Variation in Expression of Monogenic Resistance in Corn to *Exserohilum turcicum* Race 3 Under Different Temperature and Light Regimes

S. Leath, R. P. Thakur, and K. J. Leonard

Research plant pathologist, USDA-ARS, Department of Plant Pathology, Box 7616, North Carolina State University, Raleigh, 27695-7616; senior plant pathologist, Cereals Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502324, India; and research plant pathologist, USDA-ARS, Cereal Rust Laboratory, University of Minnesota, St. Paul 55108, respectively.

Paper 12154 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695-7643.
Journal Article 923 of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).
Cooperative investigations of the USDA, ARS, and the North Carolina Agricultural Research Service.
We thank D. R. Smith of Dekalb Pfizer-Genetics for providing corn germ plasm for this study.
The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.
Accepted for publication 22 September 1989 (submitted for electronic processing).

ABSTRACT

Leath, S., Thakur, R. P., and Leonard, K. J. 1990. Variation in expression of monogenic resistance in corn to *Exserohilum turcicum* race 3 under different temperature and light regimes. Phytopathology 80:309-313.

Expression of monogenic resistance in near-isogenic corn inbred lines H4460Ht_1, H4460Ht_2, and H4460Ht_3 against isolates of races 1 and 3 of *Exserohilum turcicum* was determined under different temperature and light intensity regimes. These environmental conditions influenced lesion type, number, length, and sporulation of the fungus. In general, isolates of *E. turcicum* produced more lesions at 22/18 than at 26/22 C and more lesions at 162 or 324 than at 647 μmol m^{-2} s^{-1} at 22/18 C. Lesions also were larger ($P = 0.05$) at the reduced light intensities. Virulence of three races of *E. turcicum* was clearly expressed and consistent with earlier reports at 22/18 C day/night temperature and low light intensity (324 or 162 μmol m^{-2} s^{-1}), but inconsistencies arose at 26/22 C day/night temperature and 647 μmol m^{-2} s^{-1} light intensity. Resistance of Ht_1, Ht_2, and Ht_3 often was incomplete at 22/18 C day/night temperature and low light intensity. Race 3 could be readily recognized at a 22/18 C day/night temperature regime with the differential reaction clearest at a light intensity of 324 μmol m^{-2} s^{-1}.

Epidemics of northern leaf blight of maize (*Zea mays* L.), caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs (teleomorph = *Setosphaeria turcica* (Luttrell) Leonard & Suggs) have been effectively controlled by both polygenic and monogenic resistance (6). Polygenic resistance to northern leaf blight (NLB) was identified more than 35 yr ago (7), and, subsequently, breeding procedures were developed for improving corn populations for polygenic resistance (8,13). Race-specific resistance to NLB is usually controlled by single dominant genes and is expressed as a chlorotic lesion type and by reduced pathogen sporulation (3,4). Resistance conferred by Ht_1 effectively controlled NLB for more than 15 yr. However, four physiologic races of *E. turcicum* are now known (14,15,17), and gene Ht_1, when used alone, does not provide adequate protection against NLB. Race 2, which is virulent to plants with Ht_1, has become common throughout the corn belt since it was first reported there in 1970 (10,17). Several other single gene sources of resistance to NLB exist in maize (2,5,6). Gene Ht_2 conditions a chlorotic lesion response similar to that of Ht_1, except that plants with Ht_2 exhibit extensive chlorosis that may extend the entire length of the leaf (5,6,11). The Ht_3 gene from *Tripsacum floridanum* Porter & Vassey, which has been backcrossed into elite maize inbred lines, also is characterized by extensive chlorosis (5,11). Genes Ht_2 and Ht_3 provide protection against races 1 and 2 of *E. turcicum*, but Smith and Kinsey (14) reported in 1980 that they were not effective against race 3. The first isolate identified as race 3 was from a collection made in 1976 (14). If race 3 were to increase in prevalence, NLB would not be adequately controlled by the qualitative resistance conditioned by genes Ht_1, Ht_2, and Ht_3. However, collections of race 3 have not dramatically increased in frequency in the United States since 1976, and this may be due to several factors. First, no commercial hybrids contain Ht_2 or Ht_3; therefore, there is no favorable selection pressure for *E. turcicum* race 3. Second, race 3 isolates may have poor fitness, especially in the U.S. corn belt. Third, the frequency of race 3 may be underestimated because of difficulty in distinguishing race 3 from race 1. In greenhouse studies at the University of Illinois and North Carolina State University, the first author failed to confirm virulence to inbreds with Ht_2 and Ht_3 in putative race 3 isolates. Isolates resembled race 1 (virulence formula Ht_1, Ht_2, Ht_3/0) rather than race 3 (virulence formulas Ht_1/Ht_2, Ht_3); one isolate from Estill, SC (14), which was originally described as race 3 appeared to be an unusually aggressive race 1 isolate.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1990.

