

Effects of Temperature, Dew Period, and Light on the Growth and Development of *Alternaria helianthi*

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ABSTRACT

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Alternaria blight is a serious disease of sunflower (*Helianthus annuus*) in northeastern Australia, particularly during summer. The germination of conidia of *Alternaria helianthi* was favored by temperatures between 25 and 28 C and by the presence of free water on the leaf surface. Colony growth of *A. helianthi* on PDA was greatest at 25 C. A 12-hr period of leaf wetness was required to give the maximum infection (lesions per square centimeter) at 25 and 28 C. Repeated periods of dew and high relative humidity promoted the expansion of lesions. The generation time for *A. helianthi* on sunflower leaf

disks was less than 2 days at 22 and 25 C. Artificial light had no significant effect on percent germination of conidia, but increased the number of germ tubes produced by each conidium and the growth of mycelium on PDA. The environmental conditions that favor *A. helianthi* occur in northeastern Australia during the summer, when mean daily temperatures are 25–30 C and extended periods of wet weather associated with rainbearing cyclonic depressions are common. A method of reducing losses caused by *A. helianthi*, based on time of sowing, is suggested.

Alternaria blight on sunflower, caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara, is an important disease, particularly in India and Yugoslavia where significant reductions in yield have been observed (1,2,11,12). In Australia, the pathogen was first isolated from sunflower plants in northern Queensland in 1971 (3). *Alternaria* blight quickly spread to all other sunflower growing areas in Queensland (7) and caused significant reductions in yield (4,15). Recently the disease has been recognized as a threat to sunflower production in the United States (9,13).

Few authors have studied the effect of the environment on growth and development of *A. helianthi*. Acimovic (2) reported that the minimum, optimum, and maximum temperatures for infection were about 5, 30, and 35–40 C, respectively; the generation time varied from 13 days at 5 C to 2 days at 20, 25, and 30 C, and 3 days at 35 C; sporulation occurred between 5 and 35 C and was most profuse between 15 and 20 C; and conidia formed at 5, 10, and 30 C were smaller and had fewer septa than those formed at 15, 20, and 25 C. Islam et al (10) stated that *A. helianthi* could grow on PDA between 1 and 33 C with the optimum being between 23 and 25 C. However, Islam and Maric (11) later reported that *A. helianthi* grew at temperatures from 4 to 32 C with maximum growth from 20 to 28 C, and optimum at 26 C. These authors (11) also found that sporulation was poor below 16 C and abundant between 20 and 28 C. Acimovic (2) also reported that the minimum

dew period required for infection was 1 to 2 hr. However, Islam et al (10) reported that a minimum dew period of 12 hr was required for infection.

The objectives of the present studies were to determine: the effect of temperature on conidial germination, mycelial growth on PDA, infection of sunflower leaves, and the generation time; the length of the dew period required for maximum infection; the effect of repeated dew periods on infection and lesion expansion; and the effect of light on conidial germination and mycelial growth on PDA.

MATERIALS AND METHODS

Temperature. The effect of temperature on the germination of conidia was studied by using filter paper disks (15 mm diameter) that were soaked in demineralized water and placed in petri dishes on large wet filter papers. Dry conidia of *A. helianthi* were brushed onto the disks using a small paintbrush. The plates were incubated in darkness for 6 hr at 10, 15, 20, 25, 28, and 37 C. Several drops of lactophenol trypan blue were then added to each petri dish and allowed to soak into the inoculated filter paper disks to prevent further germination and to stain both conidia and germ tubes. One hundred conidia in each of four replicates (disks) were counted and the percentage germination was calculated.

The effect of temperature on the growth of *A. helianthi* on PDA was investigated by using petri dishes containing 20 ml of medium. Five radii were marked on the base of each plate, and the plates (six replicates) were inoculated with one square of agar (10 mm²) from a 4-wk-old culture. Inoculated plates were incubated in darkness for

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7 days at 5, 10, 15, 20, 25, 30, and 37 C. The mycelial growth along each of the five radii was measured for each plate, and the mean colony diameter was calculated.

