Temperature-Induced Suppression of Alternaria Leaf Spot of Cotton in Arizona

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ABSTRACT

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Lesion formation by *Alternaria macrospora*, the causal agent of Alternaria leaf spot of cotton (*Gossypium barbadense*), is influenced by the temperature at which plants are held after infection. Forty to 100% reductions in lesion number occurred on cotton plants incubated at elevated temperatures compared with plants maintained at 30 C. Lesion formation was reduced more than 70% after exposure to 43.5 C for 2 hr. Results indicate that daily temperature maxima may be important in limiting Alternaria leaf spot in Arizona and may partially explain increased disease severity in central Arizona during cotton seasons in which daily temperature maxima are relatively low. The influence of high temperature on the pathogen in vitro was also studied. Germ tubes of *A. macrospora* explosively lysed on potato-dextrose agar (PDA) incubated at 42–46 C. After 6 hr at 42, 43.5, and 46 C, respectively, 7, 52, and 82% of the germ tubes lysed. Spore viability on PDA declined 65% after 4 hr at 46 C but remained stable for 24 hr at 42 C. Results provide indirect evidence that germ tube lysis and reduced spore viability may be mechanisms of temperature-mediated reduction in lesion number. However, other temperature-induced alterations in the host-pathogen interaction may be equally important.

Alternaria leaf spot (ALS) of cotton caused by *Alternaria macrospora* Zimm. occurs in most cotton-growing areas of the world (3,7). Although most cotton species are susceptible to the pathogen, the predominant species grown in the United States, *Gossypium hirsutum* L., is highly resistant (7). However, extra-long-staple Pima cotton (*G. barbadense* L.) is highly susceptible. In Israel, ALS reduced yields of the *G. barbadense* cultivar Pima S-5 up to 25% (3). Pima cotton has been grown commercially in Arizona for many years with only minor levels of ALS in most years (10). However, in 1982, 1983, and 1984, increased rainfall and reduced daily temperature maxima occurred and moderate to heavy levels of ALS developed in central and southeastern Arizona cotton (4,8). The role of free moisture in infection by *A. macrospora* has been well documented (2), and rain patterns may be involved in the increased disease pressure during 1982–1984. However, dews are sufficient to support severe epidemics of ALS during dry Israeli summers, and Bashi et al (2) questioned why ALS is not a perennial problem in the United States. These authors (2) proposed three explanations: 1) limited acreage cropped to sensitive varieties, 2) absence of virulent strains of *A. macrospora*, or 3) an unfavorable environment for disease development. Outbreaks of ALS during 1982–1984 indicate virulent isolates are present in Arizona (4,8), and recent acreage increases of susceptible cultivars may contribute to increased ALS levels (4). However, subsidence of disease pressure in 1985 as suceptible acreage increased may indicate that environment plays an important role. Although temperatures (25–30 C) favoring infection of cotton by *A. macrospora* (2) occur daily during the cotton season in Arizona, daily temperature maxima exceeding 40 C are common (Table 1).

The current study was undertaken to determine whether short-term exposures to high temperatures after infection, as occur in the desert valleys of Arizona, influence disease incidence. The effects of elevated temperature on the fungus in vitro also were studied.

MATERIALS AND METHODS

Cultures. *A. macrospora* isolate C-20 from *G. barbadense* collected in Safford, AZ, in 1985 was used in all in vivo studies. In vitro phenomena were confirmed with the previously described (4,5) isolates C-22, CB-1, C-3 (ATCC 58174), and C-10 (ATCC 58175). Sporulating cultures of fungal isolates were maintained on a modified V-8 medium (5) containing 5% V-8 vegetable juice (v/v) and 2% agar (w/v) at 27 C under 5,000-lux fluorescent light on a 12-hr diurnal light cycle. For long-term storage, 3-mm-diameter agar plugs of sporulating cultures were maintained at room temperature in 25-ml vials containing 5 ml of sterile distilled water.