Environmental effects have been shown to influence the expression of leaf blight (caused by *Bipolaris maydis*) resistance in maize (9,18). More recently, Thakur et al (16) showed the effects of temperature and light, pre- and postinoculation, on expression of monogenic resistance to *E. turcicum* in maize. A day/night temperature regime of 22/18 C was more conducive to NLB than a 26/22 C regime. Similarly, reduced light intensities (50 or 75% of full light) also favored disease development in controlled environment studies.

The objectives of this study were: 1) to determine if expression of resistance of race 3, as previously described (14), is influenced by environment, and 2) to identify a temperature and light regime under which *E. turcicum* virulence and aggressiveness can be consistently assessed. A preliminary report has been published (12).

MATERIALS AND METHODS

Pathogen isolates. Isolates of *E. turcicum* were maintained as conidia in 30% glycerol at −70 C. Inoculum for each experiment was obtained from cultures grown on lactose-casein hydrolysate agar for 2 wk. Cultures were flooded with water containing 0.25% Tween 20, and conidia were dislodged with a plastic spatula. Conidial concentrations were determined with the use of a hemacytometer.

Four isolates of *E. turcicum* were used to complete this study. Isolates 85-20 and 85-11 were collected from Wilkes Co. and Iredell Co., NC, respectively, and have been identified as race 1 and race 2, respectively (16). Isolate R3SC is the original race 3 culture from Estill, SC, as reported by Smith and Kinsey (14). The isolate R3NC, also identified as race 3, was recovered near Mt. Olive, NC. Isolate 85-20 (race 1), and the race 3 isolate from Estill, SC, have ATCC accession numbers 64837 and 64836, respectively. Designation of races 1 and 2 for isolates 85-20 and 85-11, respectively, were confirmed before initiation of this study.

Host plants. Three backcross derived maize inbred lines with genes Ht_1, Ht_2, or Ht_3 incorporated and their recurrent parent, H4460, were used in this study. Plants were grown in controlled environment facilities housed in the Southeastern Plant Environment Laboratory, Raleigh, NC. Six seeds were planted in 11.4-cm-diameter pots (600 cm^3 volume) in a 1:2 (v:v) mixture of peat-lite and gravel. One week after emergence, plants were thinned to four plants per pot. Plants were grown for 19 days in a greenhouse with a 26/22 C day/night temperature regime. Growing conditions after inoculation consisted of five temperature and light combinations with temperature being controlled within 0.25 C of the set point. A combination of incandescent and cool-white fluorescent bulbs provided light. A standard nutrient solution (1) was used twice daily to water all plants in the study. Pots were arranged randomly with two replications and two single plant subsamples per replication. The entire experiment was performed three times.