Five-week-old, glasshouse-grown sunflower plants (cultivar Hysun 30; Pacific Seeds, Toowoomba, Queensland, Australia 4350) were uniformly inoculated with 20 mg of conidia for 10 min at 5 r.p.m. in a turntable-type inoculation chamber (6) to study the effect of temperature on the infection of sunflower leaves. Four plants growing in separate pots (replicates) were placed in each of two dew chambers. All plants were incubated for 12 hr before being returned to the glasshouse. The experiment was repeated four times by using one dew chamber set at 26 C and the second dew chamber set successively at 19, 19.7, 22, and 29 C. This was necessary because

only two dew chambers were available. The leaf area and the number of lesions per leaf were recorded for the second and third true leaf pairs (leaves 3, 4, 5, and 6 from the base of the plant) 10 days after inoculation. The numbers of lesions per square centimeter of leaf on plants incubated at 19, 19.7, 22, and 29 C were expressed as a percentage of those obtained for plants incubated at 26 C. It was assumed that the number of lesions per square centimeter on plants at 26 C represented 100% infection, since this was reported to be the optimum temperature for infection (11).

Leaf disks were used to study the effect of temperature on the generation time of *A. helianthi* on sunflower. Disks (20 mm in diameter) were cut with a cork borer from the eighth leaf (from the base) of plants at the budding stage of growth and placed on wet

