Effects of Temperature and Light on Virulence of *Exserohilum turcicum* on Corn

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ABSTRACT

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Isolates of races 1, 2, and 4 of *Exserohilum turcicum* were tested for virulence at day/night temperatures of 22/18 or 26/22 C on seedlings of corn inbreds H4460 and B37 and their backcross lines with the *Ht1* gene for resistance. Race 2 was virulent on B37*Ht1* at both 22/18 and 26/22 C but avirulent on H4460*Ht1* at 26/22 C. Race 2 induced normal susceptible-type lesions on inbreds B37 and H4460 at both 22/18 and 26/22 C, and the number of lesions per plant was greater at reduced light intensities. Seedlings of H4460*Ht1* grown at 22/18 C before inoculation became resistant to race 2 if they were transferred to 26/22 C within the first 3 days after inoculation. Conversely, if they were grown at 26/22 C and transferred to 22/18 C within 3 days after inoculation with race 2, they developed normal susceptible-type lesions; if they were transferred later, they developed intermediate- and susceptible-type lesions. If H4460 seedlings were grown at 22/18 C before inoculation, the lengths of lesions induced by races 2 and 4 were significantly correlated with the number of days they remained at 22/18 C before transfer to 26/22 C, but if they were grown at 26/22 C and transferred to 22/18 C, the lesion lengths were not significantly affected by the number of days at 26/22 C after inoculation.

Additional keywords: northern corn leaf blight, *Setosphaeria turcica*, *Zea mays*.

Race 3 of *Exserohilum turcicum* (Pass.) Leonard & Suggs (teleomorph *Setosphaeria turcica* (Luttrell) Leonard & Suggs) is avirulent on corn plants with resistance gene *Ht3* if they are grown at day/night temperatures of 26/22 C but virulent on plants with *Ht3* grown at 22/18 C, particularly at reduced light intensity (6,8). The objectives of the present study were to elucidate the effects of temperature and light intensity on the virulence of race 2 of *E. turcicum* to corn plants with resistance gene *Ht1* and to evaluate the effects of changes from 22/18 to 26/22 C or vice versa at different times during the development of infections by *E. turcicum* on plants with and without *Ht1*.

MATERIALS AND METHODS

Pathogen isolates. Inoculum was prepared from cultures of isolates 85-20 (race 1) and 85-11 (race 2) from North Carolina and isolates 2-5 and 2-11 (both race 4) from Texas grown on lactose casein hydrolysate agar (9). Conidia were washed from cultures 10–14 days old with water containing Tween 20 at 2 drops per 100 ml, and the conidial suspensions were diluted to the desired concentrations based on counts of conidia in a hemacytometer.

Host plants. Seeds of corn inbred H4460 and backcross line H4460*Ht1* were provided by D. R. Smith, DeKalb-Pfizer Genetics; seeds of inbred B37 and backcross line B37*Ht1* were provided by W. L. Pedersen, University of Illinois, Urbana. Plants were grown in controlled environment chambers in a 1:2 mixture of peat-lite and gravel in 11.4-cm-diameter pots with four plants per pot. Air temperature was maintained within ± 0.25 C of the set point, and light during the 12-hr photoperiod was supplied by cool-white fluorescent and incandescent lamps at 50, 25, or 12 klx (647, 324, or 162 μE m^{-2} sec^{-1}). Plants were watered twice each day with a standard phytotron nutrient solution (2).

Experiment one. H4460 and H4460*Ht1* plants were started in a greenhouse at 26/22 C and moved to controlled environment chambers at day/night temperatures of 22/18 or 26/22 C and 50, 25, or 12 klx light (Table 1) at 13 days after planting. They were inoculated with race 1 or race 2 at 19 days after planting by spraying them to runoff with a suspension of 10,000 conidia/ml. They were incubated 16 hr in a moist chamber at 22 C and then returned to the chambers in which they had grown before inoculation. Disease reactions were evaluated 13 days after inoculation according to the rating scale of Pedersen et al (7): chlorotic lesions were rated resistant; narrow necrotic lesions surrounded by chlorosis were rated intermediate; and wilted necrotic lesions without chlorosis were rated susceptible. Numbers and lengths of susceptible-type lesions were determined 14 days after inoculation. Sporulation was determined on 1-cm-diameter disks cut from the centers of five lesions per replication per treatment 14 days after inoculation. The disks were incubated 4 days on moist filter paper in petri dishes at 22 C with a 12-hr photoperiod. Conidia were washed from the disks by shaking them in 5 ml of water with 2 drops of Tween 20 per 100 ml, and the conidia were counted in three 2-μl droplets of the resulting conidial suspension.

