

## The influence of light and dark on attack of bean leaves by *Alternaria zinniae*

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### Abstract

Bean leaves inoculated with spores of *Alternaria zinniae* showed small purplish brown lesions after incubation in light for 3 days. After incubation in darkness for 1–3 days necrotic spots were formed, the size of which increased with increasing length of the period of incubation in darkness. Application of culture filtrates of *A. zinniae* to the leaves caused the same symptoms as did inoculation with spore suspensions.

### Introduction

There are several examples that infection of plants by pathogenic fungi is influenced by light conditions. Infections of lettuce by *Bremia lactucae*, red radish by *Albugo candida*, wheat by *Erysiphe graminis* or *Puccinia triticina*, and bean by *Uromyces appendiculatus* were stimulated after exposure to darkness during the first days of the infection period (Sempio, 1939). Wheat varieties highly susceptible to *Puccinia triticina* became less sensitive to the pathogen after incubation in darkness for 2 days, whereas resistant varieties became less resistant (Hassebrauk, 1940). Flentje (1957) observed that lettuce seedlings hypersensitive to certain strains of *Thanatephorus cucumeris* were susceptible after exposure to reduced light intensity for 4 days or more. Numbers of lesions on leaves on *Vicia faba* caused by *Botrytis fabae* increased after 24 h incubation in darkness before infection (Sol, 1966).

It was earlier found that *Alternaria zinniae* Pape forms small distinct lesions on leaves of dwarf beans at normal light conditions (van den Heuvel, 1969). The present study deals with investigations whether the infection could be stimulated by exposure to darkness. It was also examined whether a toxic factor produced by this fungus might be involved in the disease reactions.

### Material and methods

Primary leaves of glasshouse-grown dwarf beans (*Phaseolus vulgaris* cv. ‘Irene’ and ‘Corene’) were inoculated, when 11 days old, with spore suspensions of *A. zinniae*, prepared from 4 days-old oat meal agar cultures. The suspensions, in 0.1 % Tween 80, the concentrations being adjusted to  $62.5 \times 10^3$  spores per ml, were applied to the primary leaves in two different ways: (a) by spraying the leaves to run-off (“spray” method), and (b) by pipetting 40  $\mu$ l of inoculum to each half-leaf, and then spreading it by a glass rod with a flattened end over an area of 4.9 cm<sup>2</sup> within a 25 mm diameter glass ring (“spread” method).

The leaves, still attached to their parent plants, were kept horizontal with supports of PVC during glasshouse incubation at 20°–25°C in humid-chambers. Some of these chambers were covered with black covers of cotton or plastic, whereas others were not, representing “darkness” and “light” treatment, respectively. Plants in uncovered chambers were exposed to the normal day/night light cycle.

Culture filtrates of *A. zinniae* were prepared by growing the fungus on a liquid medium according to White and Starratt (1967) in Roux bottles at 26°C for 17 days and separating the mycelial mats from the liquids by filtering through a Sartorius membrane filter MF 100. The filtrates were applied to the leaves using the spread method.

## Results

### *Effects of different periods of exposure to darkness on the infection*

Leaves were exposed to darkness for 1 day just before inoculation or for 0 to 3 days immediately after inoculation with *A. zinniae* (spray method). After 3 days of incubation the diameter of 25 lesions on each of three replicate leaves was measured (Table 1).

The diameter of the lesions appeared to correlate with the length of the darkness treatment. At longer periods of darkness, larger lesions developed. The size of the lesions after 3 days of incubation in darkness varied considerably; some lesions had a diameter of 10 mm or more (Fig. 1). Lesions formed in light were purplish brown, whereas spots formed in darkness were greyish brown, sometimes bordered by a narrow purplish brown margin.

On leaves inoculated by means of the spread method nearly the whole inoculated leaf area was necrotized after 3 days incubation in darkness, whereas normal little lesions developed when leaves were exposed to light. After exposure to darkness for 4 days or more, leaves became further necrotized and wilted and dropped prematurely.

### *Effect of culture filtrates of A. zinniae on leaves in light and darkness*

Application of undiluted culture filtrates of *A. zinniae* to the leaves caused the same symptoms as did inoculation with spore suspensions of the fungus. After incubation

Table 1. Effects of different periods of exposure to light (L) and darkness (D) on the size of lesions on bean leaves caused by *Alternaria zinniae*.

| Treatment                     |                               | Mean diameter<br>of lesions<br>(mm) |
|-------------------------------|-------------------------------|-------------------------------------|
| before inoculation<br>(1 day) | after inoculation<br>(3 days) |                                     |
| D                             | LLL                           | 0.3                                 |
| L                             | LLL                           | 0.3                                 |
| L                             | DLL                           | 0.6                                 |
| L                             | DDL                           | 2.1                                 |
| L                             | DDD                           | 2.5                                 |

Tabel 1. Invloed van verschillende incubatieperiodes in licht (L) en donker (D) op de grootte van de lesies op bonebladeren veroorzaakt door *Alternaria zinniae*.

