Effects of Temperature and Illuminance on Resistance of Inbred Lines of Corn to Isolates of *Bipolaris maydis*

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Cooperative investigations of the USDA and the North Carolina Agricultural Research Service. Journal Series Paper 9291 of the North Carolina Agricultural Experiment Station, Raleigh.

This research was made possible by USDA-SEA Grant 82-CRCR1-1135.

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The authors thank C. F. Zimmerman for excellent technical assistance.

Accepted for publication 18 October 1984 (submitted for electronic processing).

ABSTRACT

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.

Six inbred lines of corn were grown under three temperature regimes or three illuminance regimes for 1 wk before and after inoculation with *Bipolaris maydis* race O. Day/night temperatures were 30/26, 26/22, and 22/18 C with a daytime illuminance of 455 hlx (592 μmol·m^{-2}·sec^{-1} photosynthetic photon flux density). Illuminance regimes were 455, 228, and 114 hlx at 26/22 C day/night temperatures. The six lines were inoculated in all 24 possible combinations with four isolates of race O. Lesion length, infection efficiency, and sporulation per lesion were measured. In the analysis of spores per lesion, a significant interaction between isolates and lines was observed in plants grown at 114 hlx before and after inoculation but not in those grown at higher illuminances or for lesion length or infection efficiency under any environmental conditions. Lesion length and sporulation increased with increasing temperature and decreased with increasing illuminance. Infection efficiency was unaffected by temperature or illuminance. Lines differed in the extent to which their resistance was diminished by increased temperature or decreased illuminance, but there were no significant isolate × temperature or isolate × illuminance effects. The levels of specific resistance present in the lines were estimated by three methods and the consistency of the estimates was compared.

Additional key words: *Cochliobolus heterostrophus*, maize, quantitative disease resistance, *Zea mays*.

Polygenically inherited or quantitatively expressed resistance has often been assumed to be nonspecific (15,16,20,21). In several tests, however, significant pathogen isolate × host cultivar interactions have been found with diseases in which resistance of the host is quantitative (1,2,12,17). Although the levels of specificity detected may not be sufficient to define distinct pathogenic races, even a low level of specificity could indicate a potential for significant erosion of the effectiveness of the resistance through further adaptation of the pathogen population. Studies of the level and consistency of expression of isolate specificity in quantitative resistance would be helpful in predicting the durability of the resistance.

Jenns et al (6) found significant interactions between inbred lines of corn (*Zea mays* L.) and isolates of *Bipolaris maydis* (Nisik.) Shoemaker in the analyses of six individual greenhouse trials, but not in the combined analysis over all trials. Since the expression of specificity of the resistance in that study seemed to vary with trials done at different times under uncontrolled environmental conditions, we undertook the present investigation to determine whether the apparent specificity could be reproducibly detected in repeated trials under carefully controlled environmental conditions. Our second objective was to determine whether differences in temperature or illuminance would affect the interactions between lines and isolates.

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MATERIALS AND METHODS

Pathogen isolates and host lines. To obtain a wide range of virulence among isolates used in these studies, 13 conidial isolates of *B. maydis* collected from corn from a variety of locations in North Carolina in 1974 and 1975, and 37 single-ascospore isolates were screened for virulence on plants of an F_1 hybrid between two inbred lines from the open-pollinated corn cultivar Jarvis. The ascospore isolates were from crosses between isolate Hm 80, collected in 1970 in North Carolina, and isolates 934, 957, 992, 996, 1001, 1214, 1230, 1240, and 1256, which were collected in North Carolina in 1974 and 1975.

Plants for screening the isolates were grown in 10-cm-diameter clay pots containing Metro Mix, a commercial growing medium (W. R. Grace and Co., Cambridge, MA 02138), in a greenhouse at 20–36 C. A solution containing 2.5 g of 20-20-20 NPK and 2.5 g of another fertilizer containing 6% total N, 25% P_2O_5, 5% K_2O, and trace elements per liter was applied at the rate of 50 ml per pot at 14 days after planting. Plants were inoculated 16 days after planting.

