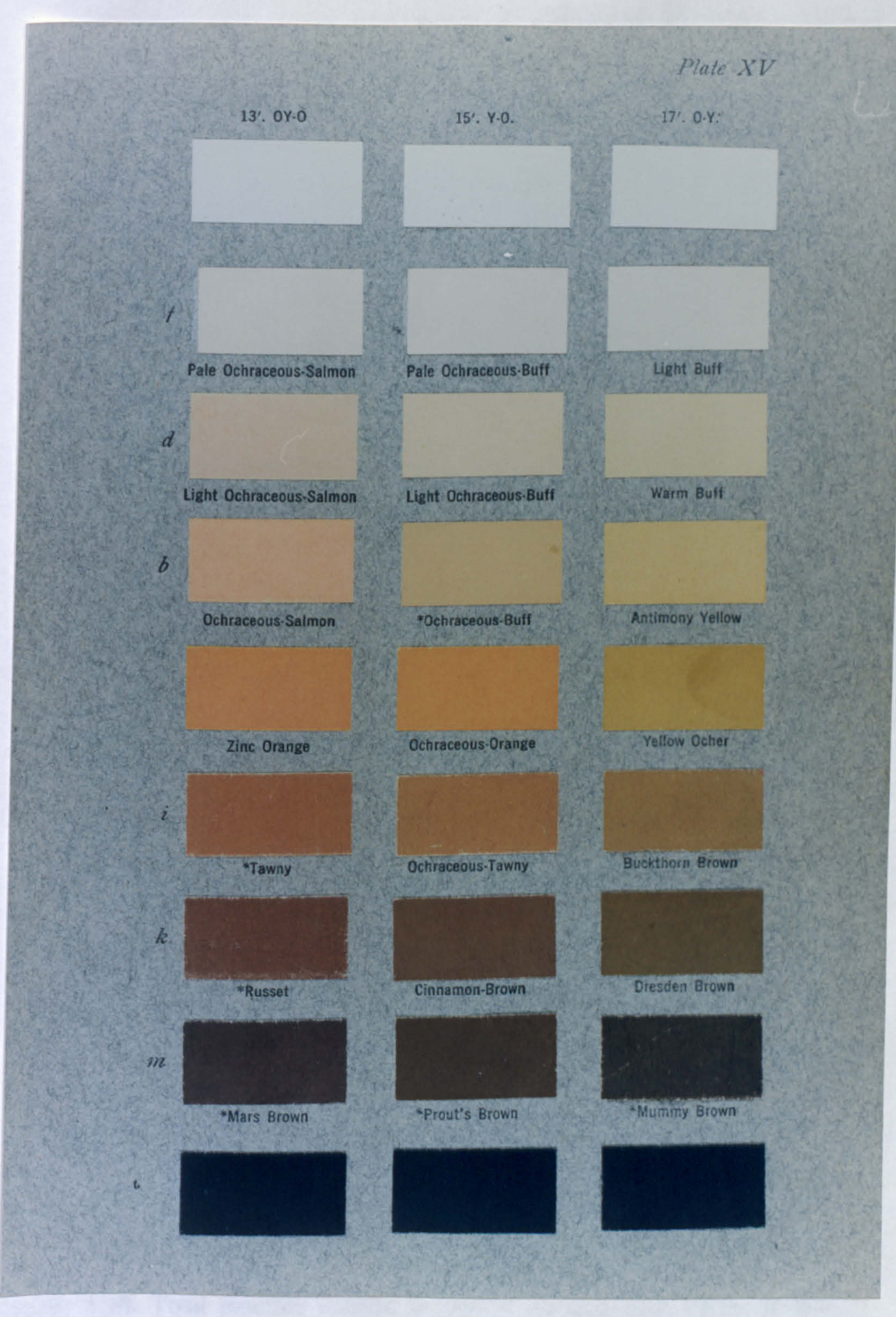



**Figure 9.9.** Ridgway plate (Ridgway, 1912).

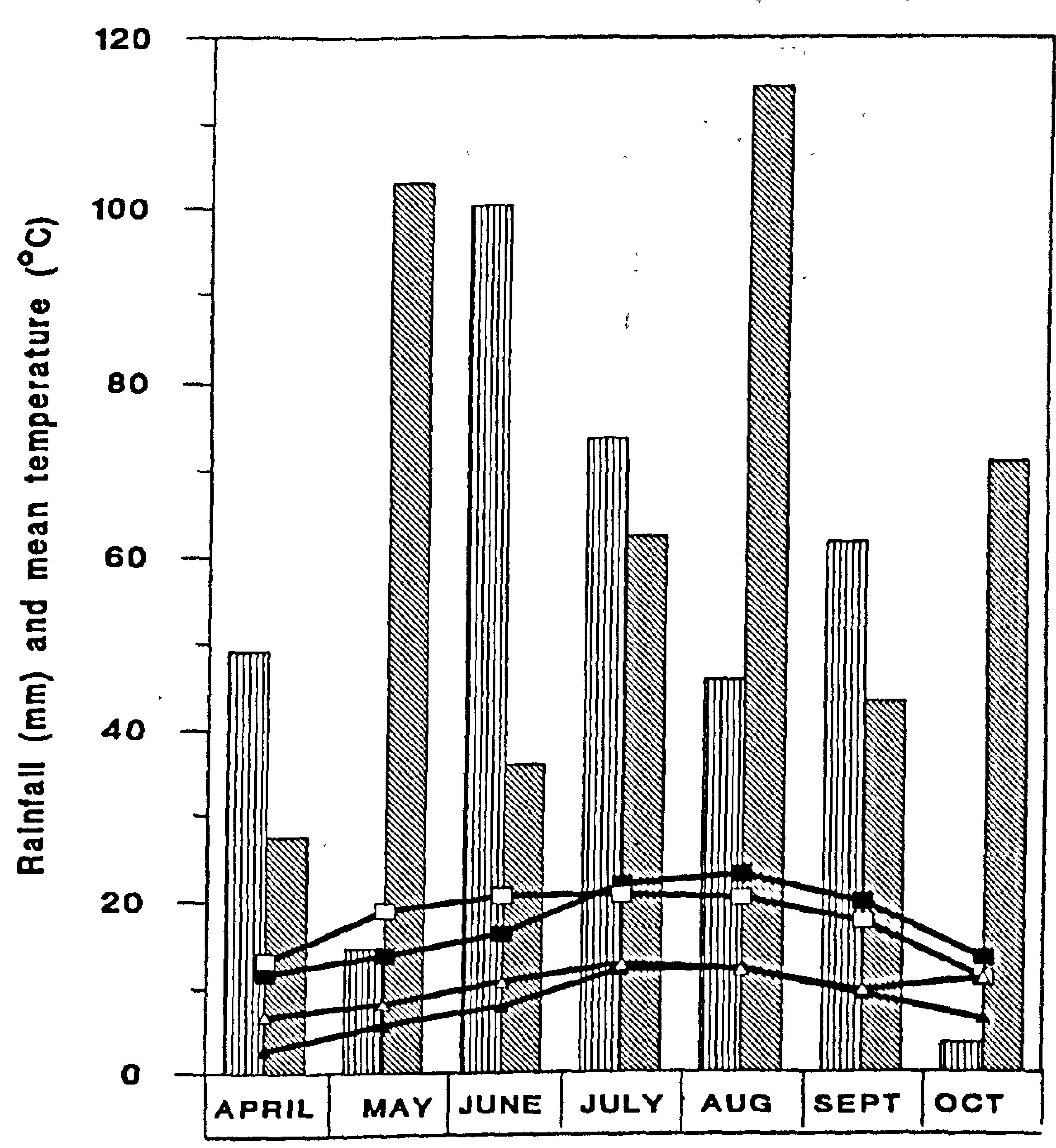



**Figure 9.10.** Total monthly rainfall in 1991 ( ▥ ) and 1992 ( ▨ ) and mean monthly maximum (■,□) and minimum (▲,△) temperatures during the period between sowing and harvest of linseed crops in 1991 (■,▲) and 1992 (□,△). In 1991, the crop was sown on 10 April and harvested on 10 October. In 1992 the crop was sown on 21 April and harvested on 17 September.