Inoculation, environmental treatment, and assessment. At 19 days after sowing, all plants were inoculated with a conidial suspension consisting of approximately 2,500 conidia/ml. Plants were inoculated with an atomizer with sufficient water to

TABLE 1. Effect of temperature and light on expression of monogenic resistance in a series of near-isogenic lines with or without Ht_1, Ht_2, or Ht_3 and their recurrent parent, H4460, against races 1 and 3 of *Exserohilum turcicum*

Isolate	Line	Temperature (C)	Light (μmol $m^{-2}s^{-1}$)	Reaction type	Lesion number/plant	Lesion length (mm)	Sporulation rating (0–5)[a]	Conidia (per cm^2)
85-20[b]	H4460	26/22	647	S	14.7	25.2	4.2	3,076
	H4460Ht_1			R	0.0	···[c]	0.1	307
	H4460Ht_2			R	0.0	···	0.3	130
	H4460Ht_3			R	0.0	···	0.7	88
R3SC	H4460	26/22	647	S	3.3	20.9	3.3	9,003
	H4460Ht_1			R	1.0	24.7	0.5	299
	H4460Ht_2			S	10.7	37.4	4.2	4,487
	H4460Ht_3			R/I	2.3	21.6	2.0	440
85-20	H4460	22/18	647	S	18.0	26.7	4.8	39,063
	H4460Ht_1			R	0.7	···	0.3	146
	H4460Ht_2			R/I	0.7	25.5	1.8	748
	H4460Ht_3			R	0.0	···	2.0	221
R3SC	H4460	22/18	647	S	11.3	24.4	4.7	18,260
	H4460Ht_1			R	0.0	···	0.8	762
	H4460Ht_2			S	13.3	32.1	4.0	12,621
	H4460Ht_3			S/I	17.0	41.8	4.5	9,087
85-20	H4460	22/18	324	S	43.7	37.0	4.3	8,991
	H4460Ht_1			R	0.0	···	0.5	49
	H4460Ht_2			R	0.0	···	0.7	188
	H4460Ht_3			R	0.0	···	1.0	6
R3SC	H4460	22/18	324	S	14.3	28.9	3.8	5,063
	H4460Ht_1			R	0.0	···	0.7	401
	H4460Ht_2			S	19.7	33.7	4.3	15,511
	H4460Ht_3			S	34.7	39.6	4.2	4,137
85-20	H4460	22/18	162	S	44.0	39.9	5.0	11,181
	H4460Ht_1			R	0.0	···	0.8	20
	H4460Ht_2			S/I	38.3	31.6	2.7	517
	H4460Ht_3			R/I	4.3	···	2.0	920
R3SC	H4460	22/18	162	S	21.7	37.8	5.0	25,953
	H4460Ht_1			R	0.0	···	1.0	417
	H4460Ht_2			S	30.3	43.8	4.8	29,757
	H4460Ht_3			S	28.7	38.8	4.2	9,483
$FSLD_{0.05}$					8.4	7.6	00.8	7,586

[a] A score of 0 indicates no conidia were visible on any of the disks; values are means of conidia from 18 lesions from three experiments with two replications with three subsamples per experiment.

[b] Isolate 85-20 has been characterized previously as race 1.

[c] Indicates no susceptible reaction types were observed and, therefore, no measurements were taken.

uniformly wet leaves as a cart holding the pots was repeatedly rotated. Plants were placed in the dark at 26 C and misted for 14 sec every 5 min during a 16-hr period to maintain 100% RH. Immediately after misting, plants were moved to appropriate chambers for environmental treatments. Full light intensity (647 μmol m^{-2} s^{-1} at 30/26 C, 26/22 C, or 22/18 C) provided three of the five treatments. Half-light and quarter-light at 22/18 C were the final treatments and were obtained by turning off 50 or 75% of the bulbs in the growth chambers in a uniform pattern. All light regimes consisted of a 12-hr day and night regime.

Disease assessments were made 14 days after inoculation. Lesions were scored R, I, or S based on lesion type as described elsewhere (16). Lesion number and lesion length also were recorded.