The experiment was run three times with seedlings planted on three successive days. In each run there were two pots of plants per treatment. Data for mean number of lesions per plant, lesion length, and log conidia per leaf disk were subjected to analysis of variance for all treatments in which susceptible-type lesions were obtained. Data for race 2 were analyzed as a split-plot design for host lines within temperature and light treatments. The effects of light intensity were determined for data from race 1 on H4460, race 2 on H4460, and race 2 on H4460*Ht1*. Temperature effects were analyzed separately for the two 50-klx light treatments at

26/22 and 22/18 C.

Experiment two. Seedlings of H4460 and H4460*Ht1* were grown at day/night temperatures of 22/18 or 26/22 C and 50-klx light and inoculated 19 days after planting with isolate 85-11 (race 2) or isolates 2-5 or 2-11 (race 4). Conditions and procedures for inoculation and moist chamber incubation were as described for experiment one, except that inoculum concentrations were 13,000, 14,000, and 9,000 conidia/ml for isolates 85-11, 2-5, and 2-11. There were 12 temperature treatments. Some plants grown at 22/18 C before inoculation were left at 22/18 C for 18 days after inoculation and removal from the moist chamber. Other plants started at 22/18 C were moved to 26/22 C at 0, 3, 6, 9, or 12 days after removal from the moist chamber. Likewise, some plants grown at 26/22 C before inoculation were left at 26/22 C for 18 days after removal from the moist chamber, but other plants started at 26/22 C were shifted to 22/18 C at 0, 3, 6, 9, or 12 days after removal from the moist chamber. There were four plants per pot and two pots per treatment. Data for mean lesion number and lesion length 18 days after inoculation were regressed against the number of days (starting at 0 for the day of removal from the moist chamber) that the plants were retained at their starting temperature before being transferred to a new temperature.

Experiment three. Seedlings of B37 and B37*Ht1* were grown at day/night temperatures of 22/18 or 26/22 C and 50-klx light and inoculated with isolate 85-20 (race 1) or isolate 85-11 (race 2) 19 days after planting. Conditions and procedures for inoculation and moist chamber incubation were as described for experiment one. Disease reactions were recorded 10 and 14 days after inoculation. There were four temperature treatments: 26/22 C before and after inoculation, 22/18 C before and after inoculation, 22/18 C until inoculation and 26/22 C starting immediately after removal from the moist chamber, and 22/18 C before inoculation and for 6 days after removal from the moist chamber followed by transfer to 26/22 C until the end of the experiment. There were four replicate pots of four plants per pot for each treatment. At 15 days after inoculation, leaf disks were cut from the centers of five lesions on each plant inoculated with race 2, and sporulation was determined on incubated leaf disks as described for experiment one. Data were log transformed for analysis of variance for a split-plot design with host lines within temperature treatments, and for Student's *t*-test of effects of constant 26/22 or 22/18 C over the entire experiment.

RESULTS

Experiment one. As expected, race 2 induced susceptible-type lesions on H4460*Ht1* at 22/18 C, although the lesions were significantly shorter and supported significantly less sporulation ($P < 0.05$) than those on H4460 (Table 1). Race 2 was avirulent on H4460*Ht1* at 26/22 C; the mean number of conidia on leaf disks from lesions of race 2 on H4460*Ht1* at 26/22 C was 242, which was similar to the means of 60–275 conidia per leaf disk for race 1 lesions on H4460*Ht1* at 22/18 or 26/22 C. Both races 1 and 2 were virulent on H4460 at all conditions tested (Table 1). The numbers and lengths of lesions of races 1 and 2 on H4460 were not significantly affected by temperature in this experiment. The regression of lesions per plant versus light intensity was significant for race 2 on H4460 ($Y = 32.1 - 24.6X$; $R^2 = 0.998$; $P < 0.05$). Lesion length and sporulation were not significantly affected by light intensity.