Effects of temperature on lesion formation. To test the effects of high temperatures after initial infection on subsequent lesion development, plants were exposed to various temperatures for 6 hr shortly after infection with *A. macrospora*. Cotton plants (*G. barbadense* cv. Pima S-6) were grown in a greenhouse in 750-ml pots containing a modified U.C. mix (peat moss, sand, vermiculite [8:6:1] plus 21 g KNO_3, 21 g K_2SO_4, 69 g treble superphosphate, 311 g dolomite lime, 104 g $CaCO_3$, and 10 g $CuSO_4$ per cubic meter) and were fertilized with 50 ml, 2,000 ppm, Miracle-Gro every 8–10 days. Plants at the four- to six-leaf stage (30–35 days old) were sprayed to runoff with suspensions containing 3,000–4,000 spores per milliliter in 0.001% Triton X-100, then immediately placed in an unilluminated humidity chamber (relative humidity 100%) maintained at 26–28 C for 15 hr. A duration of 15 hr was used because it resulted in moderate disease levels in the controls. Dew periods typically have shorter durations in the desert valleys of Arizona. Subsequently, plants were removed from the humidity chamber, allowed to dry (about 1.5 hr), and then incubated at either 30, 39, 42, or 45 C for 6 hr before being placed on the greenhouse bench. After 5 days, all leaves

Table 1. Frequency of various daily temperature maxima at Casa Grande National Monument, Casa Grande, AZ[a]

Month	Year	No. days temperature exceeded			
		37 C	40 C	43 C	46 C
May	1984	22	12	7	0
	1985	18	5	0	0
June	1984	26	19	2	0
	1985	26	24	19	5
July	1984	28	13	4	0
	1985	29	28	16	7
August	1984	25	13	2	0
	1985	30	28	9	2
September	1984	18	3	0	0
	1985	14	5	0	0
May to September	1984	119	60	15	0
	1985	117	90	44	14

[a] Data from National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

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Table 2. Number of lesions on *Alternaria macrospora*-infected cotton plants maintained at various temperatures for 6 hr immediately after infection

Experiment no.	No. of lesions per square centimeter[a]				Reduction[b] (%)
	30 C	39 C	42 C	45 C	
1	0.19 ± 0.12	···	···	0.0	100.0
2	0.82 ± 0.38	···	···	0.02 ± 0.02	98.2
3	0.77 ± 0.26	···	0.06 ± 0.06	···	92.1
4	1.29 ± 0.20	···	0.34 ± 0.19	···	73.1
5	1.10 ± 0.23	0.31 ± 0.03	···	···	71.7
6	0.89 ± 0.52	0.49 ± 0.33	···	···	45.0

[a] Experiments contained two treatments with four replicates. Differences in the number of lesions within experiments are significant ($P = 0.01$) by analysis of variance except in experiment 6.
[b] Percent reduction in the number of lesions formed at test temperatures compared with incubation at 30 C.

Table 3. Number of lesions on *Alternaria macrospora*-infected cotton plants maintained at 43.5 C for 0, 2, 4, or 6 hr immediately after infection

Hours at 43.5 C	No. lesions per square centimeter[z]	
	Experiment 1	Experiment 2
0	1.06 ± 0.39 a	0.74 ± 0.38 a
2	0.23 ± 0.15 b	0.19 ± 0.03 b
4	0.11 ± 0.08 b	0.08 ± 0.02 b
6	0.08 ± 0.06 b	0.07 ± 0.06 b

[z] Treatments contained three replicates. Numbers in the same column followed by the same letter are not significantly different ($P = 0.01$) by Tukey's Studentized range test.

were excised, photocopied, and the lesions on each leaf counted. Leaf surface areas were calculated by video image analysis. Each experiment contained two temperature treatments including the control treatment, 30 C, which is in the optimal range for host colonization by this fungus (2). Experiments were performed twice and replicated four times. Replicates consisted of individual plants.