Sporulation was assessed on three 1-cm-diameter leaf disks per plant that were incubated 4 days in moist chambers as reported earlier, but expressed as conidia per square centimeter of leaf tissue (16). Additionally, sporulation on leaf disks was rated visually on a 0–5 scale with 0 = no visible spores, 1 = some spores visible on one or two of the five disk subsamples, 2 = some spores on most of the disks, 3 = moderate sporulation on all disks, 4 = heavy sporulation on any disk, and 5 = heavy sporulation on all disks. Data on lesion number, lesion length, conidia per square centimeter, and sporulation ratings were considered as quantitative data and subjected to analysis of variance procedures. When lesion length data were considered, only data obtained from susceptible reaction types were used for analyses.

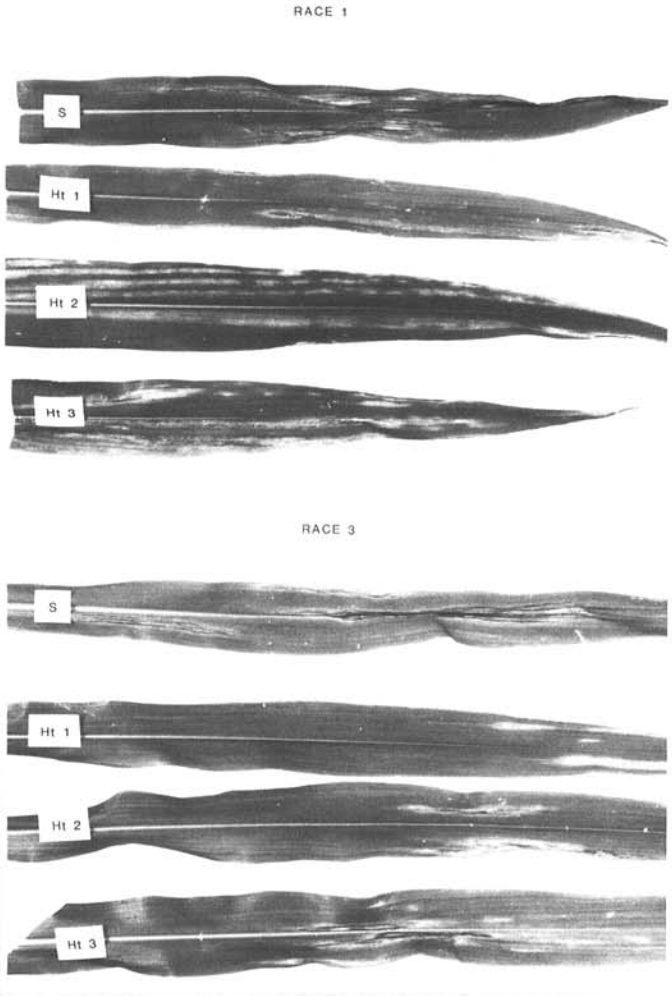

Fig. 1. Expression of monogenic resistance in four near-isogenic lines of H4460 inoculated with race 1 or race 3 of *Exserohilum turcicum* at 22/18 C with half light intensity (324 μmol m^{-2} s^{-1}).

RESULTS

At 30/26 C, neither typical susceptible- nor resistant-type lesions developed on the near-isogenic corn lines, so no data were obtained for that temperature regime.

The results of these experiments for race 2 and additional experiments on the effects of temperature and light on virulence of race 2 on corn lines with Ht_1 have been published separately (16). Lesions induced by race 2 on H4460Ht_2 and H4460Ht_3 were similar to those of race 1 (isolate 85-20) at all temperature and light conditions tested in these studies, which are described below.