**Table 9.4.** Effects of fungicide spray applications on the incidence of *A. linicola* on leaves, buds, sepals, capsules and pre-harvest seed of linseed (cv. Antares) in 1991.

| Date of sampling | Treatment[1] | Incidence (% infected) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Leaves | Stems | Buds | Sepals | Capsules | Pre-harvest seed |
| 19 Aug. | Nil | 4 | 0 | 4 | 0 | 0 | 0 |
| | B | 0 | 0 | 0 | 0 | 0 | 0 |
| | I | 4 | 0 | 0 | 0 | 0 | 2 |
| | P | 0 | 0 | 0 | 2 | 0 | 0 |
| | SED(12 d.f.) | 4.16 | 0 | 2.83 | 1.41 | 0 | 1.41 |
| 26 Aug. | Nil | 12 | 0 | 0 | 2 | 0 | 0 |
| | B | 4 | 2 | 18 | 2 | 0 | 0 |
| | I | 4 | 0 | 0 | 0 | 0 | 0 |
| | P | 0 | 0 | 0 | 0 | 0 | 0 |
| | SED(12 d.f.) | 7.12 | 1.69 | 7.87 | 2.08 | 0 | 0 |
| 3 Sept. | Nil | 4 | 4 | 4 | 0 | 0 | 0 |
| | B | 4 | 0 | 0 | 0 | 0 | 0 |
| | I | 4 | 0 | 0 | 0 | 0 | 0 |
| | P | 0 | 0 | 0 | 2 | 0 | 0 |
| | SED(12 d.f.) | 5.29 | 2.83 | 2.83 | 1.41 | 0 | 0 |
| 9 Sept. | Nil | 4 | 4 | * | 2 | 0 | 0 |
| | B | 8 | 10 | * | 0 | 0 | 0 |
| | I | 4 | 0 | * | 0 | 0 | 0 |
| | P | 0 | 0 | * | 0 | 0 | 0 |
| | SED(12 d.f.) | 5.29 | 6.68 | | 1.41 | 0 | 0 |
| 16 Sept. | Nil | *[2] | 0 | * | 1 | 1 | 0 |
| | B | * | 0 | * | 2 | 2 | 3 |
| | I | * | 0 | * | 0 | 0 | 0 |
| | P | * | 0 | * | 1 | 1 | 0 |
| | SED(12 d.f.) | | 0 | | 1.32 | 1.85 | 2.12 |
| 20 Sept. | Nil | * | 0 | * | 0 | 0 | 0 |
| | B | * | 0 | * | 0 | 0 | 0 |
| | I | * | 0 | * | 0 | 0 | 0 |
| | P | * | 0 | * | 0 | 0 | 0 |
| | SED(12d.f.) | | 0 | | 0 | 0 | 0 |

[1] B = benomyl (140 g a.i. $ha^{-1}$); I = iprodione (125 g a.i. $ha^{-1}$); P = prochloraz (72 g a.i. $ha^{-1}$). Sprays were applied on 20 June, 10, 23 July, 7 and 27 August.
[2] * : not present.

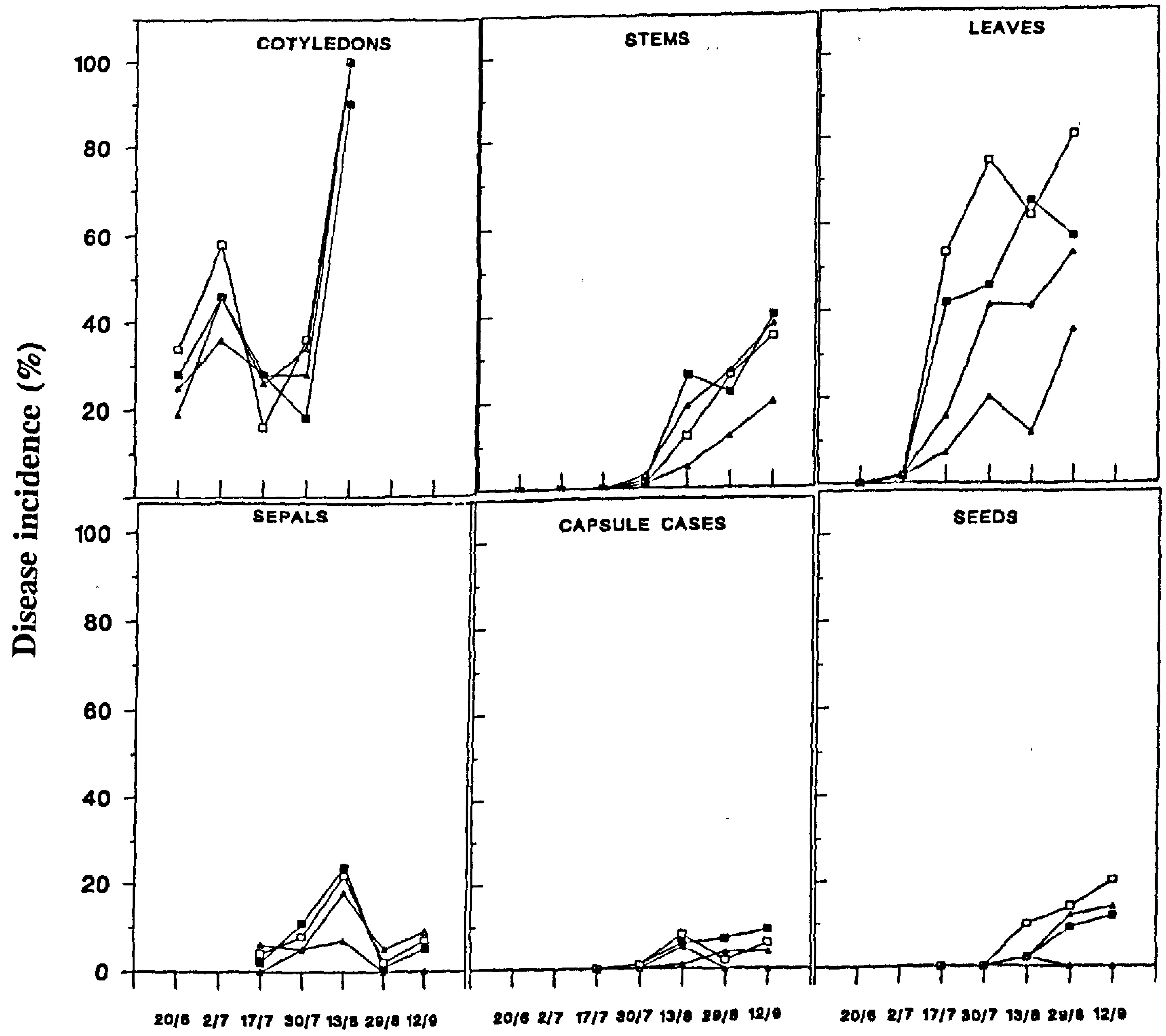



Figure 9.11. Effects of six fungicide sprays with iprodione (▲ , 250 g. a.i. $ha^{-1}$), prochloraz ( △ , 157.5 g a.i. $ha^{-1}$) or chlorothalonil ( □ , 250 g a.i. $ha^{-1}$) applied at two-weekly intervals on *A. linicola* incidence on cotyledons, SED (64 d.f.) = 11.9; stems, SED (48 d.f.) = 8.5; leaves, SED (64 d.f.) = 12.9; sepals, SED (64 d.f.) = 4.05; capsule cases, SED (48 d.f.) = 2.86 and pre-harvest seeds, SED (32 d.f.) = 4.34 in 1992. (■) : unsprayed.

cotyledons approximately 40 days after the emergence of the plants (GS 6) (Fig. 9.11). The disease spread rapidly on the cotyledons throughout the growing season. By mid-August, 90% of the plants collected from untreated plots showed symptoms of *A. linicola* infection on their cotyledons (either on one or on both cotyledons). Symptoms of *A. linicola* infection on leaves were first observed on the lower leaves at the beginning of July (GS 6-7) (Fig. 9.11). Gradually the disease spread to the middle and upper leaves and by the end of August 57% of the plants showed symptoms of *A. linicola* infection on their leaves (Fig. 9.11). Symptoms appeared on most of the leaves irrespective of their position on the stem or their age. However, only a proportion of the leaves showing brown lesions or brown tips (Fig. 9.12) appeared to be infected by *A. linicola* (Fig. 9.13).

*A. linicola* infection was detected on the sepals in mid-July and it was not until before the end of July that symptoms were first observed on the stems and capsule cases. The pathogen was detected on the pre-harvest seed by mid-August, while the seed was still white (GS 10, Fig. 1.2); by the end of the growing season, just before harvest, the incidence of *A. linicola* on the seed collected from untreated plots was 12% (Fig. 9.11). Although all the linseed tissues (cotyledons, stems, leaves, sepals, capsule cases and seeds) became infected by *A. linicola* by the end of the growing season, the disease incidence on the cotyledons and leaves was much greater than that on stems, sepals, capsule cases and seeds of plants collected from unsprayed plots (Fig. 9.11).

9.4.3. Effects of fungicide sprays on disease development

In 1991, when the fungicides iprodione, benomyl and prochloraz were applied at

**Figure 9.12.** Symptoms on leaves (flowering stems) of linseed plants (cv. Antares) caused either by pathogens (*A. linicola*, *B. cinerea*) or by natural senescence.

