Influence of Controlled Environment and Age on Development of *Alternaria macrospora* and on Shedding of Leaves in Cotton

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ABSTRACT

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Under controlled conditions disease development was 5.5–8.9 times higher in cotyledons than in leaves. For infection of both organs the minimum, optimum, and maximum wetting-period temperatures were below 10 C, from 20 to 25 C, and 35 C, respectively. Cotyledons were substantially infected after a 4-hr wetting period, and temperatures of 20 and 25 C. Much longer wetting periods were required for infection of leaves. The minimum temperature for colonization was below 10 C (the lowest temperature tested) in cotyledons and 10 C in leaves; the optimum and maximum temperatures were 20 to 30 C and 35 C, respectively. Susceptibility in cotyledons increased with age up to 20 days, and decreased thereafter. In leaves, susceptibility slightly decreased with age. Shedding of cotyledons was affected mainly by age, which obscured the effect of disease. In leaves, increase in disease severity from 0 to 16% was correlated with shedding. The epidemiological implications of cotyledon susceptibility and of shedding are discussed.

Alternaria macrospora Zimm. on cotton has been reported in most cotton-growing countries (5). The high-quality cultivar Pima (*Gossypium barbadense* L.) is especially susceptible. Although most cotton pathologists do not consider this pathogen to be a major factor in cotton production (eg, 6,10), studies in Israel showed that epidemics of *A. macrospora* decrease the yield of cultivar Pima S-5 by ~25% (2). The underestimation of the damage caused by *A. macrospora* apparently derives from the misleadingly low estimates of disease incidence in cotton foliage because infected leaves tend to shed (2). New leaves then emerge, obscure the defoliation, and give the impression of a relatively healthy crop.

A. macrospora on cotton has not been studied extensively. Reports on environmental influences on disease development mention the effect of frequent rains (3,8,10) and of periods with prolonged high humidity (1,7). However, the severity of epidemics caused by *A. macrospora* during the rainless summer months in Israel (minimum-maximum temperatures of ~20 to 33 C and dew periods lasting ~8 to 12 hr per night) suggests that the pathogen is able to penetrate and infect within the relatively short dew periods that occur at that time. The effect of dew period durations and temperature on infection are unknown. Also unknown are the effects of environmental conditions on disease development after infection. There may also be changes in plant susceptibility with age.

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We investigated these questions to gain more information on the development of this underrated disease. Special attention was paid to the reaction of cotyledons, because disease severity on these has considerable effect on the subsequent epidemic (E. Bashi, I. Sachs, and J. Rotem, *unpublished*).

MATERIALS AND METHODS

Cotton plants (cultivar Pima S-5) were cultivated in a greenhouse in 250-ml to 3-L pots filled with a mixture of sandy loam, peat, and sand (2:1:1, v/v). The small pots were used to test plant reaction at the cotyledon stage. Larger pots were used in experiments that required a longer period of growth. The inoculum was obtained from infected plants in a commercial field, maintained in petri dish cultures, and introduced by means of Schein's quantitative inoculator (11) onto a 4-cm^2 target on cotyledons and a 20-cm^2 target on leaves. The number of spores deposited on these targets ranged from 55 to 115 (± 10%) per square centimeter in different tests. In the infection experiments, the inoculated plants were kept in the dark in moist chambers at various temperatures for different periods. They were then transferred to growth chambers maintained at a constant temperature, a relative humidity of 50 to 70%, and 12-hr photoperiod with light intensity of 120 μEinsteins $cm^{-2} \cdot sec^{-1}$. Temperatures were accurate within ± 1 C. In the colonization experiments, all the inoculated plants were exposed to the same wetting period and temperature conditions in darkness. They were then incubated in growth chambers maintained at various temperatures. More details are given under Results.

The percentage of lesioned area in the inoculated area was assessed visually. Each environmental treatment was studied in several experiments. Only one experiment of each series is described here in detail. The results of other experiments of the same series are mentioned for similarities or differences from the case test.

