Effect of Environment and Host on Sporulation of *Alternaria macrospora* in Cotton

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ABSTRACT

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Prolific sporulation of *Alternaria macrospora* in cotton was induced by light (under either wet or dry conditions) between two wet periods in darkness. The wet-and-dark period after induction by light affected sporulation more than the wet-and-dark period before induction. More spores per unit area were produced on cotyledons than on leaves. On both organs, the number of spores increased and the lesioned tissue changed from greenish to chlorotic and to necrotic. These changes also were associated with lower minimum and higher maximum temperatures and extension of the optimum from 30 C in greenish leaves to a range of 25 to 30 C in chlorotic leaves, and 20 to 30 C in necrotic leaves. The earliest peak of sporulation, the longest infectious period, and the highest total production of spores occurred under relatively warm, cool, and medium temperature regimes, respectively. As a result of the induction by light, many more spores were produced under several relatively short dew periods at night interrupted by dry days than under one long and uninterrupted dew period in darkness. Proneness to sporulate increased with the increase in leaf age from 2 to 5 wk.

Additional keywords: epidemiology, humidity.

Alternaria macrospora Zimm. is a relatively little-known pathogen of the high-quality cotton *Gossypium barbadense* L. Sensitivity of plants to disease increases with an increase in yield (14,18). In fields with high yield, the pathogen is a major factor in yield loss (6). Reports about *A. macrospora* have dealt with specific aspects of epidemiology and control (5,6,13,15). The process of sporulation on host tissue has not been studied. In this respect, *A. macrospora* represents the necrotrophic (facultative) pathogens; the sporulation of these pathogens has been extensively studied in vitro (22) but rarely investigated in vivo. However, studies in vitro fail to state the influence of host (2), some environmental factors like dryness and dew (12), specific requirements for induction (4), and infectious periods. We investigated these influences to gain information on sporulation of *A. macrospora* as a representative of the necrotrophic pathogens.

MATERIALS AND METHODS

Cotton plants (*Gossypium barbadense* L. 'Pima S-5') were grown in a greenhouse in 250-ml pots filled with a mixture of sandy loam, peat, and sand (2:1:1, v/v). *A. macrospora* was isolated from infected cotton plants in a commercial field and maintained on V-8 agar in petri-dish cultures. Spores of the pathogen in water suspension were applied by means of Schein's inoculator (20) to 8-cm^2 areas on 14-day-old cotyledons and to 20-cm^2 areas on leaves of various ages. Inoculated plants were kept in the dark in dew chambers at 20 C for 20 or 24 hr and then transferred to growth chambers maintained at 25 C, 50–70% RH, and a 12-hr photoperiod with light intensity of 120 $\mu E\ cm^{-2} \times sec^{-1}$. In these and other tests, all temperatures were accurate within 1 C.

Sporulation tests were done with either detached or intact leaves. Detached leaves were placed in petri dishes over moist filter paper in darkness. Intact plants were kept moist either in plastic bags or in a dew chamber in darkness. Recovery and counting of spores were done by filtration method (2). Leaves were first shaken for 1 hr in formalin, acetic acid, and alcohol (5:5:90, v/v) to detach the spores. A given volume of the spore suspension was filtered onto an 8-μm membrane filter which was then examined microscopically.

The terms "wet light" and "wet darkness" are used to describe the environment of plants and detached cotyledons exposed to light or darkness in transparent polyethylene bags (plants) or petri dishes (cotyledons) that maintained drops of liquid water on the foliar surface. The terms "dry light" or "dry darkness" refer to environmental conditions of plants exposed to light or darkness outside the moist chamber. Other terms describe the stages of discoloration of the infected leaves: greenish, chlorotic, and necrotic.

Effect of light. Infected plants or detached cotyledons at the necrotic stage were kept first in moist conditions in darkness for production of sporophores (the initial phase), then exposed to light and/or dryness to induce formation of spores (inductive phase), and returned to moist conditions in darkness for the spore to be formed (final phase) (16). The intensity of light was approximately that of the growth chamber. All treatments were carried out in three replicates.