Experiment two. Race 2 induced resistant- and intermediate-type lesions on H4460*Ht1* plants grown continuously at 26/22 C but induced only susceptible-type lesions on H4460*Ht1* plants grown continuously at 22/18 C (Table 2). H4460*Ht1* plants grown at 22/18 C before inoculation became resistant to race 2 if they were transferred to 26/22 C within 3 days after inoculation, but only susceptible-type lesions developed on those plants that were held at 22/18 C for at least 6 days after inoculation. On H4460*Ht1* seedlings grown at 26/22 C before inoculation, race 2 induced a mixture of intermediate- and susceptible-type lesions if the plants were transferred to 22/18 C 6 or more days after inoculation; if the transfer was made within 3 days after inoculation, only susceptible-type lesions developed (Table 2). In all treatments, races 2 and 4 induced susceptible-type lesions or a mixture of intermediate- and susceptible-type lesions on H4460, and in all cases race 4 induced only resistant-type lesions on H4460*Ht1*.

Response of lesion length to temperature depended upon whether the seedlings were grown at 22/18 or 26/22 C before inoculation, and the responses differed for H4460 and H4460*Ht1*. For plants grown at 22/18 C before inoculation, increased time at 22/18 C rather than 26/22 C after inoculation resulted in significantly longer lesions on H4460 ($P < 0.0001$) but not on H4460*Ht1* (Fig. 1). For plants grown at 26/22 C before inoculation, delayed transfer to 22/18 C had no effect on length of lesions on H4460 but significantly reduced the length of race 2 lesions on H4460*Ht1* ($P < 0.02$) (Fig. 2). Similarly, delayed transfer from 26/22 to 22/18 C reduced the number of race 2 lesions that formed on either H4460 or H4460*Ht1* ($P = 0.03$).

Experiment three. Race 3 induced susceptible-type lesions on B37 and B37*Ht1* seedlings at both 22/18 and 26/22 C as well as in treatments in which inoculated plants were shifted from 22/18 to 26/22 C at either 0 or 6 days after inoculation. In all four treatments, race 1 induced susceptible-type lesions on B37 and only resistant-type lesions on B37*Ht1*. Sporulation by race 2 was significantly greater ($P < 0.05$) on leaf disks from plants grown continuously at 22/18 C (19,500 conidia/leaf disk) than on those from plants grown continuously at 26/22 C (9,500 conidia/leaf disk). Sporulation on leaf disks from plants transferred from 22/18 to 26/22 C 6 days after inoculation was intermediate (Fig. 3).

TABLE 1. Effect of temperature and light intensity on development of lesions of race 1 and 2 of *Exserohilum turcicum* on seedlings of corn inbred H4460 with or without resistance gene *Ht1*

Race[a]	Line	Temperature[b]	Light (klx)	Lesion type[c]	Lesions per plant	Lesion length (mm)	Conidia per disk×10²
2	H4460	26/22	50	S	3.3	26.8	146
		22/18	50	S	7.7	25.8	85
		22/18	25	S	19.3	35.5	24
		22/18	12	S	26.3	36.9	185
	H4460*Ht1*	26/22	50	R	···	···	2
		22/18	50	S	7.0	26.5	40
		22/18	25	S	13.0	29.2	16
		22/18	12	S	10.7	20.2	14
1	H4460	26/22	50	S	14.7	25.2	24
		22/18	50	S	18.0	26.7	308
		22/18	25	S	43.7	37.0	84
		22/18	12	S	44.0	39.9	88

[a] Race 2 is isolate 85-11, and race 1 is isolate 85-20.
[b] Day/night temperature (C) with 12-hr photoperiod.
[c] S = susceptible-type lesions; R = resistant-type lesions.

TABLE 2. Effect of high and low temperatures at various stages of infection on lesions development by *Exserohilum turcicum* race 2 on seedlings of corn inbred H4460 with *Ht1* resistance

Days after inoculation	Lesion type[a] on: Plants grown at 22/18 C and moved to 26/22 C at indicated days after inoculation	Plants grown at 26/22 C and moved to 22/18 C at indicated days after inoculation
0	R/I	S
3	R	S
6	S	I/S
9	S	S/I
12	S	I/S
18	S	R/I

[a] R = resistant-, I = intermediate-, and S = susceptible-type lesions; R/I indicates mixed lesion types with R more frequent, I/S indicates mixed lesion types with I more frequent, and S/I indicates mixed lesion types with S more frequent.