In another experiment, infected plants were maintained at 43.5 C for 2, 4, or 6 hr and compared with control plants maintained at 30 C for 6 hr. Plants in the 2- and 4-hr treatments were transferred to the 30 C chamber at the appropriate time for the remainder of the 6-hr period. This experiment was repeated once with three replicates per treatment.

In vitro studies. To assess spore survival and germ tube behavior in vitro, spores from 5-day-old cultures of *A. macrospora* were suspended in distilled water to a final concentration of about 3,000/ml. Eight 10-µl aliquots of spore suspension were applied to the potato-dextrose agar (PDA) surface in each 9-cm-diameter plastic petri dish and incubated at 27 C. After 1 hr, all spores had produced three to 10 germ tubes. Plates were then incubated at the test temperature (38, 41, 43.5, or 46.0 C) for 2, 4, 6, 8, 10, or 12 hr. At the end of each period, spores and germ tubes were fixed and stained on the agar surface with 0.1% cotton blue in lactophenol. Pieces of agar containing spores were then removed from four locations on each of two plates from each treatment and examined microscopically (600×). Germ tubes explosively lysed at temperatures higher than 38 C. Therefore, the percentage of germ tube lysis was determined for each treatment. Experiments were replicated eight times and performed twice.

Spore survival at elevated temperatures on PDA was determined by spreading 50–80 spores over the agar surface of each PDA plate and incubating at 42, 43.5, or 46 C for 4, 8, 12, 24, or 48 hr. After exposure to elevated temperatures, plates were incubated at 27 C for 24, 48, and 96 hr and examined with a dissecting microscope (40×) to determine the percentage of germinating and growing spores. Experiments were performed twice and replicated three times.

RESULTS

Exposure of cotton plants infected with *A. macrospora* to 39, 42, 43.5, or 45 C for 6 hr resulted in reductions in the number of lesions formed compared with plants maintained at 30 C for that period (Tables 2 and 3). The highest temperature tested, 45 C, resulted in 99–100% reductions in lesion number per unit area relative to the controls. Lower temperatures were less effective in limiting lesion development. However, even incubation at 39 C for 6 hr resulted in 40–70% fewer lesions. Lesion formation was reduced even when plants were exposed to elevated temperatures for periods shorter than 6 hr (Table 3). Exposure for 2 and 4 hr resulted in significant ($P < 0.01$) reductions in the number of lesions formed (Table 3).

Elevated temperatures also influenced fungal behavior in vitro. Germ tubes of five *A. macrospora* isolates exposed to 41–46 C lysed readily on PDA. Lysis appeared explosive with cellular contents extruded through lateral breaks in the germ tubes. Lysed germ tubes appeared partially or completely vacant, whereas intact germ tubes contained relatively evenly distributed cytoplasm that retained cotton blue. Germ tube lysis increased with temperature (Fig. 1) so that 7, 52, and 82% of the germ tubes underwent lysis within 6 hr at 42, 43.5, and 46 C, respectively.

Spore viability decreased on PDA with increases in temperature from 42 to 46 C during the 72-hr test period (Fig. 2). At the highest temperature, 46 C, spore viability decreased 65% within 4 hr, whereas at 42 C, spore viability remained stable for 24 hr.