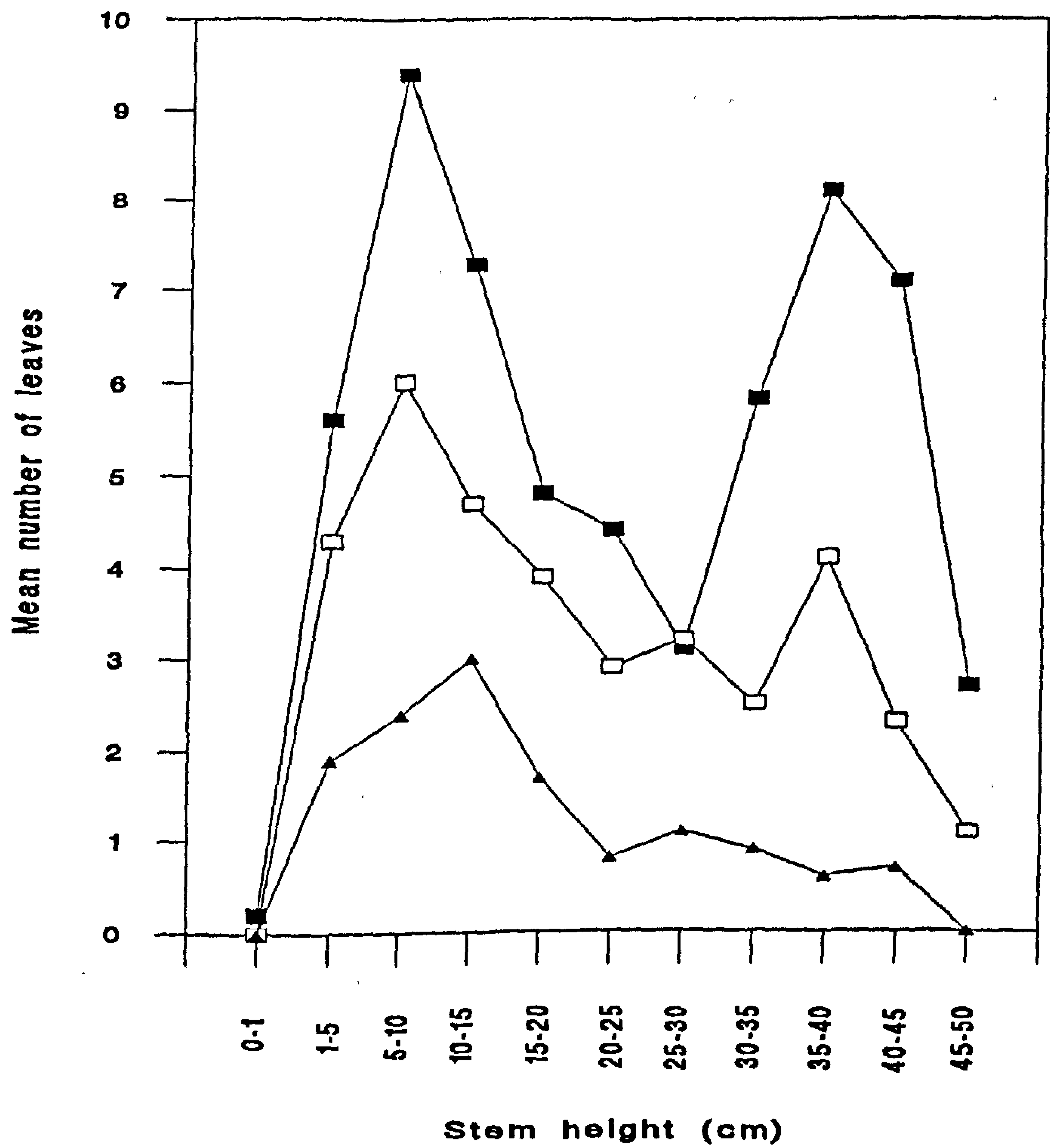



**Figure 9.13.** Mean numbers of leaves present [■ ,SED (40 d.f.) = 1.3], brown leaves [□ , SED (40 d.f.) = 0.89] and leaves infected by *A. linicola* [▲,SED (40 d.f.) = 0.64] at different heights on the stem of linseed plants (cv. Antares) sampled from unsprayed plots on 17 July 1992. Leaves present at stem heights between 25 and 50 cm were from the flowering stems.

half the recommended dose and the disease appeared at a very low incidence late in the season, prochloraz was the most effective fungicide in decreasing the incidence of *A. linicola* on plants, followed by iprodione (Table 9.4). However, in 1992 when the fungicides iprodione, chlorothalonil and prochloraz were applied at the recommended dose and the disease appeared early in the growing season, iprodione was more effective than prochloraz in decreasing the incidence of *A. linicola* in the crop (Fig. 9.11).

In 1991, no *A. linicola* infection was detected on leaves, stems, buds and pre-harvest seeds during the growing season on plots sprayed with prochloraz (Table 9.4). However, a low incidence of *A. linicola* infection (1 - 2%) was detected on sepals on samples collected on 19 August (GS 11), 3 September and 20 September (GS 12). Only one sample taken on 16 September (GS 12) from plots treated with prochloraz had 1% *A. linicola* infection on the sepals and capsule cases (Table 9.4). Although the incidence of *A. linicola* infection was 1% on the unsterilized post-harvest seed, no *A. linicola* infection was detected on the surface-sterilized seed (Fig. 9.14). Iprodione spray applications were very effective in decreasing the incidence of *A. linicola* on stems, buds, sepals and capsule cases but had no effect on the disease incidence on leaves and seeds (pre- or post-harvest) (Table 9.4 & Fig. 9.13). The percentage of infected leaves in plots treated with iprodione was the same as in the unsprayed plots. *A. linicola* was detected only on one pre-harvest seed sample taken on 19 August (GS 11) from the iprodione-treated plots (2% incidence). The percentage of *A. linicola* infection on the surface-sterilized and on the unsterilized post-harvest seed taken