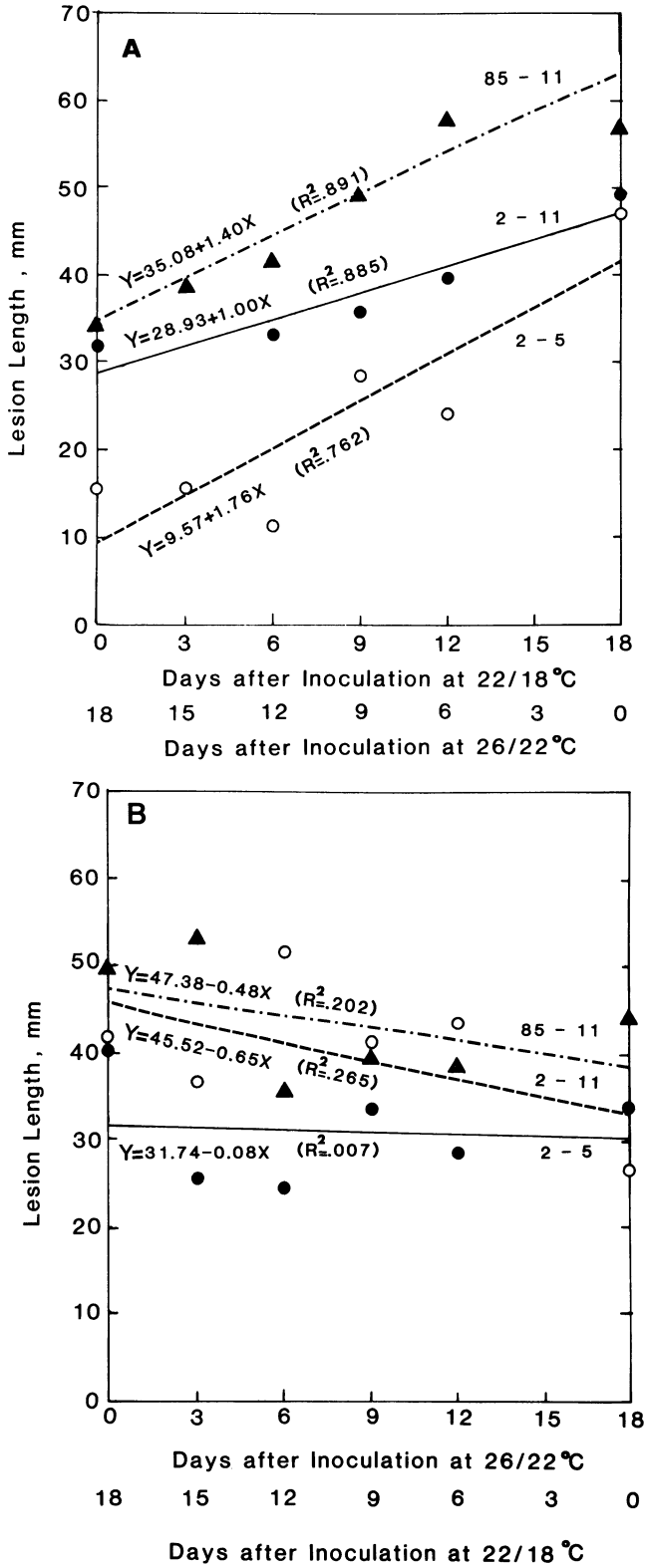

Fig. 1. Effect of temperature on length of lesions of race 2 (isolate 85-11 [▲]) and race 4 (isolates 2-5 [○] and 2-11 [●]) of *Exserohilum turcicum* on corn inbred H4460. **A,** Plants were grown at day/night temperatures of 22/18 C until inoculation 19 days after planting and then transferred to 26/22 C at 0, 3, 6, or 12 days after inoculation; one set was retained at 22/18 C until lesions were measured 18 days after inoculation. For all three isolates, the regression coefficients are significantly greater than 0 ($P < 0.05$). **B,** Plants were grown at day/night temperatures of 26/22 C until inoculation and then transferred to 22/18 C at 0, 3, 6, or 12 days after inoculation; one set of plants was retained at 26/22 C until lesions were measured at 18 days after inoculation. Regression coefficients are not significantly greater than 0.