Inoculum for each isolate consisted of an aqueous suspension of 10,000 conidia per milliliter that was applied in 10 5-μl droplets to the fourth leaf with a 100-μl hypodermic syringe. Conidial suspensions were prepared from 2-wk-old cultures of isolates of *B. maydis* grown in the dark at room temperature on potato-dextrose agar (PDA) containing 10 g of sucrose per liter. The cultures were flooded with a solution containing two drops of Tween-20 per 100 ml of distilled water and scraped to release the conidia. Suspensions were filtered through two layers of cheesecloth, and spore concentrations were determined in a hemacytometer (Spiers-Levy Eosinophil Counter, C. A. Hausser and Son, Philadelphia, PA 19104). After inoculation, the plants were incubated in a mist chamber in the greenhouse for 16 hr. Four days after inoculation the lengths of lesions on the fourth leaf were measured with a ruler to the nearest millimeter. Based on the results of this trial, four isolates representing a wide range of virulence were selected for the temperature and illuminance studies.

Six inbred lines from the open-pollinated cultivar Jarvis, which had been used in a previous study and which represented a range of susceptibility to *B. maydis* (6), were selected for the temperature and illuminance studies. Cultivar Jarvis has been maintained as a randomly mating population. It has been shown to be genetically heterogeneous with respect to grain yield and other agronomic traits (9,14) as well as for levels of resistance to *B. maydis* (6).

Temperature. Plants of cultivar Jarvis inbred lines 2, 27, 68, 80, 316, and 349 were grown in 15.2-cm-diameter plastic pots in a mixture of Peat Lite (a commercial mixture made by W. R. Grace) and No. 16 gravel (1:2, v/v). The plants were irrigated twice each day with a standard phytotron nutrient solution (3). Experiments were conducted in the phytotron of the Southeastern Plant Environment Laboratory, Raleigh, NC 27695. Plants were grown for 2 wk in the air-conditioned phytotron greenhouse with a 9-hr day at 26 C and a night temperature of 22 C. Then they were thinned to one per pot and transferred to walk-in controlled-environment chambers at the 9-hr day/15-hr night temperatures of 30/26, 26/22, or 22/18 C.

Air temperatures in the chambers were maintained at ±0.25 C of the set point as measured with a #24, type "T," welded-bead thermocouple in a shielded, aspirated housing. The chambers were equipped with a combination of cool-white fluorescent and incandescent lamps, which provided an illuminance of 430 to 480 hlx (average photosynthetic photon flux density of 592 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) at pot level during the 9-hr day period. Day lengths were kept effectively long by interrupting the dark period from 2300 to 0200 hours with 41-hlx illumination from incandescent lamps. Top-to-bottom air flow was indicated by a Hastings air velocity meter (Hastings Co., Hampton, VA 23361) to average 20 m/min. Relative humidity was measured on a Weather Measure TOP 21-10 hygrometer (Weather Measure Co., Sacramento, CA 95841) and maintained at 70% or more at all temperatures. Carbon dioxide concentrations were measured on a Beckman IR gas analyzer (Beckman Instruments, Atlanta, GA 30340) and controlled at 300–400 ppm by injection of commercial grade CO_2.

Isolates of *B. maydis* were grown in petri plates in the dark at room temperature for 10 days on PDA containing 10 g of sucrose per liter. Plates were opened and allowed to dry in a microvoid (Air Control Inc., Norristown, PA 19006) for 48 hr. Conidia were collected by using a cyclone spore collector and stored at 4 C until used for inoculation. Conidia for all trials were from the same pool.