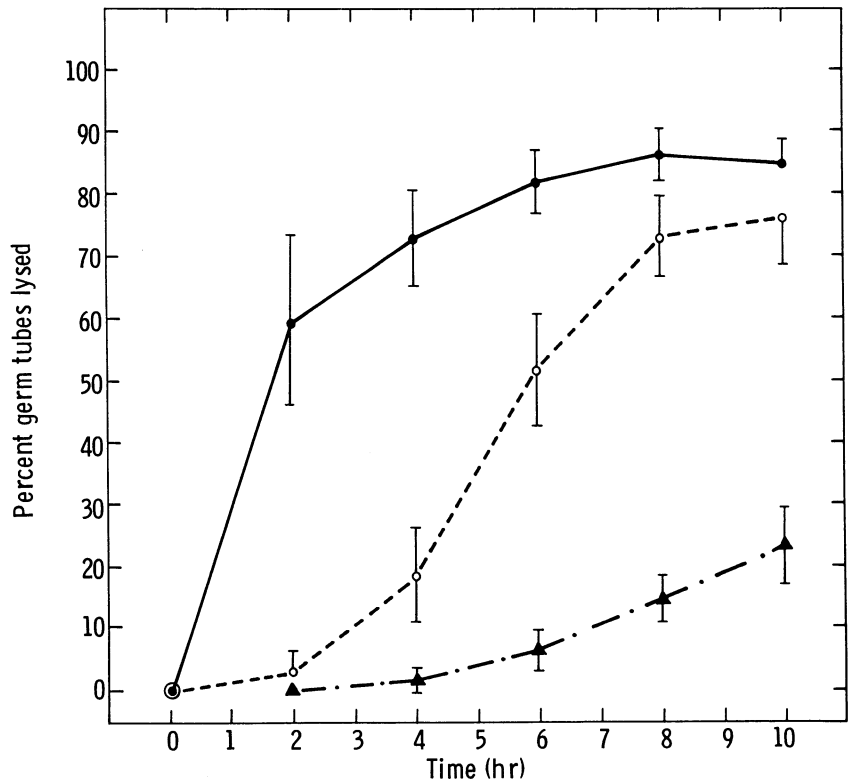

Fig. 1. Percentage of *Alternaria macrospora* germ tubes lysed at 41 C (– · –), 43.5 C (– – –), and 46 C (——) after incubation on potato-dextrose agar for 0–72 hr.

DISCUSSION

Forty-eight hours lapse between infection of cotton by *A. macrospora* and expression of the initial ALS symptoms (2,4). This stage in disease development has not been studied in detail, but it is apparently a period of host tissue colonization (2). Results of the current study indicate that this early pre-symptomatic stage in disease development is highly susceptible to disruption by elevated temperatures (39–46 C). When plants with presymptomatic infections are incubated at 43.5 (110 F) for just 2 hr, there is at least a 70% reduction in the number of lesions formed (Table 3). Furthermore, the level of disease suppression increases with temperature (Table 2) and exposure time (Table 3). Daily temperature maxima between 39 and 46 C are typical in central Arizona during the cotton season (Table 1). Thus, although the presence of free moisture (dew) and moderate temperatures at night may favor infection in the Arizona desert (2), elevated temperatures during the day following infection may limit development of ALS symptoms. The influence of daily temperature maxima may be particularly important in Arizona early in the season (June and July) before canopy closure, when hot, dry winds and water stress are common. Establishment of the disease early in the season is considered an important component leading to severe ALS epidemics in Israel (2).

In central Arizona, temperatures in the optimal range for infection by *A. macrospora* (20–30 C) occur most nights in years with either high or low ALS incidences. However, daily temperature maxima were lower during 1984, a year in which ALS was heavy, than in 1985, when disease pressure greatly declined (Table 1). During June and July 1985, the daily temperature maximum exceeded 43 C 35 times, whereas in 1984, temperatures only exceeded 43 C six times. Disease development is rapidly disrupted at 43.5 C (Table 3). Therefore, low daily temperature maxima may have contributed to the prevalence of ALS in 1984 and may be indicative of periods favoring disease development in Arizona. This supports the suggestion (2) that environmental constraints limit ALS in Arizona in most years.

Rapid lysis of *A. macrospora* germ tubes in vitro at temperatures higher than 38 C (Fig. 1) may partly explain the reduction in lesion formation caused by exposing infected plants to these temperatures. Germ tube lysis increases with increased temperature and exposure time (Fig. 1). Thus, germ tube lysis may be a mechanism through which temperature-mediated reductions in lesion establishment result. However, the observed phenomenon may be due to other alterations in the host/pathogen interaction.