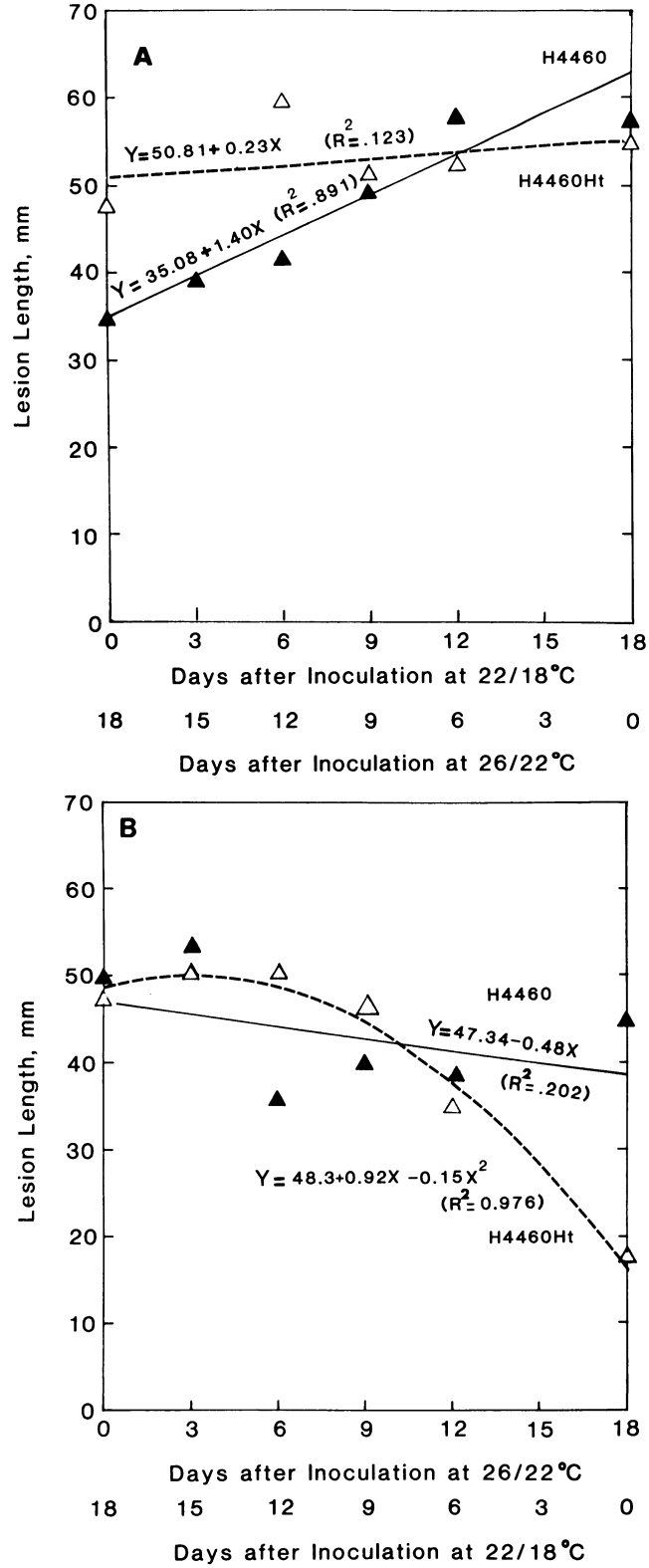

Fig. 2. Effect of temperature on length of lesions of race 2 (isolate 85-11) of *Exserohilum turcicum* on corn inbreds H4460 (▲) and H4460*Ht1* (△). **A,** Plants were grown at day/night temperatures of 22/18 C until inoculation 9 days after planting and then transferred to 26/22 C at 0, 3, 6, or 12 days after inoculation; one set was retained at 22/18 C until lesions were measured 18 days after inoculation. The regression coefficient for H4460*Ht1* is not significantly different from 0, but it is significantly different from the regression coefficient for H4460 ($P < 0.05$). **B,** Plants were grown at day/night temperatures of 26/22 C until inoculation and then transferred to 22/18 C at 0, 3, 6, or 12 days after inoculation; one set of plants was retained at 26/22 C until lesions were measured 18 days after inoculation. The quadratic effect of the regression for H4460*Ht1* is significantly different from 0 ($P < 0.05$), but the linear effect is not.