Reactions of isolate 85-20 on near-isogenic lines of H4460 at full light intensity at either 26/22 C or 22/18 C were consistent with race 1's described virulence formula of $Ht_1Ht_2Ht_3/0$; i.e., isolate 85-20 induced susceptible-type lesions on H4460 but resistant-type lesions on lines with Ht_1, Ht_2, or Ht_3 (Table 1). The resistant-type lesions of race 1 on H4460Ht_2 and H4460Ht_3 exhibited the extensive chlorosis and "bleeding" often associated with these resistance genes (Fig. 1). At the lowest light intensity, however, the resistance of Ht_2 to race 1 broke down, and a mixture of intermediate- and susceptible-type lesions were observed. A similar breakdown of Ht_2 resistance to race 2 isolate 85-11 occurred at the lowest light intensity. Resistance of Ht_3 to races 1 and 2 was slightly negated at the lowest light intensity, with some intermediate-type lesions occurring.

The reported virulence formula of Ht_1/Ht_2Ht_3 for race 3 was expressed with isolate R3SC at 22/18 C with the clearest

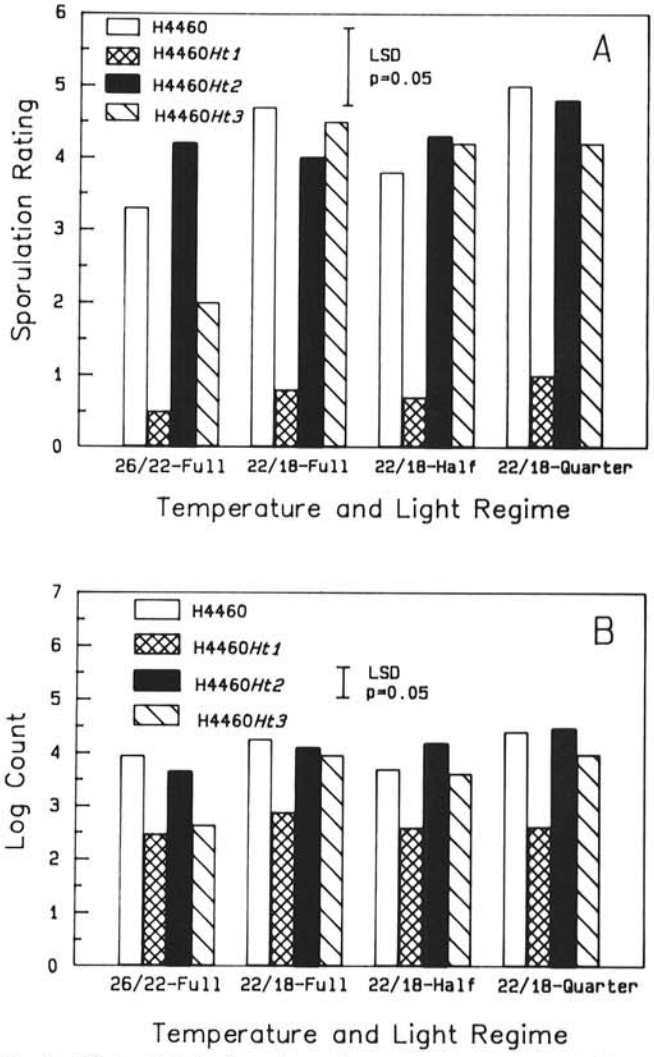

Fig. 2. Effect of light intensity and temperature on sporulation of *Exserohilum turcicum* race 3 (R3SC) on four near-isogenic maize lines, where **A** represents a visual estimate (0–5) of number of conidia and **B** represents counts (log 10) expressed in conidia/cm^2.

expression of virulence on H4460Ht_3 occurring at reduced light intensity (Table 1 and Fig. 1). The virulence of isolate R3SC to H4460Ht_1 was not expressed at 26/22 C; only resistant- to intermediate-type lesions were induced on H4460Ht_3 at 26/22 C. Isolate R3NC, which had also been reported to be race 3, could not be distinguished from race 1 in these tests.