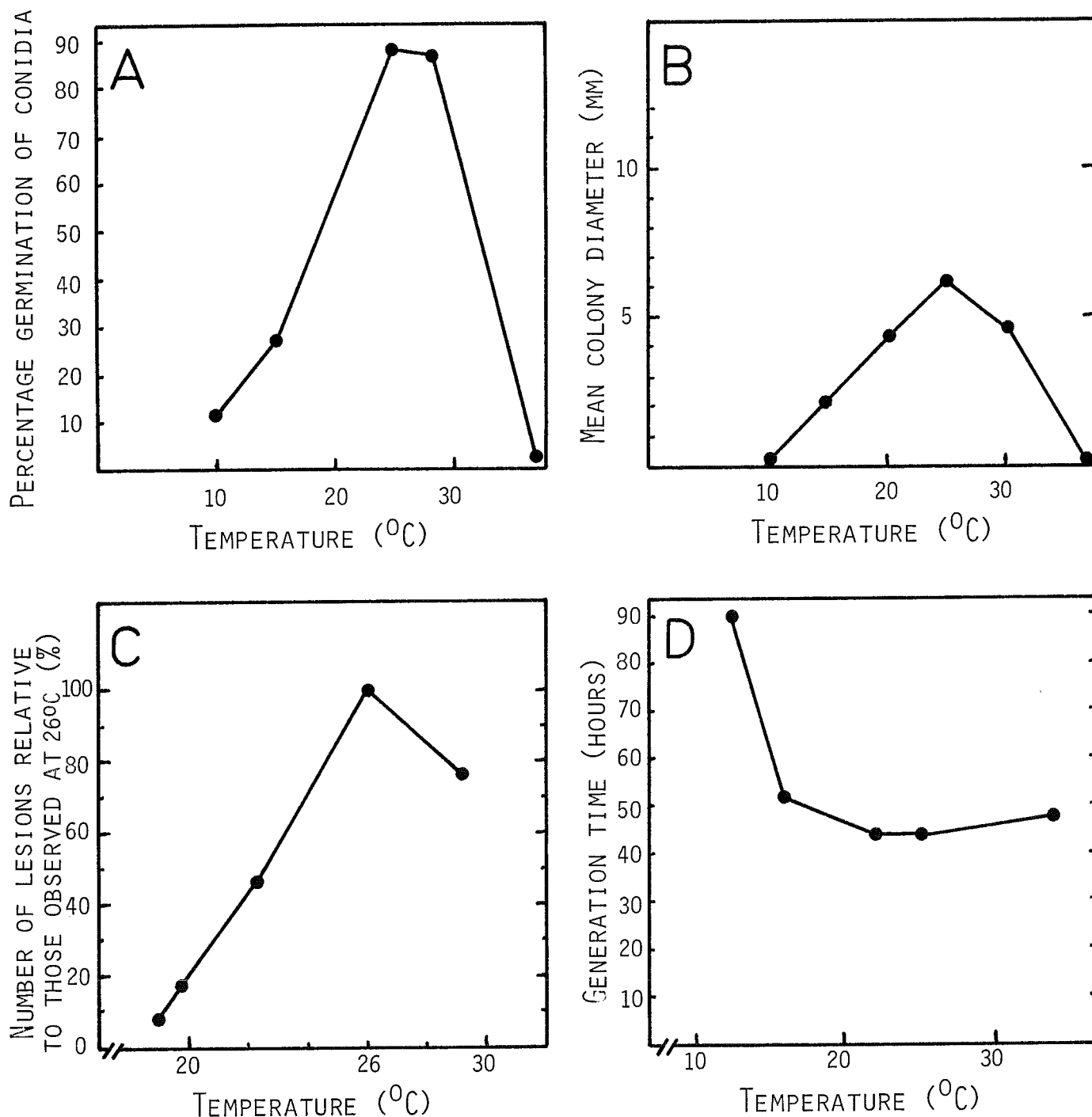


Fig. 1. The effect of temperature on *Alternaria helianthi*. A, Percentage germination of conidia on wet filter paper after 6 hr; B, Colony diameter on PDA after 7 days of incubation in darkness; C, Number of lesions on sunflower leaves incubated at different temperatures relative to those formed at 26 C; D, Generation time on leaf disks mounted on wet filter paper.

filter papers in petri dishes. Four separate drops of a suspension of conidia of *A. helianthi* (40–50 conidia per milliliter) were placed on each of four leaf disks in each dish. The inoculated disks were incubated in darkness at 12, 16, 22, 25, and 32 C. Observations with a stereomicroscope ($\times 60$) were made at 2- to 4-hr intervals for 4 days to determine when conidia first appeared on the lesion surface.

Dew period. Thirty-two glasshouse-grown plants (6 wk old) were inoculated in an inoculation chamber (6). Sixteen plants were placed in a dew chamber at 25 C, and 16 plants were placed in a dew chamber at 22 C. Four plants were removed from each chamber 9, 12, 15, and 18 hr after inoculation. The mean number of lesions per square centimeter on the second and third leaf pairs (leaves 3, 4, 5, and 6) was determined 1 wk after inoculation.

Eyal et al (8) found that the area of necrosis caused by the *Septoria* state of *Leptosphaeria nodorum* Muller on wheat was markedly affected by the length of the postinoculation dew period. Eight sunflower plants at the budding stage of growth were, therefore, inoculated with a conidial suspension (20 mg conidia per 100 ml of water applied with an atomizer) to investigate the effect of repeated dew periods and high relative humidity on the growth of lesions. Inoculated plants were placed in a glasshouse bay equipped with a Walton SW5 humidifier, which maintained 70–100% RH with 10- to 15-hr dew periods each day. Two days after inoculation, four plants were removed from the humid environment and placed in an adjacent glasshouse bay at 30–70% RH and no free moisture on the leaf surface. Ten days after inoculation, the diameters of necrotic lesions and the width of the associated chlorotic halos were determined. Ten lesions on each of four leaves per plant (leaves 9, 11, 13, and 15 from the base of the plant) were measured.

Light. Experiments were conducted to compare the effects of light and darkness on the germination and mycelial growth of *A. helianthi* on artificial substrates. Conidia were brushed onto wet filter paper disks in petri dishes and incubated at 26 C under light (3,500 lux) and dark (wrapped in aluminum foil) for 6 hr. The percent germination of conidia was determined after counting 100 conidia in each of eight replicates. The mean number of germ tubes per conidium was determined for 200 conidia from each treatment.

In a second experiment, *A. helianthi* was grown on PDA

amended with sunflower seed extract (10 g seed/100 ml of water, in a Waring blender for 60 sec, then passed through cheesecloth), and kept at 26 C. There were six replicates of each of two treatments, that is, light (3,500 lux) and dark (wrapped in aluminum foil). Colony growth along five radii marked on the base of each plate was measured 10 days after inoculation.

RESULTS

The optimum temperature for germination of conidia of *A. helianthi* on wet filter paper was between 25 and 28 C (Fig. 1A). The optimum temperature for growth on PDA was 25 C (Fig. 1B). The results of experiments with whole sunflower plants showed that at 19, 19.7, 22, and 29 C, the numbers of lesions produced by *A. helianthi* (after a 12-hr dew period) were 9.4, 18.5, 46.1, and 75.8%, respectively, relative to those produced on plants incubated at 26 C (Fig. 1C). The generation time for *A. helianthi* on sunflower leaf disks was <48 hr when incubated at 22 and 25 C in water droplets (Fig. 1D).

Incubation of inoculated plants for a 12-hr dew period gave maximum infection of sunflower leaves (lesions per square centimeter). Dew periods >12 hr did not significantly increase or decrease the level of infection. At the cooler temperature (22 C) an 18-hr dew period was required to give the same level of infection as that achieved in 12 hr at 25 C (Fig. 2). Necrotic lesions and the associated chlorotic halos that developed on plants kept in a wet, humid environment for 10 days were 81 and 69% larger, respectively, than necrotic lesions and chlorotic halos produced on plants that were removed from the wet, humid environment 2 days after inoculation (Table 1).