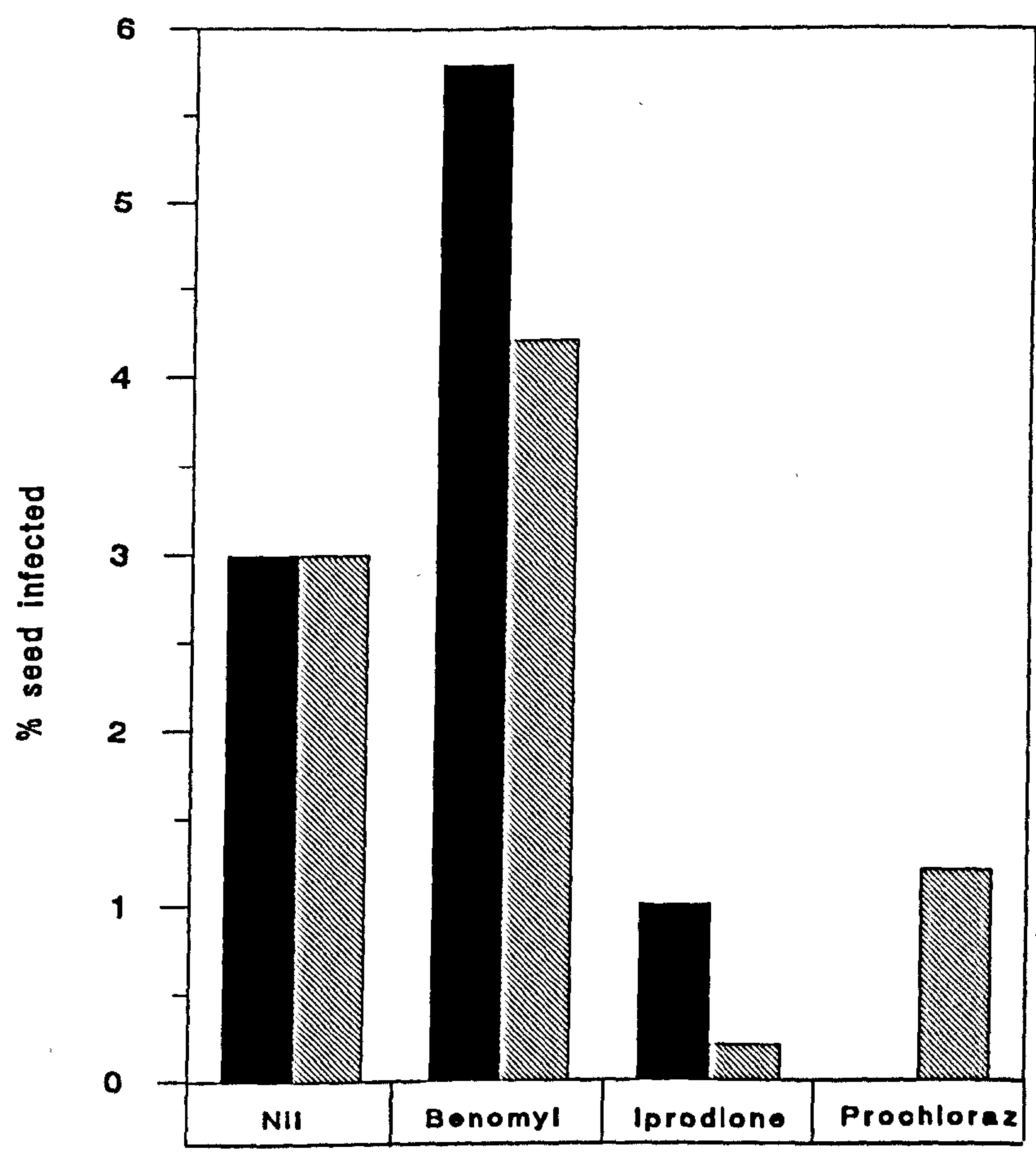



**Figure 9.14.** Effects of five fungicide sprays with iprodione (125g a.i.ha$^{-1}$), prochloraz (72 g a.i. ha$^{-1}$) or benomyl (140 g a.i. ha$^{-1}$) applied at two-weekly intervals in 1992 on the incidence of *A.linicola* on surface-sterilized (■) [SED (12 d.f) = 1.03] and unsterilized (▨) [SED (12 d.f.) = 0.99] post-harvest seed tested after 8 months of storage at -15°C.

from plots sprayed with iprodione was also low (1 and 0.2%, respectively) (Fig. 9.14).

In 1992, none of the fungicides used was effective in decreasing the incidence of the disease on the cotyledons and by the end of the growing season 100% of the plants sampled from plots sprayed with fungicides appeared to have their cotyledons infected by *A. linicola* (Fig. 9.11). However, iprodione decreased the incidence of *A. linicola* on stems and leaves by 50 and 38%, respectively, compared with the unsprayed plots. No *A. linicola* infection was detected at the end of the growing season on sepals, capsule cases and pre-harvest seed collected from plots sprayed with iprodione (Fig. 9.11). Iprodione also decreased the incidence of *A. linicola* on post-harvest seed by 31 and 61% on surface-sterilized and unsterilized seed, respectively (Fig. 9.15). Prochloraz was less effective than iprodione in decreasing *A. linicola* infection on stems and leaves (Fig. 9.11). Moreover, in some cases the disease incidence on sepals, capsule cases and pre-harvest seed sampled from plots sprayed with prochloraz was greater than on those taken from unsprayed plots (Fig. 9.11).

Post- and pre-harvest seed samples collected from the unsprayed, prochloraz- or chlorothalonil-sprayed plots had approximately the same incidence of *A. linicola* (Fig 9.12 & Fig. 9.15). Although no *A. linicola* infection was detected on the pre-harvest seed samples (Fig. 9.12) taken from the iprodione-treated plots, there was 10 and 6% of *A. linicola* infection on the surface-sterilized and unsterilized post-harvest seed, respectively (Fig. 9.15). Post-harvest seed samples collected from the prochloraz-treated plots had a slightlygreater incidence of *A. linicola* infection (11% on both surface-sterilized and unsterilized

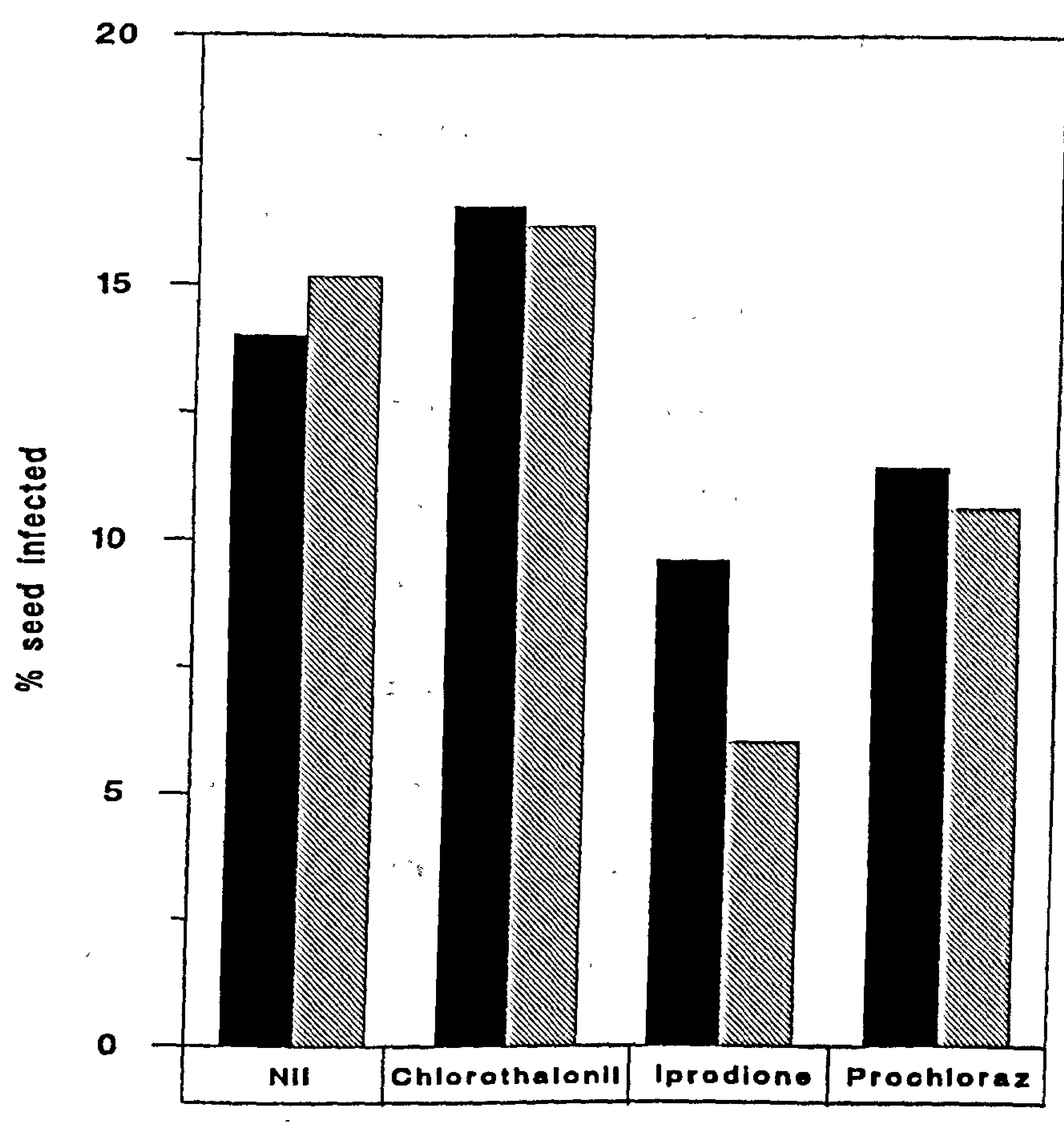



**Figure 9.15.** Effects of six fungicide sprays with iprodione (250 g a.i. $ha^{-1}$), prochloraz (175.5 g a.i. $ha^{-1}$) or chlorothalonil (250 g a.i. $ha^{-1}$) applied at two-weekly intervals in 1992 on *A. linicola* incidence on surface-sterilized (■), [SED (12 d.f.) = 4.5] and unsterilized (▧), [SED (12 d.f.) = 4.7] post-harvest seed tested after 14 months of storage at 15°C.

seed) than the seeds sampled from plots sprayed with iprodione (10 and 6% on surface-sterilized and unsterilized seed, respectively) (Fig. 9.15).

When benomyl and chlorothalonil were used in 1991 and 1992, respectively, they were not only less effective than prochloraz and iprodione in controlling *A. linicola* but also in some cases the disease incidence was greater in plots sprayed with these fungicides than in the unsprayed plots (Table 9.4 & Fig. 9.11). Neither benomyl nor chlorothalonil was effective in decreasing the incidence of *A. linicola* on post-harvest seed. Seed samples taken from the benomyl-treated plots had a greater incidence of *A. linicola* (6 and 4% on surface-sterilized and unsterilized seed, respectively) than the seed sampled from unsprayed plots (3% on both surface-sterilized and unsterilized seed) (Fig. 9.14). Similarly, the *A. linicola* infection on post-harvest seed samples collected from plots sprayed with chlorothalonil was greater (16% on both surface-sterilized and unsterilized seed) than on seeds sampled from unsprayed plots (14 and 15% on surface-sterilized and unsterilized seed) (Fig. 9.15).