Spore counts and visual sporulation ratings on leaf disks from lesions were consistent with the reaction type assessments, except that sporulation was low on leaf disks from susceptible-type lesions on H4460Ht_2 plants infected with race 1 at 22/18 C and 162 μmol m^{-2} s^{-1} light intensity. In all other cases, sporulation was high on leaf disks taken from lesions scored susceptible and low on leaf disks from lesions scored resistant (Table 1 and Fig. 2). There were apparent differences in degrees of susceptibility of near-isogenic lines to race 3 as judged by spore counts. For example, at 22/18 C and full light intensity, sporulation of race 3 on H4460Ht_3 was only 50% of that on H4460 and 75% of that on H4460Ht_2. In susceptible-type lesions induced by races 1 and 3, sporulation was much greater on leaf disks taken from plants at 22/18 C than on those from plants at 26/22 C (Table 1, Fig. 2).

Isolates of *E. turcicum* produced significantly more lesions on H4460 at reduced light intensity than at full light intensity at 22/18 C, and the lesions also were significantly longer at reduced light intensity (Figs. 3 and 4). Temperature effects on lesion number or length were not significant, except in cases in which reaction type changed at different temperatures. For example, isolate R3SC induced significantly more susceptible-type lesions on H4460Ht_3 at 22/18 C than intermediate-type lesions on H4460Ht_3 at 26/22 C (Fig. 3).

DISCUSSION

Through the use of controlled environment chambers, it was possible for us to verify the existence of race 3 of *E. turcicum* with virulence to corn genotypes with genes Ht_2 and Ht_3 for NLB resistance. The inadequacy of greenhouse conditions to consistently distinguish race 3 from race 1 can be seen from our inability to verify race 3 virulence in our earlier greenhouse tests and from our demonstration that isolate R3NC, which had previously been identified as race 3 in greenhouse tests, was avirulent on near-isogenic corn lines H4460Ht_2 and H4460Ht_3. One reason that greenhouse tests are unreliable for identification of race 3 may be the sensitivity of race 3 virulence to high temperature. Race 3 isolate R3SC was not virulent on H4460Ht_3 at the 26/22 C regime. Greenhouse temperatures may often exceed 26 C, particularly in the summer. Another difficulty stems from the sensitivity of Ht_2 resistance to low light intensity. During the winter, both race 1 and race 3 may appear to induce susceptible-type lesions on lines with Ht_2 in greenhouses without supplemental light.

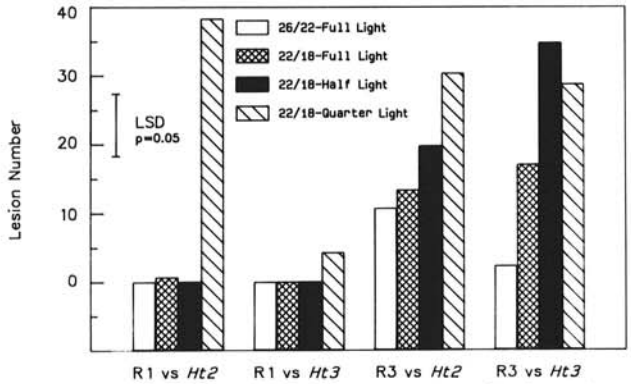

Fig. 3. Effects of light and temperature on number of lesions/plant as an indicator of monogenic resistance in maize to race 1 and race 3 of *Exserohilum turcicum*.

Because race 3 virulence to Ht_3 is not expressed at 26/22 C temperature regimes, isolates of race 3 collected in field surveys could easily be misidentified as race 1. The overall result could be an underestimation in the frequency of race 3 in populations of *E. turcicum* and an inability to monitor changes in virulence patterns in field populations.