RESULTS

Wetting-period conditions. Infection at 10, 15, 20, 25, 30, 33, and 35 C during the 20-hr wetting period was determined in 3-wk-old plants (cotyledons and one leaf). At the end of this period, the plants (10 replicate plants per treatment) were incubated for 7 days in a growth chamber at 20 C. Disease developed to a much greater extent in the cotyledons (Fig. 1-A) than in the leaves (Fig. 1-B). In cotyledons given a wetting-period temperature of 15–30 C, symptoms appeared 2 days after inoculation; in the leaves, they appeared after 2 days only in plants given a wetting-period temperature of 20 C. Lesions in cotyledons expanded rapidly during the first 4 days, and slowly thereafter. Leaf lesions expanded slowly over the entire period. For both organs the optimum, minimum, and maximum wetting-period temperatures were 25, 10 (the lowest temperature tested), and 35 C, respectively. The minimum and maximum wetting-period temperatures were confirmed in two additional tests designed and executed as the previously described one. In one of these tests the optimum was 25 C, but the difference between the effects of 20 and 25 C were negligible. In the second test, there was somewhat more development at 20 C than at 25 C. It may be concluded that the optimum wetting-period temperature is in the range of 20 to 25 C.

Seven replicates per treatment were used to test the combined effect of wetting-period durations of 4 to 20 hr and temperatures ranging from 15 to 30 C in plants with cotyledons (evaluated on the 3rd day) and five or six leaves (evaluated on the 6th day). As shown in Fig. 2-A, cotyledons maintained at 25 C reached 100% disease severity after a wetting period of 9 hr, whereas at the other temperatures tested this occurred only after a wetting period of 20 hr. Up to a wetting period of 12 hr, the differences in disease severity on the leaves at various temperatures were small. Temperature had an effect only at a wetting period of 20 hr. Infection was somewhat higher at 20 C than at 25 C. An additional test confirmed these wetting-period duration and temperature effects. Based on the average for all treatments described in Fig. 2, the severity of disease in cotyledons was 8.9 times higher than in the leaves.

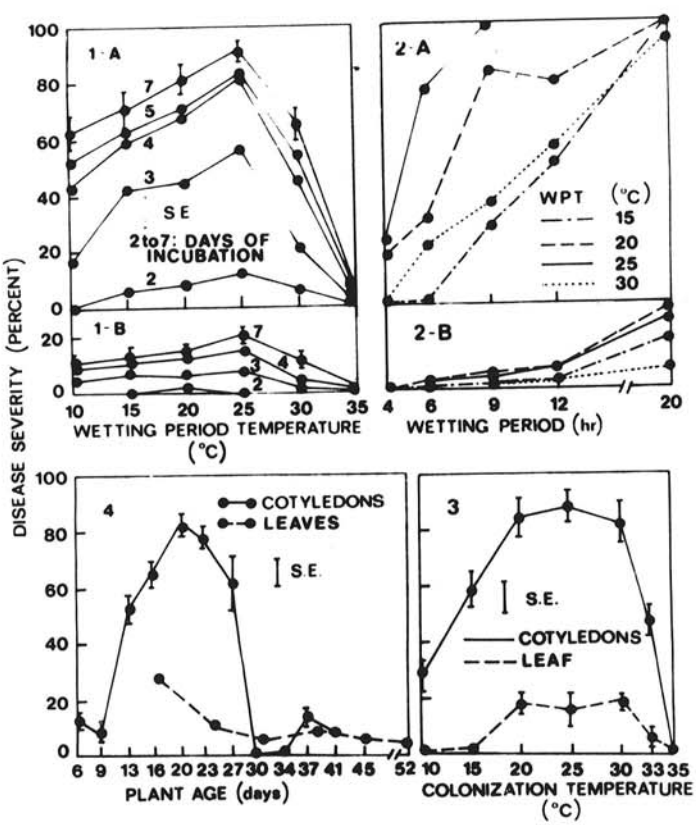
Colonization temperature. Three-week-old plants (cotyledons and one leaf) were inoculated, maintained in a dark moist chamber at 20 C for 16 hr, and then incubated for 5 days at 10, 15, 20, 25, 30, 33, or 35 C (10 replicate plants per treatment). Disease developed well on cotyledons in the 10–30 C range, with optimum and maximum development at 25 and 35 C, respectively. In the leaf, minimum and maximum development was at 10 and 35 C, respectively. In both organs, the optimum temperature for colonization was in the 20–30 C range. Based on the average for all treatments, the level of disease in the cotyledons was 6.1 times higher than in the leaves.