9.4.4. Effects of fungicide sprays on yield, crop height and number of capsules

Yield and yield responses to fungicide sprays were greater in 1991 than in 1992. In 1991, plots sprayed with fungicides gave a higher yield than the unsprayed plots (Table 9.5). In 1992 plots sprayed with prochloraz or chlorothalonil gave higher yields than the unsprayed or the iprodione-sprayed plots (Table 9.5). Although iprodione was the fungicide which was the most effective in decreasing the disease incidence on plants, the yield from plots sprayed with iprodione was less than that from unsprayed plots. However, in 1992 differences between yields were not significant (Table 9.5).

**Table 9.5.** Effects of fungicide sprays applied at two-weekly intervals on yield of linseed (cv. Antares) in 1991 and 1992.

| Treatments | Yield (tonnes $ha^{-1}$) | |
|---|---|---|
| | **1991** | **1992** |
| Nil | 2.24 | 0.96 |
| Iprodione | 2.50 | 0.93 |
| Prochloraz | 2.67 | 1.18 |
| Benomyl | 2.59 | * |
| Chlorothalonil | * | 1.06 |
| SED (12 d.f.) | 0.082 | 0.086 |

* Not applied.

In 1992, the mean crop height and the mean number of capsules per plant were smaller in plots sprayed with iprodione or prochloraz than in unsprayed or chlorothalonil-sprayed plots (Table 9.6).

## 9. 5. Discussion

There were considerable differences in the incidence of the disease, epidemic development and the efficacy of fungicide sprays against *A. linicola* between the 1991 and 1992 growing seasons. Seed from the same origin with no detectable *A.linicola* infection on it was sown in both years. Hence it is likely that the differences in disease development in the crop were due to the differences in the weather conditions between the two growing seasons.

In 1991, the crop was sown earlier than in 1992 but it emerged at approximately the same date as in 1992. This was mainly because the temperature and the total rainfall for the period between sowing and emergence were lower in 1991 (mean maximum and minimum temperatures 12.3$^0$C and 2.9$^0$C, respectively; total rainfall 14.8 mm) than in 1992 (mean maximum and minimum temperatures 14.4$^0$C and 7.6$^0$C, respectively; total rainfall 43 mm). However, the emergence was greater in 1991 (100%, 27 days after sowing) than in 1992 (56%, 34 days after sowing). According to Mercer *et al.* (1991a), seed infection by *A. linicola* can decrease emergence by 50%, especially when the soil moisture and temperature favour the growth of the *A. linicola* mycelium from the seed coat to the seedlings. Therefore, it is possible that in 1992 a very low, but not detectable *A. linicola* infection on the seed was favoured by the temperature

**Table 9. 6.** Effects of fungicide sprays on crop height (cm) and number of mature capsules of linseed plants (cv. Antares) sampled on 29 August 1992.

| Treatments | Mean crop height (cm) | Mean number of capsules/plant |
|---|---|---|
| Nil | 48.3 | 11.0 |
| Prochloraz | 45.5 | 9.3 |
| Iprodione | 46.9 | 9.9 |
| Chlorothalonil | 48.5 | 11.8 |
| SED (12 d.f.) | 2.07 | 1.55 |

and the frequent rainfall during the period between sowing and emergence and it decreased emergence. Moreover, some seedlings failed either to emerge or to grow because of the severe flea beetle damage.

In both years, no *A. linicola* infection was observed on the young seedlings at the beginning of the growing season, probably because the pathogen was either absent from the seed used for sowing or present, but at a very low level and any infection of the plants could not be detected on the small number of plants sampled. The low maximum and minimum temperatures and the dry weather conditions in May 1991 did not favour the development of the disease on the plants which grew free of any symptoms for most of the growing season. However, the frequent rain during June and July 1991 might have favoured *A. linicola* infection, although the disease was not detected before mid-August, though at a very low level. The dry weather conditions between flowering and harvest (August - September) did not favour further development of the disease which remained at a low, almost undetectable, incidence for the rest of the growing season.

By contrast, in 1992 the wet weather conditions and the high temperatures that occurred at the beginning of the growing season favoured the infection of the plants by *A. linicola*. Moreover, although June was dry, the overhead irrigation of the crop might have favoured not only the sporulation of *A. linicola* on the infected plant tissues but also the splash dispersal of the conidia and subsequently the development of the disease in the crop. Irrigation, especially sprinkling, increased the incidence of *A. sesami* on sesame (Culp & Thomas, 1964), *A. brassicicola* on cabbage (Anon, 1951), *A. chrysanthemi* on chrysanthemum (Sobers, 1965), *A. dianthi* on carnation (Bickerton,1943) and *A. solani* on potato (Guthrie, 1958). However,

rain was more important than sprinkling for the development of *A. alternata* on wheat and sprinkling alone failed to increase the disease incidence (Conner, 1989). In 1992, the disease was detected for the first time by the end of July and at higher levels than in 1991. The wet weather conditions that followed flowering (i.e. from the end of July to the beginning of September) increased the disease incidence on the plants and just before harvest all the plants appeared to have cotyledons infected by *A. linicola*. These results are in accordance with those reported by Fitt & Ferguson (1993) that *A. linicola* can be damaging to linseed crops when the weather in the period between flowering and harvest (July & September) is wet.

The results of the present study also showed that *A. linicola* can spread vertically up the crop canopy and that all the plant tissues (cotyledons, stems, leaves, buds, sepals, capsule cases and seeds) are susceptible to *A. linicola* infection. However, cotyledons and leaves seem to be more susceptible than stems, although they are all present on the plants for most of the growing season. The first symptoms of *A. linicola* infection appeared on the cotyledons and lower leaves, but symptoms developed throughout the growing season on all the leaves irrespective of their position on the stem. This observation contrasts with other studies on the vertical development of the disease on the crop (Mercer *et al.*, 1991a; Mercer & Hardwick, 1991). According to those studies, although the first symptoms of the disease were observed on the cotyledons and lower leaves, the plants appeared to grow without any symptoms on the middle or upper leaves for the rest of the growing season. Dark chocolate - brown lesions appeared on the sepals and capsule cases just before harvest, especially when the weather during that period was wet. The present study also showed that as the symptoms caused

by *A. linicola* on linseed plants are not distinctive and can be confused with those caused by *B. cinerea* or natural senescence, visual assessments of the disease incidence on the plants are not reliable. The identification of *A. linicola* infection is possible only by inducing sporulation of the pathogen on the infected plant tissues.

Fungicide applications to linseed crops to control *A. linicola* and increase yield may give variable results between different years, even in the same region, depending on the weather conditions and cultural practices. In 1991, when the fungicides were applied at half the recommended dose and the weather conditions did not favour the development of the disease in the crop, prochloraz was better than iprodione or benomyl in decreasing the disease incidence and in controlling the *A. linicola* infection on the seed (pre- and post-harvest seed). By contrast, in 1992 when the fungicides were applied at the recommended dose and the weather conditions were favourable for the development of the disease, iprodione was more effective than prochloraz or chlorothalonil in decreasing the disease incidence and in controlling the seed infection at least on the pre-harvest seed. Although benomyl and chlorothalonil increased yield they were the least effective fungicides and in some cases they increased the disease incidence, probably because they controlled some other fungi antagonistic to *A. linicola.*

In 1991, the increase in the disease incidence on the post-harvest seed samples taken from unsprayed, benomyl- or iprodione-sprayed plots may have occurred because the last pre-harvest seed sample was examined on 20 September and the crop was not harvested until 10 October. The wet weather during the period between 20 September and 10 October (total rainfall 110 mm) might had favoured the infection

of the seed by the pathogen. In 1992, there was no great difference in the disease incidence between the pre-harvest and the post-harvest seed samples taken from the unsprayed, prochloraz- or chlorothalonil-sprayed plots. However, it is not known why some *A. linicola* infection was detected on the post-harvest seed samples (10 and 6% on surface-sterilized and unsterilized seed, respectively) collected from the iprodione-sprayed plots although no infection was detected on the pre-harvest seed. The yield response to control of *A. linicola* by fungicide sprays was greater in 1991 when the disease incidence was very low throughout the growing season compared with that in 1992. The lower yield in 1992, even in the unsprayed plots, compared with that in 1991 was mainly associated with the low emergence of the plants.