Exposure to low light intensity (3,500 lux) had no effect on the percentage germination of conidia of *A. helianthi* but increased the number of germ tubes produced per conidium (Table 2). The growth of colonies of *A. helianthi* on artificial media was greater when the fungus was grown under lights.

DISCUSSION

The data obtained show that the germination of conidia, growth, and infection of sunflower by *A. helianthi* are favored by temperatures between 25 and 28 C, and extended periods of leaf wetness.

Epidemics of *Alternaria* blight of sunflowers are most common and severe in areas of northeastern Australia that experience extended periods of wet weather in summer accompanied by mean

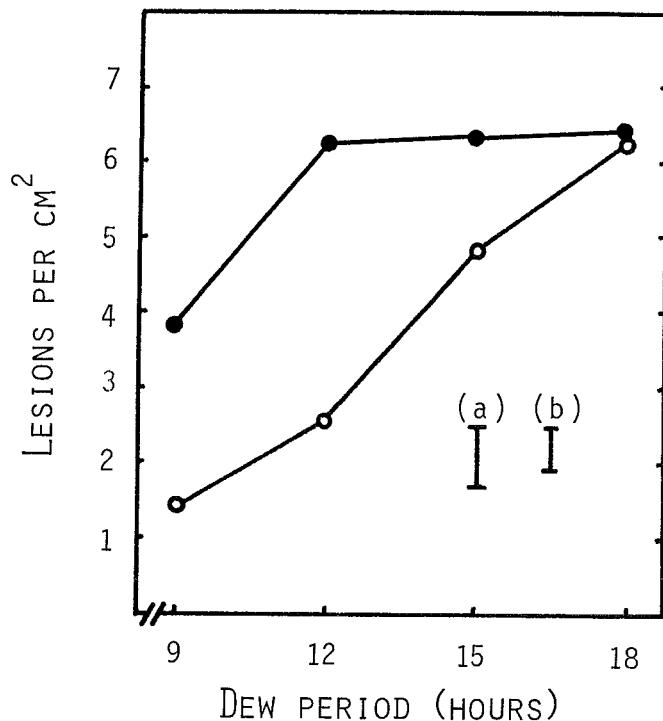


Fig. 2. The effect of duration of the dew period on infection of sunflower leaves by *Alternaria helianthi* at 25 C (—●—) and 22 C (—○—). Vertical bars represent LSD ($P = 0.05$) for comparing (a) dew periods and (b) temperature of the dew chamber.

TABLE 1. The effect of regular dew periods and high relative humidity on the size of lesions caused by *Alternaria helianthi* on sunflower leaves 10 days after inoculation

Treatment	Necrotic lesion diameter (mm)	Chlorotic halo width (mm)
70–100% RH 10–15 hr dew/day	1.72	1.22
30–70% RH No dew	0.95	0.72
LSD ($P = 0.05$)	0.10	0.70

TABLE 2. The effect of light on the germination of conidia and the growth of *Alternaria helianthi* on artificial media

Treatment	Germination ^a (%)	Mean number of germ tubes per conidium ^a	Mean radius of colony (mm) ^b
Light (3,500 lux)	90.87	4.94	28.50
Dark	89.12	3.88	21.57
LSD ($P = 0.05$)	5.03	0.41	1.02

^aSix hours of incubation at 26 C on wet filter paper.

^bTen days incubation at 26 C on PDA supplemented with a sunflower seed extract.

daily temperatures between 25 and 30 C. These extended periods of wet weather are associated with rain-bearing cyclonic depressions. Several authors have related the severity of *Alternaria* blight to the occurrence of wet humid conditions. Kolte and Tewari (12) reported that maximum disease severity on sunflower in India was correlated with the monsoon season. In Japan, Tubaki and Nishihara (14) noted that the severity of *Alternaria* blight rapidly increased during the rainy season.

The results of studies reported in this paper indicate that severe epidemics of *Alternaria* blight might be avoided in northeastern Australia by growing sunflower during autumn when temperatures are suboptimal for the development of *A. helianthi* (but still suitable for growing sunflowers) and when the probability of extended periods of wet weather (tropical cyclones) is low. The control of *Alternaria* blight of sunflowers in eastern Australia using time of sowing as a strategy in disease control has been discussed by Allen et al (5).

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