The effect of temperature during the colonization period was investigated in three additional tests, all of which confirmed the earlier results with regard to differences in susceptibility between cotyledons and leaves, and the minimum and maximum temperatures. However, different optimum temperatures were obtained in the three tests: 20, 30 C, and 20 to 30 C. Thus, it may be concluded that the optimum temperature for colonization is between 20 and 30 C.

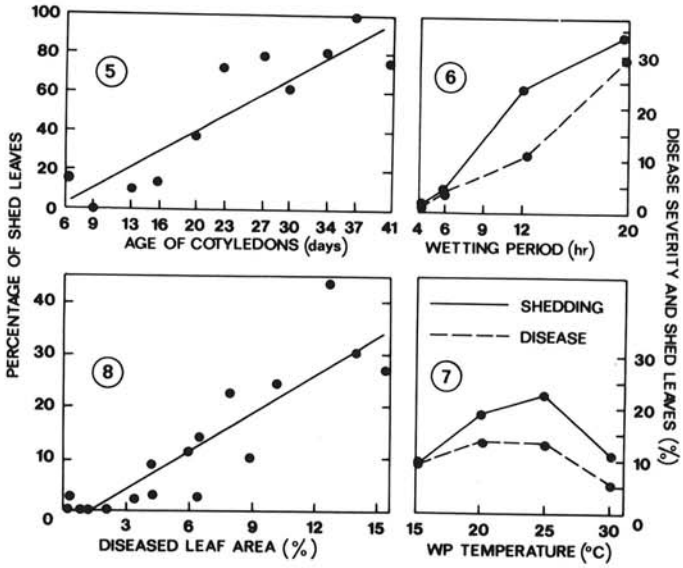
The effect of plant age on susceptibility. The influence of plant age on susceptibility to infection was tested with cotyledons ranging from 6 to 41 days of age and first leaves ranging from 16 to 52 days of age (eight replicates per treatment). The inoculated plants were kept for 20 hr in a moist chamber at 20 C. As assessed 5 days after inoculation, the susceptibility of cotyledons increased from the age of 6–9 days to a maximum at the age of 20 days, and decreased thereafter. The susceptibility of the first leaves decreased slowly with age (Fig. 4). An additional test with leaves showed that when a successful infection occurs, and the incubation time is long, more necrosis tends to develop on old than on young leaves.

The effect of various factors on shedding. Old cotyledons are shed whether or not they are infected (Fig. 5). Because of the dominant effect of age on the shedding of cotyledons (correlation coefficient $r = 0.896$) no correlation was found between shedding and disease severity in cotyledons at different ages.