In the first test, cotyledons were kept for 20 hr in wet darkness (initial phase), exposed for 0.5 to 24 hr to either wet or dry light or dry darkness (inductive phase), then kept for 20 hr in wet darkness (final phase). Controls were cotyledons kept in wet darkness during the inductive period.

In the second test, the treatments differed in duration of the dark, light, and dark phases which lasted for 5, 10, and 24 hr in wet darkness. Between these phases, all treatments, except control, were exposed to wet light for 5 hr.

In the third test, we checked whether the total of 5 hr of wet light exerts its inductive effect when divided into shorter fractions. The total wet period in darkness was 48 hr and was interrupted by one (5 hr), two (2 × 2.5 hr) and three (3 × 1.75 hr) light periods.

Effect of leaf discoloration. Fourteen- and 30-day-old plants with cotyledons and true leaves, respectively, were sprayed with approximately 1,000 spores/1 ml of water, kept 24 hr in a dew chamber at 20 C, and then kept in a growth chamber at 25 C. From several hundred inoculated plants, we selected plants with 8 to 10 lesions of 2–3 mm that on the 4th, 11th, and 16th day represented the category of greenish, chlorotic, and necrotic leaves, respectively. On these days, the plants were atomized with water, covered with plastic bags, and exposed for 24 hr in dark growth chambers maintained at 5, 10, 15, 20, 25, 30, 35, and 40 C (10 replicate plants per treatment).

Infectious period. The effect of temperature regimes of 20–10 (12-hr day-night periods), 25–15, and 30–20 C on recurrent production of spores was tested during a period of 45 days. Plants

with cotyledons or leaves were inoculated when they were 7 or 30 days old and kept in the standardized conditions in the growth chamber during the day and in dew chambers at night. Sampling during the first 30 days and the following 15 days was carried out on every second and fifth day, respectively. Sporulation was assessed on 10 replicate cotyledons or leaves detached from five plants that were removed from further experimentation. Each day spores not collected for sampling were removed by short but vigorous sprinkling with water and the plants were left for further experimentation. Leaves that shed at the end of the infectious period were left in situ and examined for spores.

Effect of wetting period. The treatments, in 10 replicates, included a continuous wetting period (CWP) without induction by light, and an interrupted wetting period (IWP) when wet periods in the dew chamber at night were interrupted by dry periods in the growth chamber during the day. Preliminary tests showed that spores did not disperse under the nearly windless conditions of the growth chambers. The 12-hr dew-period temperatures were 15 and 25 C. The dry day temperature was 25 C.

Effect of age. The experiment was performed with 85-day-old plants in which leaves six, seven, eight, and nine were approximately 5, 4, 3, and 2 wk old, respectively. The infected plants (four replicates per treatment) were kept 7 nights and days in alternating 12-hr periods of wet darkness and dry light. Spores were collected after each wet night by rinsing the leaves with water without detaching them from plants.

RESULTS

Effect of light. Figure 1A shows that sporulation in controls not exposed to induction by light increased with the increase of the incubation period in wet darkness from about 1 to 18 $\times$ 10^3 spores/cotyledon. A slightly higher number of spores was produced in cotyledons exposed during the inductive phase to conditions of dry darkness. Exposure to wet or dry light during the inductive phase was associated with production of many more spores. A minimum of 3 hr of light was required to result in prolific sporulation.

The second experiment showed that under an equal duration of the inductive phase the number of spores produced in plants depended on the specific duration of the initial and final phase in wet darkness. Controls exposed to these phases without induction by light produced up to 20$\times$ 10^3 spores/cotyledon (Fig. 1B). Figure 1C shows that in irradiated plants exposed to equally long durations of wetness (initial plus final phase) the duration of the final phase affected sporulation more than did the duration of the initial phase (see total wetting durations of 34 and 39 hr). However, the maximum number of spores was produced when each phase was extended to 24 hr.