Spore viability also declined on PDA at temperatures higher than 38 C. However, viability reduction was too slow to be significant in situ except at temperatures higher than 45 C. *Alternaria* spores are less sensitive to temperature in a desiccated state, which probably reflects the conditions under which these pathogens are typically dispersed in deserts (9). Therefore, high daily temperature maxima probably do not significantly reduce the inoculum potential of *A. macrospora* in the Arizona desert valleys.

Temperatures tested in the current study reflect typical daily temperature maxima occurring in central Arizona during June and July (Table 1). However, actual leaf surface temperatures in the field will vary greatly depending upon variables such as light, host nitrogen metabolism, water stress, position within the canopy, and relative humidity; these factors also independently influence the incidence and severity of disease (1). Light-dependent host defenses influence the outcome of *Alternaria*/host interactions (6). Because high light intensities occur simultaneously with daily temperature maxima, these factors may act in concert against the pathogen.

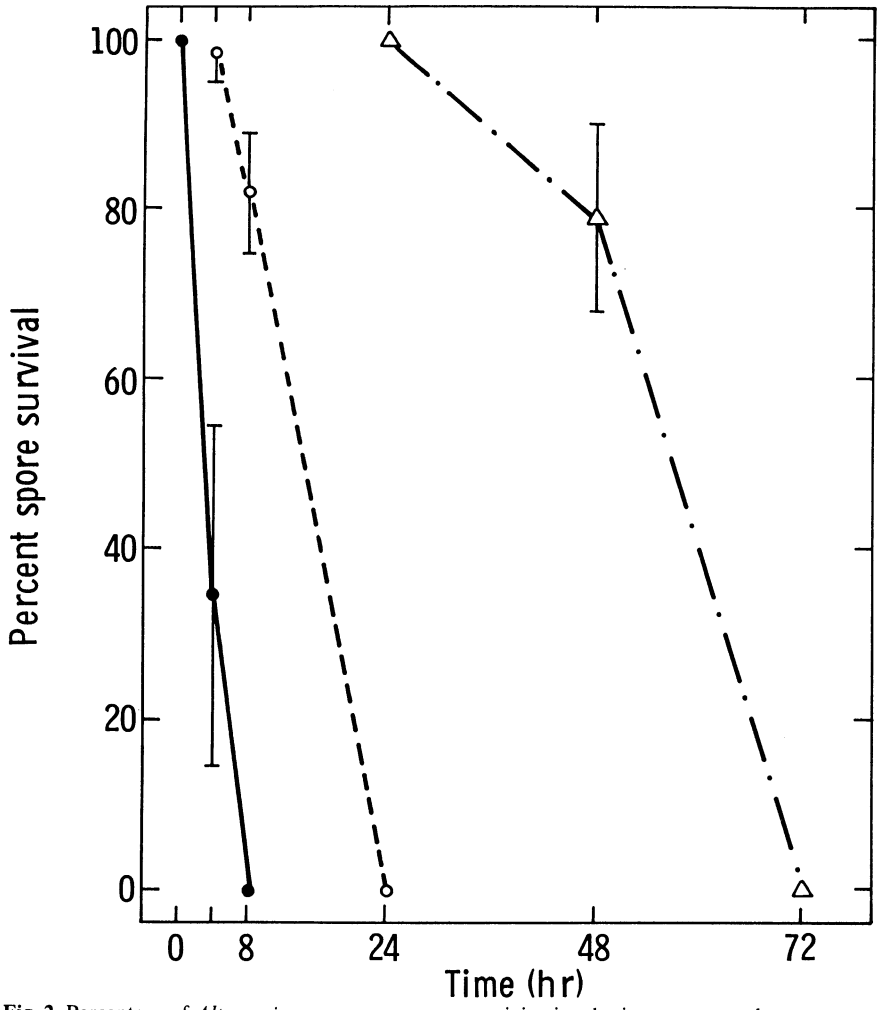

Fig. 2. Percentage of *Alternaria macrospora* spores surviving incubation on potato-dextrose agar at 41 C (– · –), 43.5 C (– – –), and 46 C (——) for 0–72 hr.

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