DISCUSSION

Race 2 was virulent on backcross line B37*Ht1* at both 22/18 and 26/22 C but was avirulent on backcross line H4460*Ht1* at 26/22 C, which indicates that the ability of race 2 to overcome resistance conditioned by the gene *Ht1* depends not only on temperature, but also on the genetic background of the host. The avirulence of race 2 on H4460*Ht1* at 26/22 C was unexpected because there was no evidence that resistant-type lesions were induced by race 2 on other inbreds and hybrids with *Ht1* in previous field and greenhouse experiments (1,10). On the other hand, we previously found race 3 of *E. turcicum* to be avirulent on either H4460*Ht3* or B37*Ht3* at 26/22 C, even though race 3 consistently induced susceptible-type lesions on inbreds H4460 and B37 at 26/22 C (6,8). We also found that B37*HtN* segregated for resistance to race 4 of *E. turcicum* at 26/22 C, even though it was susceptible to race 4 at 22/18 C, and even though race 4 consistently induced susceptible-type lesions on B37 at 26/22 C (8). Thus, virulence genes corresponding to *Ht1*, *Ht3*, and possibly *HtN* show temperature-sensitive responses on at least some corn genotypes. It is interesting that race 2 induced larger lesions and sporulated more profusely on H4460 than on H4460*Ht1* even at the conducive temperature of 22/18 C.

Seedlings of H4460*Ht1* were resistant to race 2 either if they were grown continuously at 26/22 C or if they were transferred from 22/18 C to 26/22 C at 0 or 3 days after inoculation, but not if they were transferred to 26/22 C at 6 or more days after inoculation. Thus, the resistance response of H4460*Ht1* to race 2 was effective if activated up to 3 days after inoculation but ineffective if activated 6 or more days after inoculation. The resistance mechanism was reversible; H4460*Ht1* seedlings kept at 26/22 C for 12 days after inoculation with race 2 still developed intermediate- and susceptible-type lesions when transferred to 22/18 C for 6 more days.

In susceptible corn plants and corn plants with monogenic resistance, *E. turcicum* spreads slowly through the leaf mesophyll and chlorenchyma for the first 2 or 3 days after inoculation (4,5).

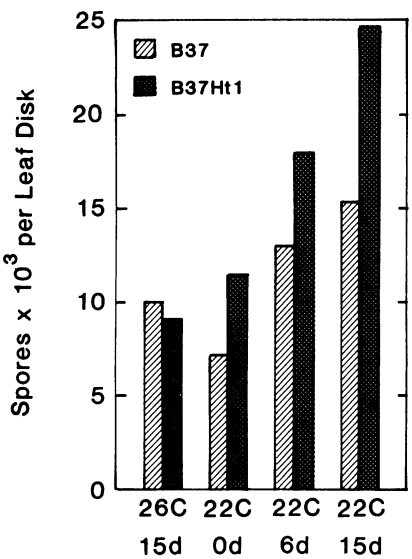

Fig. 3. Effect of temperature during lesion development on sporulation of race 2 of *Exserohilum turcicum* in leaf disks cut from the lesions on B37 and B37*Ht1* corn seedlings at 15 days after inoculation and incubated 4 days in moist chambers. Seedlings were grown at day/night temperatures of 26/22 or 22/18 C. One-third of the plants grown at 22/18 C were transferred to 26/22 C immediately after inoculation (22 C, 0 d), and one-third were transferred to 26/22 C at 6 days after inoculation (22 C, 6 d). Treatments designated 26 C, 15 d and 22 C, 15 d were kept continuously at 26/22 or 22/18 C, respectively, both before and after inoculation. For these two treatments, sporulation was significantly greater at 22/18 than at 26/22 C ($P < 0.05$). Over all temperature treatments, sporulation on B37 did not differ significantly from that on B37*Ht1*.

In susceptible plants, the fungus then penetrates the xylem and ramifies rapidly through the xylem for 3 or 4 days before it reemerges into the mesophyll and induces wilting and necrosis. The critical time for expression of resistance of H4460*Ht1* to race 2 in seedlings transferred from 22/18 to 26/22 C in our experiment two corresponds to the time at which *E. turcicum* enters the xylem. H4460*Ht1* plants infected with race 2 expressed resistance if they were transferred to 26/22 C at 3 days after inoculation but not if they were retained at 22/18 C for 6 days after inoculation, at which time the fungus would have established itself in the xylem according to Hilu and Hooker (4) and Jennings and Ullstrup (5). In experiment two there was a dramatic reduction in lesion length on H4460*Ht1* plants grown at 26/22 C and kept at 26/22 C for 12 days after inoculation with race 2. Transfer to 22/18 C at 12 days after inoculation allowed only 6 days for normal lesion development at conducive temperatures. According to Hilu and Hooker (4), 6 days after entry of the mycelium of *E. turcicum* into the xylem corresponds to an early stage of rapid expansion of lesions following emergence of hyphae from the xylem.