Other studies on the effects of fungicide sprays against *A. linicola* have also shown variable results depending on the region and the weather conditions. Although trials in Northern Ireland with single sprays of iprodione or prochloraz either had no effect or increased the disease incidence, weekly sprays decreased the incidence of *A. linicola* on the seed (Mercer *et al.*, 1991a; 1991b). In the North of England, sprays with iprodione applied during the period between flowering and harvest slightly decreased the disease incidence on the seed (Hardwick & Mercer, 1989). By contrast in the SE of England in 1988, sprays with a mixture of iprodione and benomyl decreased the incidence of *A. linicola* on the seed from 40 to 8% and increased yield by up to 30% (Fitt & Ferguson, 1993). However there was no significant decrease in the disease incidence when sprays of iprodione, benomyl or prochloraz were applied in 1989 and 1990 (Fitt & Vloutoglou, 1992).

The failure of the fungicides, especially that of iprodione or prochloraz, to control *A. linicola* might have been due to the presence of *A. linicola* strains resistant

to these fungicides, although this case was not investigated in the present study. However, some of the fungicides used in this study are very effective in controlling diseases caused by other *Alternaria* species on other crops. Humpherson-Jones & Maude (1982b) reported that sprays with iprodione or prochloraz were very effective in controlling *A. brassicae* and *A. brassicicola* and increasing yield on oilseed rape, although the effects of iprodione were more persistent than those of prochloraz. Sprays with chlorothalonil decreased disease damage caused by *A. solani* on potato crops, although they had no effect on yield (Easton *et al.*, 1975).

The results of this study suggest that application of prophylactic fungicide sprays to linseed crops to control seed infection by *A. linicola* and increase yield cannot be recommended, mainly because the results depend on the weather conditions and the incidence of the disease on the crop. Moreover, although multiple sprays of iprodione or prochloraz may decrease the incidence of *A. linicola* infection on the pre-harvest seed, they are considered to be uneconomic as linseed is a low input break crop. Seed treatment with prochloraz, suggested by Mercer *et al.* (1991a), seems to be the only effective method for decreasing the incidence of the disease on the young seedlings although its effect on *A. linicola* is fungistatic rather than fungitoxic. Biological agents have also been tested against *A. linicola* with some promising results. In Northern Ireland sprays with spore suspensions of different isolates of *Epicoccum nigrum* and *Trichoderma viride* were as effective as those with iprodione but only when the crop was sprayed weekly (Mercer *et al.*, 1991b; 1992a).

It seems that traditional cultural practices such as selection of healthy seed for sowing, effective burial of the infected debris and crop rotation can minimize

the primary inoculum and subsequently delay the introduction of the disease in new growing areas. Additionally, a better understanding of the life cycle of *A. linicola* and of the environmental conditions that favour the infection of linseed plants may indicate periods when the spread of the disease is most likely and application of fungicides most effective.

# CHAPTER X. GENERAL DISCUSSION

This study demonstrates for the first time the life cycle and the seasonal cycle of *A. linicola* on linseed in the UK (Fig. 10.1 & 10.2, respectively). The life cycle of *A. linicola* can be very short; 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), germination, penetration and infection of linseed plants by *A. linicola* can occur within 8 h. The first symptoms may appear on the linseed plants within 4 days and conidia of the pathogen can be produced on the infected linseed tissues at the end of the first wet night.

This short life cycle suggests that under prolonged periods of favourable conditions, conidia originating from a few initial infections can potentially produce a great number of new lesions and damage the crop within a relatively short time. If sources of inoculum are present and conditions are favourable, healthy linseed tissues can be infected early in the growing season by air-borne conidia. Conidia of *A. linicola* may be produced on infected linseed throughout the growing season to infect new plant tissues as they emerge. In the final stage of the development of the disease in a linseed crop, the pathogen may infect the seeds through the capsule cases. However, severe epidemics caused by *A. linicola* on linseed crops have not been reported recently in the UK. This is probably due to the use of treated seed for sowing and to the rotation of the crops in commercial fields, both of which may decrease the available primary inoculum of the pathogen. Moreover, the occurrence of conditions that are marginal for infection, especially the low average temperatures, in the UK early in the growing season (April-May) may lengthen the life cycle of

*A. linicola* and delay the build-up of inoculum in linseed crops.

The stages in the life cycle of *A. linicola*, namely conidial germination, penetration of the plant tissues, infection and subsequent symptom development, production of conidia on the infected linseed tissues and dispersal of inoculum by the wind (as air-borne conidia) (Fig. 10.1) are all affected by environmental factors such as leaf wetness, temperature, light and by their interactions. However, these experiments suggest that the presence of free moisture on the linseed plant tissues, produced by rain, dew or periods of high relative humidity, is the key component in the life cycle of *A. linicola*.

The results of this study suggest that germination of *A. linicola* conidia deposited on plant tissues is a very rapid process, greatly affected by water availability on the plant surfaces and by light. In the presence of water and in darkness, conidia of *A. linicola* germinated within 2 h by producing one to several germ tubes. Occasionally, *A. linicola* conidia germinated by producing secondary conidia. The formation of secondary conidia, also reported for other *Alternaria* species, has been attributed to the occurrence of conditions unfavourable for germination (Rotem, 1994). No germination occurred in the absence of free moisture or in the presence of light. Germination of *A. linicola* conidia stopped under unfavourable conditions (interrupted leaf wetness or short light periods) and resumed when the conditions became favourable again. In contrast with leaf wetness or light, temperature does not seem to be a factor limiting the germination of *A. linicola* conidia. Germination occurred over a wide range of temperatures (5-25$^{0}$C), although low temperatures (e.g. 5$^{0}$C) delayed the onset of germination and decreased the rate of germination and germ tube elongation.

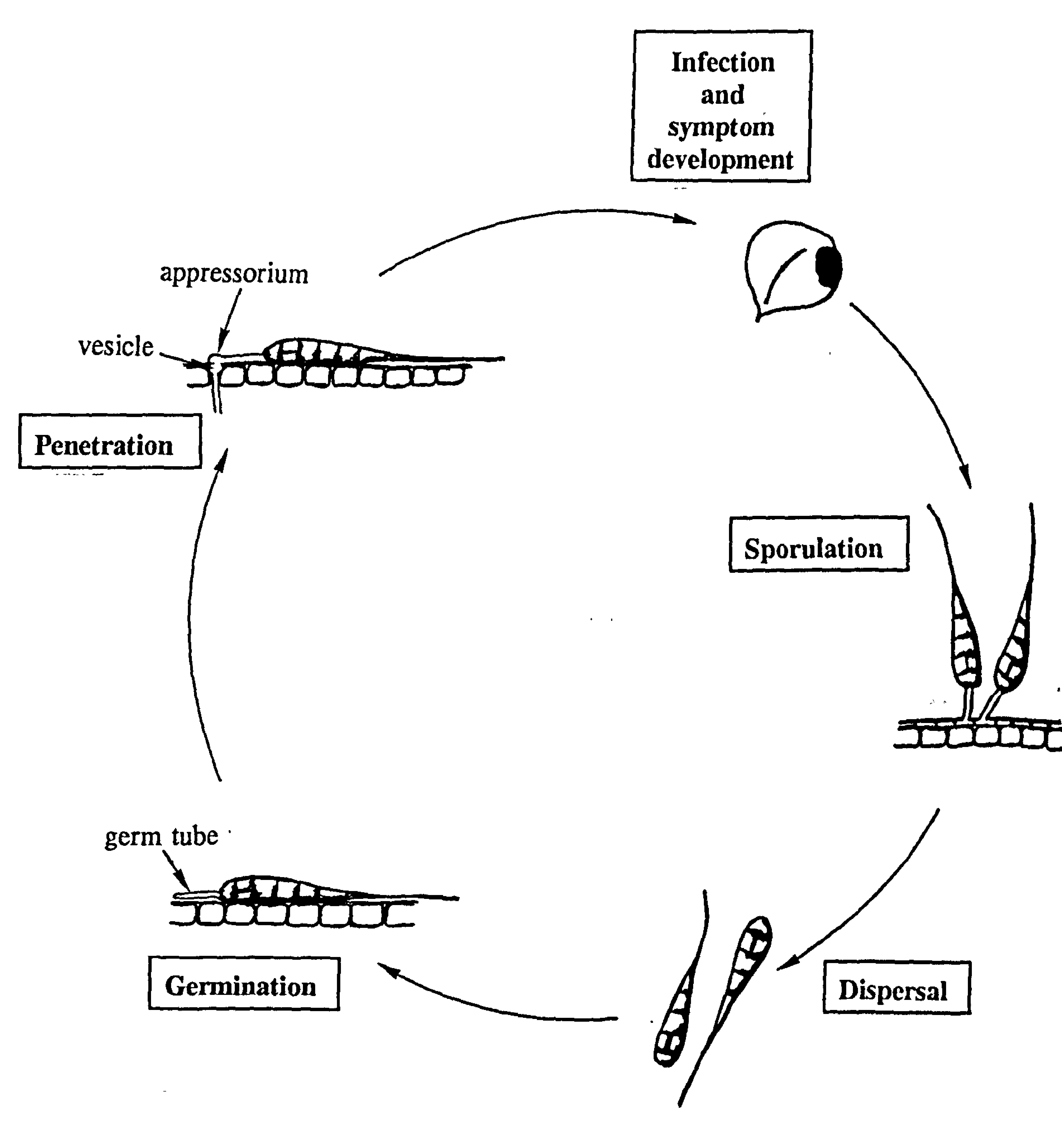



**Figure 10.1.** Proposed life cycle of *A. linicola* on linseed.

Results suggest that not only conidial germination but also penetration of the linseed tissues by *A. linicola* is a rapid process. In the presence of leaf wetness and in darkness, penetration of the plant tissues started within 12 h and the route of penetration was generally directly through the epidermal cells, with or without formation of appressoria. Occasionally, the hyphae penetrated the tissues indirectly through stomata. After the fungus entered the host, the hyphae grew intercellularly and colonized the linseed tissues.