Figure 1D shows that the inductive light period can be divided into several fractions. Part A of this figure represents the control, with only one 5-hr induction by wet light. Part B shows that, when the induction period was divided into two parts of 2.5 hr each, most spores were formed after the second dark phase. Part C shows that, when the inductive phase was divided into three fractions, vigorous sporulation started after the second period of light and the third dark phase and increased further after additional light and dark periods.

Effect of leaf discoloration. Figure 2 shows that the number of produced spores increased from greenish to chlorotic and to necrotic organs and was higher in cotyledons (Fig. 2A) than in leaves (Fig. 2B). No spores were produced at 40 C. The minimum, optimum, and maximum temperatures for sporulation were influenced by the discoloration of the leaf or cotyledon tissue. In both organs, the minimum temperature for sporulation decreased from 15 C in the greenish organs to 10 C in the chlorotic organs and to 5 C in the necrotic organs. The optimum temperature extended from 30 C in the greenish organs to a range of 25 to 30 C in the chlorotic organs and 20 to 30 C in the necrotic ones. At 35 C spores were produced on cotyledons but not on true leaves.

Infectious period. Numbers of spores collected and counted during the sampling days are presented in Figure 3. The numbers of spores removed by sprinkling on the other days were estimated by interpolation. Including the interpolated data, we calculated the daily rate of sporulation and the total number of spores produced over the infectious period. Cotyledons (Fig. 3A) produced more spores than leaves (Fig. 3B). On each organ the earliest beginning of sporulation, its highest daily rate, the shortest duration, and the lowest total production coincided with the warmest temperature regime (30–20 C day-night temperature). Sporulation in 25–15 C regime started later but produced the highest total number of spores over the infectious period. The lowest daily production but the longest infectious period occurred at the lowest temperature regime of 20–10 C.

Effect of wetting period. Figure 4 shows the effect on sporulation of CWP and IWP under a dew-period temperature of 25 C (A) and