In experiment two, there was a statistically significant correlation between length of lesions of races 2 and 4 on H4460 seedlings and the number of days the seedlings were kept at 22/18 C after inoculation before transfer to 26/22 C. The fact that lesions on plants at 22/18 C were larger than those on plants at 26/22 C seems inconsistent with Hilu and Hooker's (3) report that 30 C was optimal for mycelial growth of *E. turcicum* on leaf disks cut from lesions on susceptible corn seedlings. Evidently, intact leaves restrict the mycelial growth of *E. turcicum* more at high temperatures than excised leaf disks do. This effect was persistent in H4460 seedlings grown at 26/22 C and later shifted to 22/18 C; the seedlings remained less compatible to rapid growth of races 2 and 4 of *E. turcicum* after the seedlings were moved to 22/18 C. Even a shift to 22/18 C immediately after inoculation did not result in lesions as long as those on H4460 seedlings grown at 22/18 C before and after inoculation.

Hilu and Hooker (3) reported that sporulation by race 1 in resistant-type lesions on seedlings with *Ht1* was 2.5% as great as that in susceptible-type lesions on seedlings without *Ht1* after 72 hr of incubation. This is similar to our values of 1.2% as much sporulation by race 1 on H4460*Ht1* as on H4460 over all temperature and light conditions, or 1.7% as much sporulation by race 2 in resistant-type lesions on H4460*Ht1* at 26/22 C as in susceptible-type lesions on H4460*Ht1* at 22/18 C.

The sensitivity of virulent races of *E. turcicum* such as race 2 or race 3 (6,8) to "defeated resistance" at high temperatures may have important epidemiological implications. If this effect depends on the presence of modifier genes, it may be possible to achieve some useful resistance from the "defeated genes" during periods of warm weather by selecting for these modifier genes.

LITERATURE CITED

1. Berquist, R. R., and Masias, O. R. 1974. Physiologic specialization in *Trichometasphearia turcica* f. sp. *zeae* and *T. turcica* f. sp. *sorghi* in Hawaii. Phytopathology 64:645-649.
2. Downs, R. J., and Thomas, J. F. 1983. Phytotron Procedural Manual for Controlled Environment Research at the Southeastern Plant Environment Laboratory. N. C. Agric. Res. Serv. Tech. Bull. 244. 44 pp.
3. Hilu, H. M., and Hooker, A. L. 1963. Monogenic chlorotic lesion resistance to *Helminthosporium turcicum* in corn seedlings. Phytopathology 53:909-912.
4. Hilu, H. M., and Hooker, A. L. 1964. Host-pathogen relationship of *Helminthosporium turcicum* in resistant and susceptible corn seedlings. Phytopathology 54:570-575.
5. Jennings, P. R., and Ullstrup, A. J. 1957. A histological study of three Helminthosporium leaf blights of corn. Phytopathology 47:707-714.
6. Leath, S., Thakur, R. P., and Leonard, K. J. 1987. Effects of temperature and light on reaction of corn to race 3 of *Exserohilum turcicum*. (Abstr.) Phytopathology 77:1737.
7. Pedersen, W. L., Perkins, J. M., Radtke, J. A., and Miller, R. J. 1986. Field evaluation of corn inbreds and selection for resistance

to *Exserohilum turcicum* race 2. Plant Dis. 70:376-377.
8. Thakur, R. P., Leonard, K. J., and Jones, R. K. 1989. Characterization of a new race of *Exserohilum turcicum* virulent on corn with resistance gene *Ht*N. Plant Dis. 73:151-155.
9. Tuite, J. 1969. Plant Pathological Methods. Burgess Publishing Co., Minneapolis. 239 pp.
10. Turner, M. T., and Johnson, E. R. 1980. Race of *Helminthosporium turcicum* not controlled by *Ht* genetic resistance in corn in the American corn belt. Plant Dis. 64:216-217.