Plants were inoculated 21 days after planting when they were in the six-leaf stage. Ten 5-μl drops of an aqueous suspension containing 10,000 conidia per milliliter were applied to the fourth leaf of each plant with a 100-μl syringe. A quantitative inoculator (18) was used to spray three 3-cm-diameter circular areas of the fifth leaf of each plant for 2.2 sec at 138 kPa (20 psi) with an aqueous suspension of 40,000 conidia per milliliter. After every three plants, a glass microscope slide, coated with a thin layer of water agar, was similarly sprayed with the inoculum suspension. The average number of conidia of each isolate arriving on each sprayed area of the leaf was estimated by counting the average number of conidia on the same area of the slides sprayed with that isolate. An average of approximately 30 conidia was applied to each 3-cm-diameter target area.

After inoculation, the plants were incubated in a controlled-environment chamber at 24 C in darkness for 18 hr. During the incubation period a fine mist of deionized water was sprayed for 14 sec every 5 min at a rate of about 10.6 L/hr from each of four nozzles near the ceiling. After incubation, the plants remained in the chamber for 2 hr while the humidity returned to normal.

After the plants had dried, they were returned to the controlled-environment chambers at 30/26, 26/22, and 22/18 C. Lesions that developed from the droplet inoculations on the fourth leaves were measured 4 days after inoculation. On the third day after inoculation, lesions on the fifth leaves were counted, and percentage infection efficiency was calculated as 100 × (number of lesions/estimated number of conidia deposited per target area). Plastic bags were then secured over these leaves to induce sporulation. Seven days after inoculation, the leaves enclosed in bags were removed and stored at 4 C until sporulation was measured.

To measure sporulation, areas of the fifth leaf with lesions were excised and placed in a test tube with 2 ml of a solution of two drops Tween-20 per 100 ml water. The tube was agitated with a Vortex mixer and the concentration of conidia in the resulting suspension was determined with a hemacytometer.

Three trials, each involving 216 plants, were performed. Thirty-six plants of each of two lines were inoculated on each of three successive days during each trial. In the first trial, lines 2 and 68 were inoculated on the first day, lines 80 and 349 on the second day, and lines 27 and 316 on the third day. In the second trial, the pairs were 27 and 80, 316 and 68, and 2 and 349 and in the third trial 316 and 349, 2 and 27, and 68 and 80. There were three replications of each treatment for each pair of lines inoculated on a given day, but the limited size of the inoculation chamber required that the complete set of inoculations for each trial be conducted over 3 days. Seeds of each line were randomly assigned to pots at planting, and plants were randomly assigned positions in the controlled-environment chambers and in the incubation chamber.

Illuminance level. The experiments testing the effects of illuminance were done in a manner similar to that described for temperature. The controlled-environment chambers in which plants were grown before and after inoculation were all at day/night temperatures of 26/22 C. The illuminances during the 9-hr day period were 455, 228, or 114 hlx (592, 296, and 148 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ photosynthetic photon flux density, respectively) and during the 3-hr interrupted-night period were 41, 20, or 10 hlx.

In the three illuminance trials, 12 plants of each of the six lines were inoculated on each of three successive days. Since the 12 plants of each line were from three different illuminance regimes and were each inoculated with one of four different isolates, there was no replication in a single day's inoculation. The means for each trial were used in the analysis, so that there were three replications in time for each treatment.

In one trial, both length and width of lesions were measured on all the plants inoculated on a single day and kept in the chamber at 228 hlx. The correlation between lesion length and area was calculated for elliptical lesions, which represents a good approximation of the shape of most lesions of *B. maydis*.

RESULTS

Mean lesion lengths for the 50 isolates of *B. maydis* that were screened ranged from 2.9 to 8.7 mm (Fig. 1). Four isolates, 80 × 1001-6, 80 × 934-5, 1214, and 1230 were selected for use in the experiments at different temperatures and levels of illuminance. Mean lesion lengths for the four selected isolates were 2.9, 4.5, 6.8, and 8.7 mm, respectively. The coefficient of correlation between

lesion length and lesion area (calculated as area = π·[length/2][width/2]) was 0.90 ($P > |R| = 0.0001$).