Controlled environment studies showed that infection by *A. linicola* and subsequent symptom development on linseed plants is affected by leaf wetness, temperature, light and their interactions. Infection and development of symptoms on linseed plants occurred only in the presence of leaf wetness or high relative humidity. In contrast with germination, the infection process was temperature-dependent. Low temperatures prolonged the period of leaf wetness required for infection to occur. Although the minimum period of leaf wetness for the infection of linseed plants was 8 h at 25°C, a longer period of leaf wetness (10 h) was required for infection at 15°C; under these conditions the incubation period was approximately 4 days. However, periods of leaf wetness on linseed crops during the summer are often shorter than those required for infection of linseed by *A. linicola* to be completed. Nevertheless, the results of these experiments 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. Light is another environmental factor which affects the infection of linseed plants by *A. linicola;* controlled environment studies have shown that both disease incidence and disease severity

decreased as the ratio light period : dark period increased. For some *Alternaria* species light affects the infection process by inhibiting the production of toxins which are involved in the pathogenesis of these species (Haggblom & Hiltunen, 1992). However, it is not known if toxins are produced by *A. linicola* and if light affects the infection process by inhibiting the production of these toxins.

Controlled environment and field studies suggest that both conidial germination and infection of linseed plants by *A. linicola* are influenced by differences between linseed plant tissues. Cotyledons were the plant tissue which was most susceptible to infection by *A. linicola* and symptoms developed on them more rapidly and more severely than on other plant tissues (leaves, buds, sepals, capsule cases or seeds). Differences between cotyledons and leaves in susceptibility to infection by *A. brassicae* or *A. macrospora* infections of brassica and cotton plants, respectively, have been attributed to differences either in the wax content or in age between these two plant tissues. Although it is not known whether the susceptibility of cotyledons to infection by *A. linicola* is due to the wax content or to age, this susceptibility may have important implications for the disease development under field conditions. Cotyledons can be infected early in the growing season and therefore can support the early stages of the disease epidemic. Moreover, cotyledons killed by the pathogen are present on the plants for most of the growing season, providing inoculum for the infection of new plant parts (leaves, stems, buds, sepals, etc.).

These results suggest that air-borne *A. linicola* conidia deposited on plant tissues in crops during the day-time are unlikely to germinate and infect linseed plants before the following night due to the inhibitory effect of light on both the germination

and infection process. The inhibitory effect of light on spore germination has also been reported for a wide range of rust species including *Puccinia graminis* f.sp. *tritici* (Givan & Bromfield, 1964b; Knights & Lucas, 1980; Knights & Lucas, 1981), *Puccinia recondita* (Givan & Bromfield, 1964a) and *Hemileia vastatrix* (Nutman & Roberts, 1963). However, germination, penetration and infection of linseed plants by *A. linicola* might not be completed during the first wet night, especially at low temperatures (e.g. 5°C) or in short nights. In these cases, although germination and germ tube elongation stop during the following day, they will resume during the second wet night. This process will be repeated until the germ tubes penetrate the host.

Controlled environment studies suggest that sporulation of *A. linicola* on infected linseed tissues is affected by leaf wetness, light, temperature and their interactions. Sporulation on infected plant tissues occurred only in the presence of free moisture on the plant surfaces. However, in the absence of a single period of leaf wetness sufficiently long for production of conidia, *A. linicola* was able to use successive short periods of leaf wetness. In contrast with conidial germination and infection, sporulation of *A. linicola* was favoured by short periods of light. *A. linicola*, like many *Alternaria* species, is a "diurnal sporulator" with the two phases of the sporulation process, formation of conidiophores and production of conidia, affected by light in two different ways : light stimulates formation of conidiophores, but inhibits production of conidia (Leach, 1967). Unlike other *Alternaria* species (Houston & Oswald, 1946; Zimmer & McKeen, 1969), *A. linicola* appears to have the same requirements for light for sporulation *in vitro* and *in vivo*. *In vitro* most isolates sporulated only after exposure to diurnal NUV-light. However, for some isolates

exposure to NUV-light did not seem to induce sporulation unless the mycelium was also wounded and grown on a medium rich in $CaCO_3$ (S-medium) at high relative humidity.

The stimulating effect of light on sporulation of *A. linicola* on linseed plants was influenced by two other environmental factors : leaf wetness and temperature. Intermittent periods of leaf wetness, especially dry conditions during the light period, and low temperatures (e.g. 10°C), delayed the onset of sporulation and decreased the number of conidia produced, even under favourable light conditions. *A. linicola* seems to be well adapted to the conditions in linseed crops in the UK and to be able to sporulate when there are daily fluctuations in temperature, light or wetness.

Field studies showed for the first time that inoculum dispersal is another critical phase in the life cycle of *A. linicola* and that wind is the main agent for dispersal of *A. linicola* conidia. Dispersal by rain may also occur, but it does not add substantially to the number of conidia dispersed by wind. Aylor (1990) divided the fungal pathogens into those that actively release spores and those that produce spores which are removed passively from the host by wind. *A. linicola,* like all *Alternaria* species, belongs to the second category of fungi. Conidia of *A. linicola* were mainly dispersed from the source by strong wind, regardless of whether the source was infected linseed plants, stem debris left on the soil surface at the end of the season, volunteer linseed plants or secondary host plants (weeds). The dispersal of *A. linicola* above linseed crops followed a seasonal periodicity similar to that of other *Alternaria* species (Meredith, 1966; Schenk, 1968; Strandberg, 1977; Allen *et al.*, 1983; Mortensen *et al.*, 1983). In the SE of England numbers of *A. linicola* conidia dispersed above linseed crops were likely to be largest on the first dry day

following periods of rain at times between 12:00 and 13:00 h, when the average wind speed was sufficient (2-3 m $sec^{-1}$) for the detachment of the conidia from their conidiophores. Field studies showed that most of these conidia were transported by the wind for short distances (up to approximately 40 m) from the foci of their production within a linseed crop. However, the small number of conidia that escaped from the crop canopy might have been involved in long-distance dispersal of the pathogen.

The seasonal cycle of *A. linicola* has two phases : a) the spread of the disease vertically up the linseed plants and between plants within the crop (epidemic development) and b) the transmission of the disease from one growing season to the next (Fig. 10.2). This study suggests that the seasonal cycle of *A. linicola* is started early in the growing season by the seed-borne phase of the pathogen. If infected seed is sown, then the resting hyphae of *A. linicola*, situated in the outer layer of the seed coat, are activated during the imbibition of water by the seed and infect the young seedlings (Mercer *et al.*, 1991a). Under field conditions the first lesions appeared on the cotyledons, initially beneath the remains of the seed coat. Gradually, these lesions coalesced to form large necrotic areas. Re-infections of the same plant or infections of neighbouring plants occurred throughout the growing season mainly by means of air-borne conidia produced on the infected plant tissues. Following the infection of the cotyledons, lesions appeared on the lower leaves, but gradually the middle and upper leaves became infected. All the plant tissues (cotyledons, leaves, stems, buds, sepals, capsule cases) were susceptible to *A. linicola* infection and lesions appeared on them throughout the growing season. The disease appeared to follow the extension of the stem and the pathogen infected new plant tissues as they emerged.