Leaves were less prone than cotyledons to shed, at least within the age range tested in this study. Factors associated with disease development in leaves were also associated with shedding. To illustrate this we used data on the effects of wetness-period durations of 4 to 20 hr, and temperatures of 15 to 30 C on infection (see Fig. 2), but compared disease level on the sixth day after inoculation with shedding measured on the 10th day. To show association between factors affecting disease and shedding we determined the average effects exerted by wetness-period temperatures of 15, 20, 25, and 30 C at durations of 4 to 20 hr. As shown in Fig. 6, both the disease severity and shedding increased with prolongation of the wetness period. Shedding and disease severity likewise responded similarly to wetness-period temperature (Fig. 7). The relationship between disease severity and shedding was analyzed when these two parameters were plotted against each other. In leaves with disease severity ratings ranging from 0 to 16%, the increase in lesioned area was correlated with an increase in shedding, with a correlation coefficient of $r = 0.887$ (Fig. 8). No further increase in shedding was observed in two plants with disease severity of 30 and 40%, but the sample thus infected was too small for inclusion in the regression analysis.



Figs. 1–4. Development of *Alternaria macrospora* in cotton, as related to environment and age. **1,** The effect of wetting-period temperature on infection of **A)** cotyledons and **B)** leaves during incubation periods ranging from 2 to 7 days. **2,** The combined effect of duration and temperature of wetting periods on infection in cotyledons **A)** 3 days after inoculation and **B)** in leaves 6 days after inoculation. Standard errors of the values for leaves ranged from 0.03 to 2.9%. **3,** The effect of temperature on colonization. **4,** The effect of age on susceptibility of cotyledons and leaves. All effects were evaluated according to disease severity. S.E. means standard error.



Figs. 5–8. Shedding of leaves in cotton infected by *Alternaria macrospora*. **5,** Relationship between age and shedding in cotyledons ($r = 0.896$) 6 days after inoculation according to data presented in Fig. 4. **6 and 7,** Association between factors affecting infection and the subsequent shedding of leaves: **6,** wetting periods; **7,** wetting-period temperature. **8,** Relationship between disease severity and shedding in leaves ($r = 0.887$). Disease severities for association with shedding were taken from data presented in Fig. 2 B.

DISCUSSION

Environmental effects on infection and colonization of *A. macrospora* have not been previously studied in cotton. They have, however, been tested for a strain pathogenic on *Anoda crustata*, which appears to require higher temperatures and longer wetting periods (4) than the strain pathogenic on cotton.

Alternaria macrospora on cotton develops well under a wide range of temperatures (Figs. 1–3). This explains the wide geographical distribution of this pathogen on cotton (5). It is not clear why this cotton blight is of little importance in the U.S. The reasons for it may derive from small acreage cropped with the sensitive *G. barbadense*, unfavorable environment for the pathogen in habitats in which *G. barbadense* is grown, and/or an absence of a virulent strain. In any case the use of *A. macrospora* strain for biological control of weeds (4) should be viewed with caution.

In the field, cotyledons support the early stage of the epidemic (E. Bashi, I. Sachs, and J. Rotem, *unpublished*). Our study shows that cotyledons are several times more susceptible than the leaves to *A. macrospora*. It is likely that because of their susceptibility, the cotyledons become infected under wetting-period durations marginal for infection of the leaves (Figs. 1–3). Such marginal conditions often exist in the field early in the season, when the night temperature is relatively low, as in Israel in April. The lack of a dense canopy early in the season may prevent the creation of a favorable microclimate (9). Under these conditions, the extreme susceptibility of cotyledons may compensate for environmental deficiences. In the later stage of crop development the pathogen has to develop on leaves with a much lower susceptibility to disease. The absence of susceptible cotyledons at this stage of crop development is compensated for by a more favorable microclimate in the dense canopy and more favorable temperatures.

Old cotyledons shed, whether diseased or not (Fig. 5), and it then becomes difficult to establish a relationship between disease and shedding. Shedding of leaves is less affected by age, and is correlated with disease severity (Fig. 8). The largest quantities of spores of *A. macrospora* are produced on shed leaves (J. Rotem and E. Bashi, *unpublished*). Therefore, shedding of the diseased leaves increases sporulation, ie, the inoculum pressure in the field. Thus, every generation of leaves produced after shedding of the diseased ones is exposed to increased inoculum pressure.

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