Fig. 1. Bean leaves with lesions caused by *Alternaria zinniae*. Leaves were incubated in light (A) or darkness (B) for 3 days immediately after inoculation.

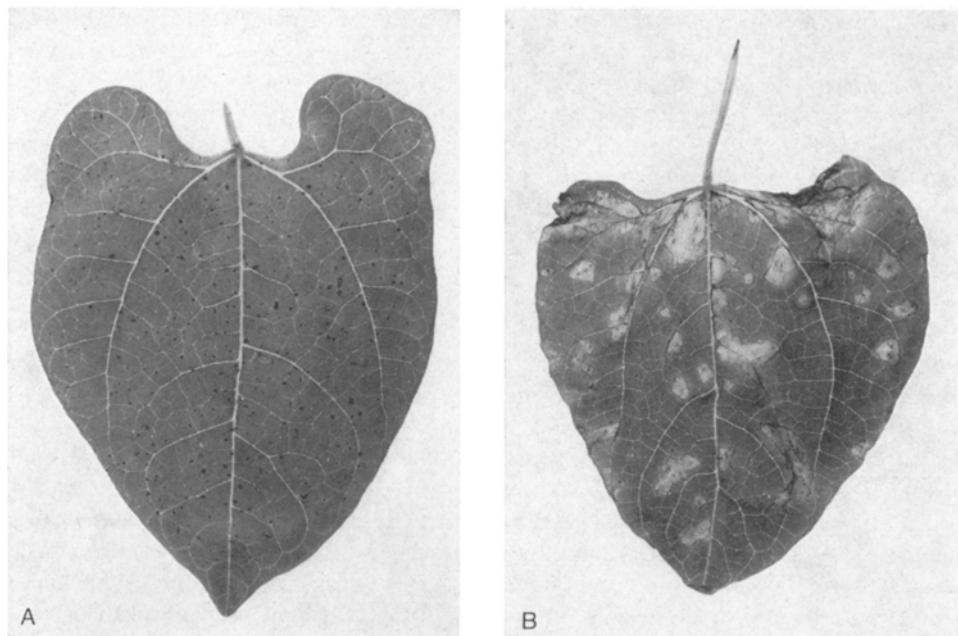


Fig. 1. Bonebladeren met lesies veroorzaakt door *Alternaria zinniae*. De bladeren waren direct na inoculatie gedurende 3 dagen in licht (A) of donker (B) geïncubeerd.

in light for 3 days small purplish brown lesions developed; after exposure to darkness large necrotic spots were formed. Sometimes, however, no symptoms appeared.

### Discussion

The reaction of bean leaves to infection with *A. zinniae* in light resembles a hypersensitivity reaction; the reaction in darkness is a more or less susceptible one, dependent on the length of the dark period. Application of culture filtrates of *A. zinniae* to the leaves had the same effect as inoculation with spores of *A. zinniae*. The enhanced susceptibility of bean leaves to this fungus seems to be due to an increased sensitivity to a toxic substance produced by *A. zinniae*.

Research is in progress to make clear whether the toxic factor in the culture filtrate is zinniol, a toxin isolated and characterized by White and Starratt (1967) and Starratt (1968). Subsequently, research is necessary on the nature of the increase of the toxin sensitivity in darkness.

### Acknowledgments

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## Samenvatting

### *De invloed van licht op aantasting van bonebladeren door Alternaria zinniae*

Bonebladeren, geïnoculeerd met sporen van *Alternaria zinniae*, vertoonden na 3 dagen incubatie in het licht kleine paarsbruine lesions (Fig. 1A). Na incubatie gedurende 1 tot 3 dagen in het donker ontstonden necrotische vlekken (Fig. 1B), waarvan de grootte toenam met de lengte van de incubatieperiode in het donker (Tabel I).

Toediening van cultuurfiltraten van *A. zinniae* aan de bladeren veroorzaakte dezelfde symptomen als inoculatie met sporensuspensies.

De grotere vatbaarheid van bonebladeren voor deze schimmel in het donker is waarschijnlijk te wijten aan een verhoogde gevoeligheid voor een toxische stof, mogelijk zinniol, gevormd door *A. zinniae*.

## References

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