Thakur et al (16) reported that race 2 was virulent on H4460Ht_1 at 22/18 C but avirulent at 26/22 C. They showed that H4460Ht_1 plants grown at 26/22 C developed susceptible-type lesions with abundant sporulation by race 2 if they were transferred to 22/18 C within 3 days after inoculation with race 2. We do not know whether this also will be true for race 3 on H4460Ht_3. If it is, that would allow accurate identification of race 3 on plants grown under greenhouse conditions but transferred to cooler temperatures in controlled environment chambers after inoculation. Such a procedure would allow for better *E. turcicum* race identification than is possible in standard greenhouses and would not require utilization of growth chambers for extended periods. Until the reliability of this procedure is verified, determinations of race 3 should be completed under consistently cool temperatures and moderate light intensities (about 364 μmol m^{-2} s^{-1}). Data already obtained on distribution and frequency of race 3 based on greenhouse evaluations should be regarded as inaccurate and very conservative.

The increased length of lesions of *E. turcicum* at reduced light intensity is consistent with Jenns and Leonard's (9) demonstration that length and sporulation of southern leaf blight (SLB) (*B. maydis*) lesions increased with reduced light intensity. Warren (18) also showed a similar effect for lesions of *Colletotrichum graminicola* on corn. Thus, there appears to be a general response of corn toward increased susceptibility to necrotrophic fungal foliar pathogens at reduced light intensity.

The increased number of lesions induced by *E. turcicum* at reduced light intensities differs from the results of Jenns and Leonard (9) with SLB. They found no increase in infection efficiency of *B. maydis* on corn plants at reduced light intensity. This may result from a difference in infection processes in NLB and SLB. *B. maydis* lesions develop very rapidly and may be visible 1 or 2 days after inoculation, whereas lesions of *E. turcicum* have a 7- to 12-day incubation period. Thus, the number of lesions produced by *B. maydis* may be determined before the inoculated plants are returned to the light following incubation in the dark in a moist chamber. The number of lesions induced by *E. turcicum*, however, is affected by conditions during the prolonged incubation

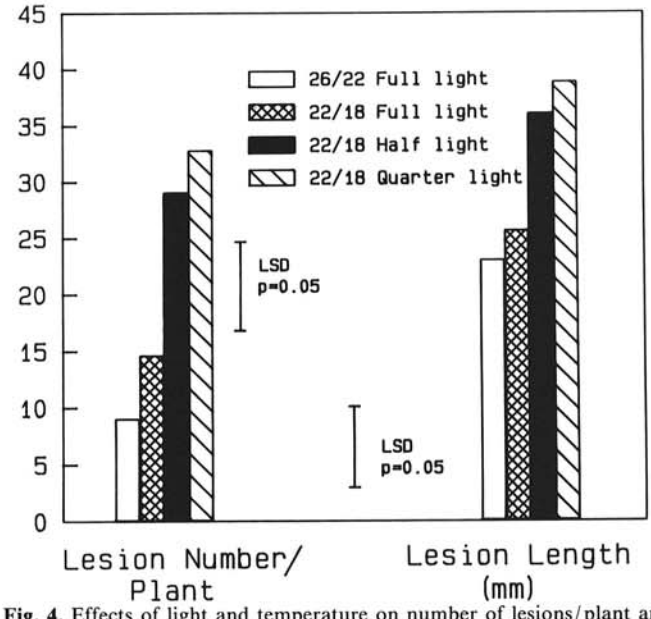

Fig. 4. Effects of light and temperature on number of lesions/plant and lengths caused by *Exserohilum turcicum* on susceptible maize inbred H4460. Values are means from 48 plants from three repetitions of the experiment and four isolates.

period during which the fungus colonizes the vascular system of infection sites in the leaves.