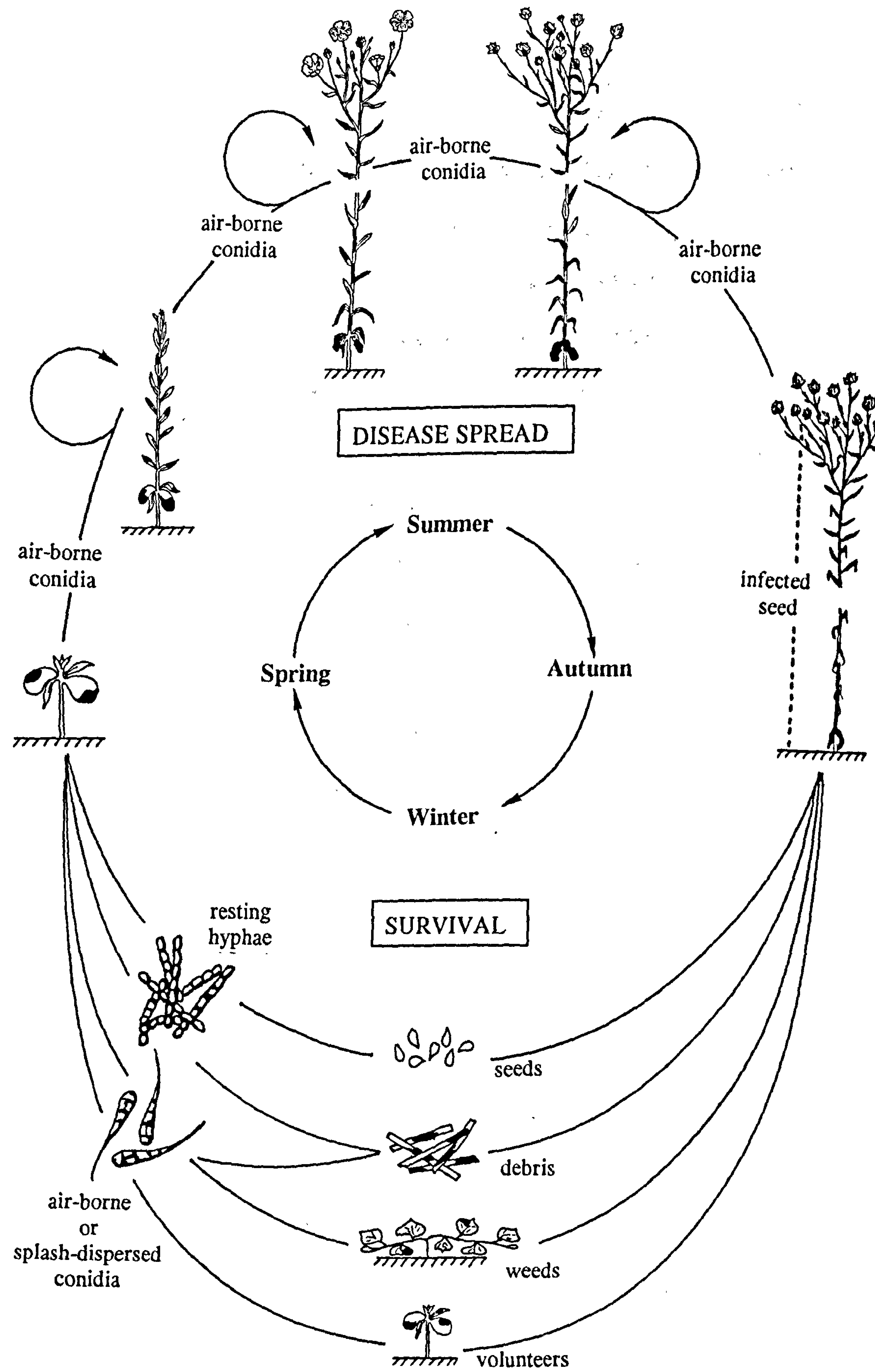



**Figure 10.2.** Proposed seasonal cycle of *A. linicola* on linseed. The drawings of the linseed plants were based on the description of the linseed main stem growth stages (Turner, 1987).

The development of the disease was favoured by wet weather and high temperatures, conditions which favoured all the phases of the life cycle of the pathogen. In the SE of England, early in the growing season (April-May) *A. linicola* can infect linseed plants during the long nights, despite the low temperatures that occur during this period. However, the low night temperatures and the long length of the days early in the growing season (April-May) may limit the sporulation of the pathogen on the infected linseed tissues. As a result, the build-up of inoculum and subsequent development of the disease on the crop may be very slow during this period. This is possibly the reason why severe disease epidemics are not caused by *A. linicola* in linseed crops early in the growing season.

The higher temperatures that occur in the SE of England during the period between June and September compared with those earlier in the growing season compensate for the short night periods so that not only the infection of linseed plants by *A. linicola* but also the sporulation of the pathogen on the infected tissues are very rapid processes, with the first conidia produced at the end of the first wet night. The occurrence of these conditions seems to be the main reason not only for the increase in the disease incidence on linseed crops but also for the large numbers of conidia produced and dispersed during the period between late June and early September. However, dispersal of *A. linicola* conidia during this period may be restricted by prolonged periods with rainy days, due to the inhibitory effect of leaf wetness on dispersal or by extended dry periods, due to the inhibitory effect of dryness on sporulation. Furthermore, the results of field experiments on disease spread showed that epidemics caused by *A. linicola* may develop slowly within a linseed crop during the growing season and that they are likely to be restricted to short distances from

the inoculum source. However, it is not known whether the *A. linicola* conidia that escaped from the crop canopy spread the disease over longer distances.

The final and most critical phase for the development of the disease on linseed plants is the infection of the seed by the pathogen. This study demonstrates for the first time not only the significance of the seed-borne phase of *A. linicola* as a source of primary inoculum, but also the ability of the pathogen to survive between growing seasons on infected linseed stem debris, volunteer linseed plants and weeds. Infected seed was the main source of primary inoculum as the pathogen was effectively transmitted from infected seed to seedlings. However, the efficiency of the transmission was temperature-dependent. Potential sources of primary inoculum for the infection of linseed crops by *A. linicola* can be infected linseed stem debris, volunteer linseed plants or weeds. However, infected stem debris and volunteer plants are likely to have a marginal role in the infection of the following linseed crop as not only are the fields deep-ploughed immediately after harvest but also crops are rotated. More important as a source of primary inoculum might be weeds infected by *A. linicola*, which grow in the neighbouring fields during the period between harvest of the linseed crop and sowing of the following crop (September-April).

The results of this study suggest that once *A. linicola* is established in a linseed crop, options for management of the disease are very limited. Although application of multiple fungicide sprays, especially of iprodione, to linseed crops may decrease the incidence of *A. linicola* in developing seeds, it is considered to be uneconomic as it might increase crop yield little. Moreover, disease control by fungicide sprays applied to linseed crops may be less efficient when other sources of primary inoculum (infected debris,volunteer linseed plants, weeds) than the seed are present in the

field and the weather conditions favour the short life cycle of the pathogen. It was not possible to make any conclusions on the effects of multiple applications of fungicide sprays of iprodione, prochloraz or benomyl on *A. linicola* on the linseed crop in 1991 because the disease incidence on the crop was very low throughout the growing season. However, the disease may have appeared on the crop early in the growing season in 1992 and at a greater incidence than in 1991 because inoculum was carried over on the debris, volunteer linseed plants or weeds from the previous season.

Multiple applications of fungicide sprays of iprodione to the crop in 1992 were slightly more effective than those of prochloraz or chlorothalonil in decreasing the disease incidence and in controlling the seed infection by *A. linicola*. However, none of the fungicides used increased crop yield. Similarly, in field trials in Northern Ireland multiple sprays of iprodione decreased the incidence of *A. linicola* in the seed, although the same fungicide was not effective when applied as a seed-treatment (Mercer & Hardwick, 1991; Mercer et al., 1991b). Nevertheless, some of the fungicides tested in this study (iprodione, prochloraz, chlorothalonil) have been successfully used to control other *Alternaria* diseases (Humpherson-Jones, 1982b; Easton *et al.*, 1975; Rotem, 1994). The reasons for the failure of prochloraz or chlorothalonil to control *A. linicola* on linseed crops in this study are not clear. However, it is possible that either they controlled fungi antagonistic to *A. linicola* and therefore favoured the development of the disease on the crop (e.g. chlorothalonil) or that fungicide-resistant strains of the pathogen were present in the field. Although iprodione-resistant strains of *A. linicola* have been reported in the past (Mercer *et al.*, 1988) there is no evidence that resistance to prochloraz has occurred in field populations of *A. linicola*.

Information provided in this study on the life cycle and seasonal cycle of *A. linicola* suggests that the pathogen is very flexible in its responses to the environmental conditions and that once introduced into an area, it is able to survive and persist, even in the absence of linseed crops. Once the pathogen has become established in an area, it is either difficult or uneconomic to control it by applications of fungicide sprays to the crop. Seed treatment with prochloraz, used by the seed industry, and crop rotation are likely to be the most effective methods for decreasing the amounts of primary inoculum for the infection of linseed crops by *A. linicola.*

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