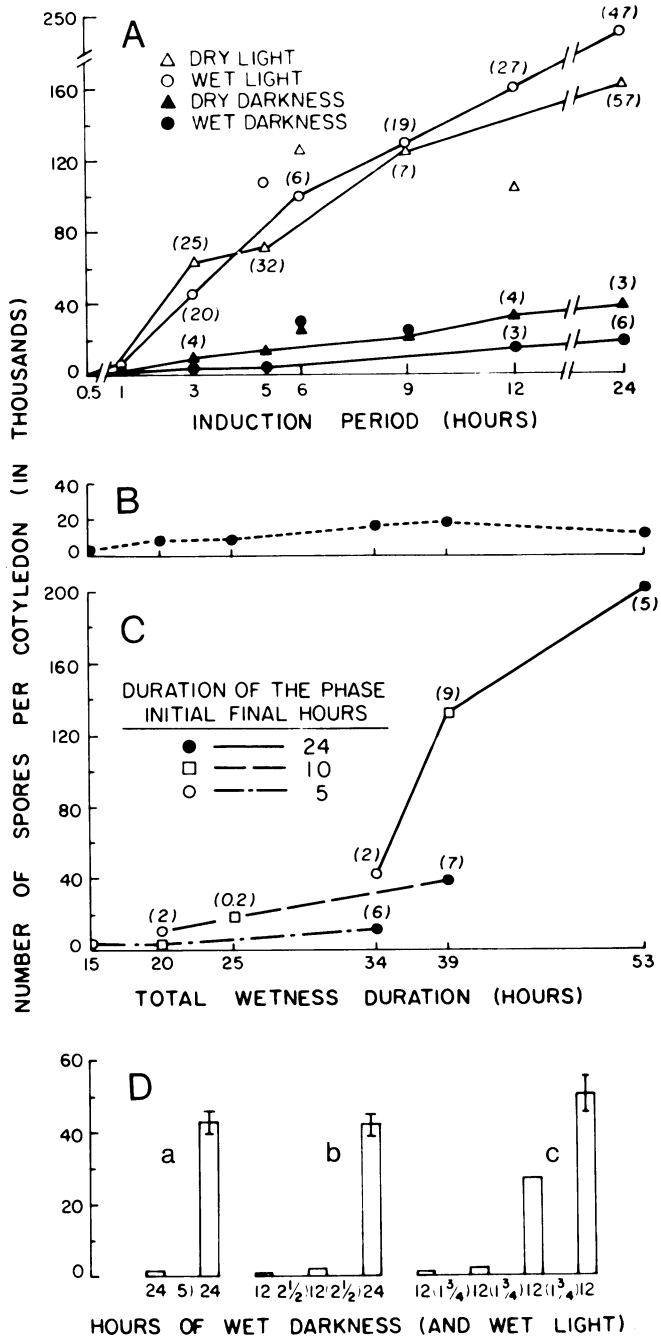


Fig. 1. Effect of light and darkness on sporulation of *Alternaria macrospora* in cotton. Values in parentheses or bars are standard errors. **A,** Effect of wet and dry light and darkness periods. **B, C,** Number of spores produced in various combinations of the initial and final phase in wet darkness without **(B)** and with **(C)** induction by wet light. **D,** Effect of wet light (total of 5 hr) applied in one (a), two (b), and three (c) fractions between periods of wet darkness (total 48 hr).

15 C (B). Under both night temperatures, fewer than 10×10^3 spores/cotyledon were produced in plants exposed to CWP. In the IWP treatments, more spores were produced at the dew-period temperature of 25 than at 15 C. At both night temperatures, the number of spores decreased from an IWP of 2×12 hr treatment (two nights with 12 hr of wet darkness) to 3×8 hr and 3×6 hr. In the 3×6 hr treatment, relatively high numbers of spores were produced at the dew-period temperature of 25 but not at 15 C. Few and no spores were produced during 6 nights with 4-hr dew in each at 25 and 15 C, respectively.

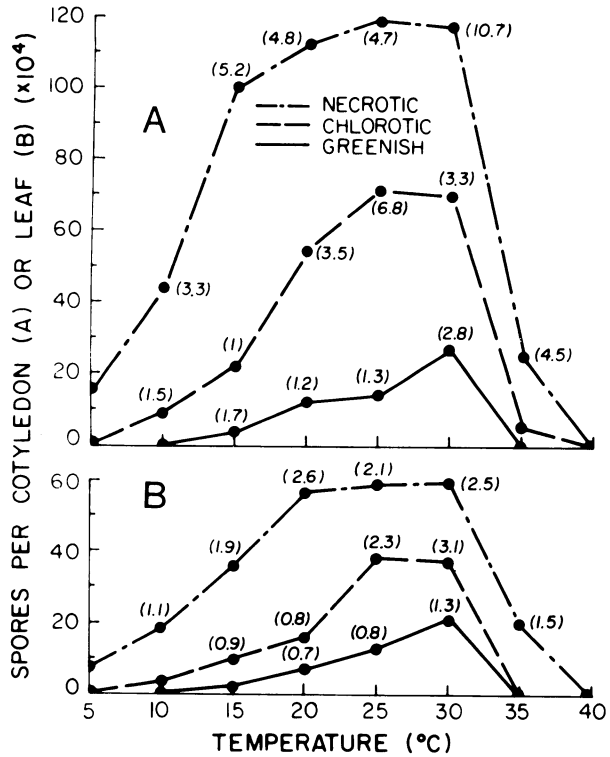


Fig. 2. Effect of the wet-period temperature on sporulation of *Alternaria macrospora* in greenish, chlorotic, and necrotic cotyledons **(A)** and leaves **(B)** of cotton. Values in parentheses are standard errors.