The response of *E. turcicum* lesion length to postinoculation temperature was contrary to results of Jenns and Leonard (9) for *B. maydis* lesions. They found that lesion length and sporulation of *B. maydis* were directly correlated with temperature from 22/18 C to 30/26 C, but infection efficiency was unaffected by temperatures in this range. In our study, *E. turcicum* did not induce any recognizable lesions at 30/26 C. The *E. turcicum* lesions induced at 22/18 C were at least as long and produced at least as many spores as those at 26/22 C. These results are consistent with the geographical adaptations of NLB and SLB, in that SLB normally is endemic in the southeastern United States but NLB is common in the north central states but rarely occurs in the Southeast. Prolonged periods of high temperatures can prevent increase of NLB. As shown by Thakur et al (16) for race 2 and by the results of our study with race 3, exposure to prolonged periods of moderately high temperatures (e.g., 26 C) could suppress the development of virulent races on corn genotypes with Ht_1 or Ht_2.

LITERATURE CITED

1. Downs, R. J., and Thomas, J. F. 1983. Phytotron procedural manual for controlled environment research at the Southeastern Plant Environment Laboratories. NC Agric. Exp. Stn. Tech. Bull. 244 (Rev.). 44 pp.
2. Gevers, H. O. 1975. A new major gene for resistance to *Helminthosporium turcicum* leaf blight of maize. Plant Dis. Rep. 59:296-299.
3. Hooker, A. L. 1963. Monogenic resistance in *Zea mays* to *Helminthosporium turcicum*. Crop Sci. 3:381-383.
4. Hooker, A. L. 1963. Inheritance of chlorotic-lesion resistance to *Helminthosporium turcicum* in seedling corn. Phytopathology 53:660-662.
5. Hooker, A. L. 1977. A second major gene locus in corn for chlorotic-lesion resistance to *Helminthosporium turcicum*. Crop. Sci. 17:132-135.
6. Hooker, A. L., and Perkins, J. M. 1980. Helminthosporium leaf blights of corn—the state of the art. Pages 68-87 in: Proc. Annu. Corn Sorghum Res. Conf., 35th. Chicago, IL.
7. Jenkins, M. T., and Robert, A. L. 1952. Inheritance of resistance to the leaf blight of corn caused by *Helminthosporium turcicum*. Agron. J. 44:136-140.
8. Jenkins, M. T., Robert, A. L., and Findley, W. R., Jr. 1954. Recurrent selection as a method for concentrating genes for resistance to Helminthosporium leaf blight in corn. Agron. J. 46:89-94.
9. Jenns, A. E., and Leonard, K. J. 1985. Effects of temperature and illuminance on resistance of inbred lines of corn to isolates of *Bipolaris maydis*. Phytopathology 75:274-280.
10. Jordan, E. G., Perkins, J. M., Schall, R. A., and Pedersen, W. L. 1983. Occurrence of race 2 of *Exserohilum turcicum* on corn in the central and eastern United States. Plant Dis. 67:1163-1165.
11. Leath, S., and Pedersen, W. L. 1986. Effects of the Ht_1 Ht_2 and/or Ht_3 genes in three maize inbreds on quantitative resistance to *Exserohilum turcicum* race 2. Plant Dis. 70:529-531.
12. 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 77:1738.
13. Miles, J. W., Dudley, J. W., White, D. G., and Lambert, R. J. 1980. Improving corn population for grain yield and resistance to leaf blight and stalk rot. Crop Sci. 20:247-251.
14. Smith, D. R., and Kinsey, J. L. 1980. Further physiologic specialization of *Helminthosporium turcicum*. Plant Dis. 64:779-781.
15. Thakur, R. P., Leonard, K. J., and Jones, R. K. 1988. Characterization of a new race of *Exserohilum turcicum* virulent on corn with resistance gene HtN. Plant Dis. 73:151-155.
16. Thakur, R. P., Leonard, K. J., and Leath, S. 1989. Effects of temperature and light on virulence of *Exserohilum turcicum* on corn. Phytopathology 79:631-635.
17. 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.
18. Warren, H. L. 1975. Temperature effects on lesion development and sporulation after infection by races O and T of *Bipolaris maydis*. Phytopathology 65:623-626.