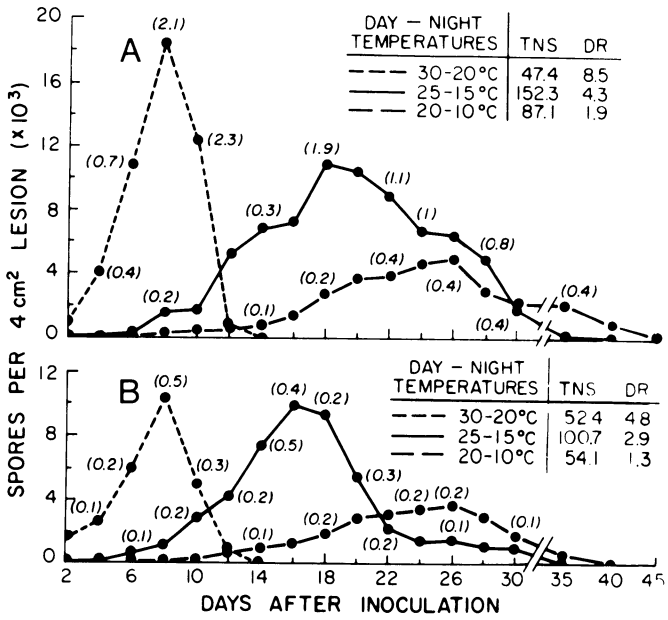


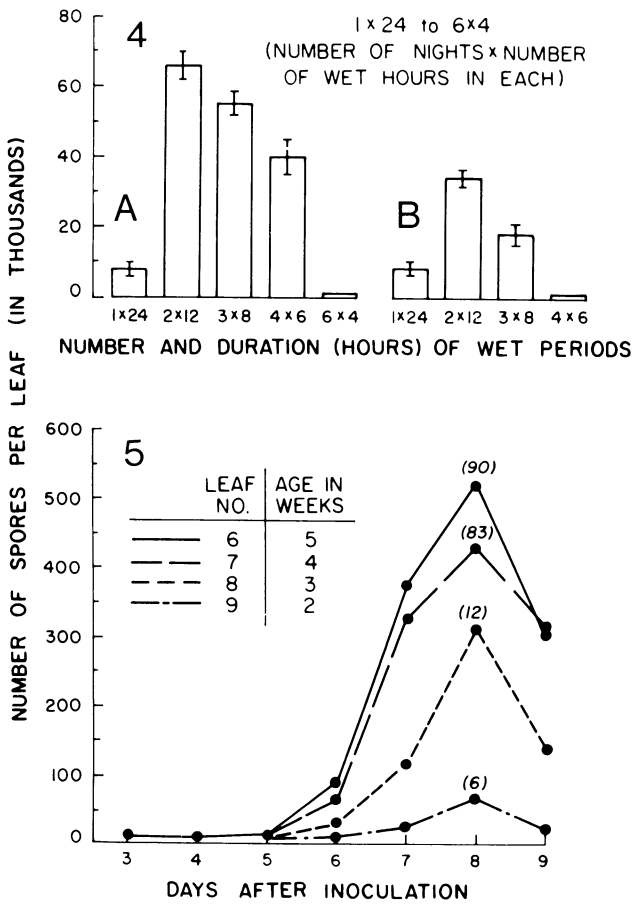
Fig. 3. Infectious period and number of spores produced by *Alternaria macrospora* on cotyledons **(A)** and leaves **(B)** of cotton. TNS and DR indicate the total number of spores and daily rate of sporulation, respectively.

Effect of age. Figure 5 shows that the number of spores produced decreased from the oldest to the youngest leaf. The total number of spores over the entire period (in millions) decreased from 1.3 to 1.2, 0.6, and 0.1 in leaves approximately 5, 4, 3, and 2 wk old, respectively.

DISCUSSION

Sporulation of necrotrophic pathogens in vivo often differs from sporulation in vitro. For instance, radiation that induces sporulation of many fungi in culture (22) is less obligatory in vivo (10,16). Among *Alternaria* and related dematiaceous fungi, *Stemphylium botryosum* f. sp. *lycopersici*, which requires induction in vitro, sporulates on tomatoes without any induction (3). In *Alternaria solani* on potatoes (4) and *Helminthosporium turcicum* on corn (12), induction by light could be replaced by dryness. *Alternaria dauci* on carrots requires induction by light (24) as does the same pathogen in vitro. In *A. macrospora*, induction by light is as necessary for prolific sporulation in cotton as it is in culture. Similarly to *A. solani* on potatoes (4), sporulation of *A. macrospora* in vivo is induced by wet as well as by dry light.

In the field, induction by light acts in conjunction with the effect of interrupted wetting periods. In *S. botryosum* f. sp. *lycopersici*, which sporulates in vivo without induction, a similar number of spores is produced under continuous and interrupted wetting periods of the same total duration (1). In *H. turcicum* on corn (12) and *A. solani* on potatoes (1), which require induction by light or dryness, the interrupted wetting period regime secures more prolific sporulation because it includes the element of induction. This is also the case with *A. macrospora*, which produces many



Figs. 4 and 5. 4, Number of *Alternaria macrospora* spores produced under a temperature of 25 C **(A)** and 15 C **(B)** in one continuous wet period of 24 hr in darkness and in several shorter wet periods totalling 24 hr, but interrupted by dryness and light. Bars are standard errors. **5,** Number of *Alternaria macrospora* spores in cotton leaves 2–5 wk old during 3 to 9 days after inoculation. Numbers in parentheses are standard errors.

more spores in the interrupted than in the continuous wetting period regimes. In the interrupted regimes, the number of wet nights and the wetting duration of each night affects sporulation according to the temperature of the dew period. For instance, many and few spores were produced in four nights each with 6-hr dew, at 25 and 15 C, respectively.

The effects of temperature also were related to the greenish, chlorotic, or necrotic state of the infected organs. The effect of these stages of pathogenesis upon sporulation of various pathogens is well known, although the mechanism of action is obscure (16). In the case of *A. macrospora* in cotton, the peak of sporulation at 30 C in greenish leaves extends to 25 to 30 C in chlorotic leaves and 20 to 30 C in necrotic leaves. With the advance in necrotization, there is a decrease in the minimum and an increase in the maximum temperature for sporulation. The occurrence of various optimum temperatures for sporulation on yellow and necrotic leaves was reported for *Pseudoperonospora cubensis* in cucumbers (8). The extension of a temperature peak to a plateau is a common phenomenon in the process of infection (17). However, to the best of our knowledge, such an effect has not been reported for the process of sporulation. We assume that the lack of difference in action among several temperatures in the plateau situation derives from the inability of the system to produce more than a given maximum of spores, even at a more suitable temperature.

In our experiments, higher temperature regimes were associated with production of more spores at early stages of disease development, but the total number of spores was higher and the infectious period was longer at medium and low temperatures. Similar phenomena were noted for *Colletotrichum lagenarium* on cucumbers (21), *P. cubensis* on cucumbers (9), *H. turcicum* (12) and *Phyllosticta maydis* (7) on corn, and *Pyricularia oryzae* on rice (11). Cohen and Rotem (10) assumed that the relatively higher temperatures facilitate the sporulation directly but that a prolonged exposure to these temperatures results in a faster depletion of nutrients.

In *A. macrospora*, sporulation also was affected by age, and 5-wk-old leaves produced about five times more spores than 2-wk-old leaves. This experiment indicates that, similar to an age-conditioned proneness to infection (5), there is an age-conditioned proneness to sporulation. Such a phenomenon has been described for *Phytophthora infestans* in potatoes (19).

The sporulation factors that condition epidemic development are the number of spores produced per lesion and the length of the latent and the infectious periods (23). Compared to other pathogens (10), *A. macrospora* does not produce many spores per lesion. However, it has a short latent period and a long infectious period. The spores are resistant to environmental extremes (J. Rotem, *unpublished*) frequently present in the cotton growing zones. The ability to sporulate under interrupted wetting periods and under a wide range of temperatures make *A. macrospora* a most efficient pathogen. However, the damage caused by *A. macrospora* usually is underestimated. Estimates of disease are low because the infected leaves shed while new leaves emerge, obscure the defoliation, and give the impression of a relatively healthy crop (6).

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