The frequency distribution of mean lesion lengths for isolates approached normality, whereas those of percentage infection efficiency and sporulation per lesion were strongly skewed toward the right. The null hypotheses that the data for percentage infection efficiency and mean sporulation per lesion were random samples from normal distributions were tested by using a modified version of the Kolmogorov-Smirnov *D*-statistic (19). The null hypothesis was rejected in both cases at $P < 0.01$. The distributions of log-transformed data for these two variables were closer to the normal distribution, so log-transformed data were used in all analyses of percentage infection efficiency and sporulation.

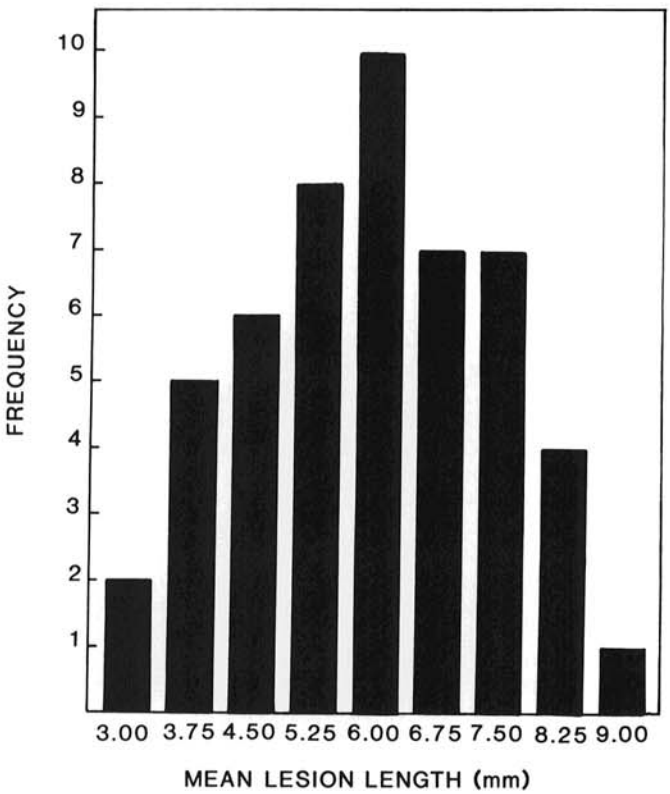

Fig. 1. Frequency distribution of mean lesion length produced on a hybrid of two inbred lines derived from the open-pollinated corn cultivar Jarvis by 50 isolates of *Bipolaris maydis* race O.

Temperature. The analysis of variance for lesion length showed that the effects of lines, temperatures, and the line × temperature interaction were significant (Table 1). For percentage infection efficiency, the effects of trials and the line × temperature interaction were significant. For spores per lesion, the effects of trials and temperatures were significant.

Percentage infection efficiency was not affected by the temperature treatments, but lesion length and sporulation per lesion both increased with increasing temperature (Fig. 2). Lesion length was significantly correlated with sporulation per lesion ($r = 0.49$, $P = 0.001$) and with infection efficiency ($r = 0.13$, $P = 0.05$). Infection efficiency and sporulation per lesion were not significantly correlated.

Illuminance level. The analysis of variance for lesion length showed that the effects of isolates, lines, trials, and illuminance levels were significant (Table 2). For percent infection efficiency, isolate and line effects were significant. For sporulation per lesion, the effects of lines, illuminance levels, trials, and the line × illuminance interaction were significant. Analysis of the sporulation data separately for each level of illuminance revealed a significant interaction ($P = 0.003$) between isolates and lines at 114 hlx but not at 228 or 455 hlx.

Percentage infection efficiency was not affected by illuminance level before and after inoculation, but lesion length and sporulation per lesion decreased with increasing illuminance (Fig. 3).

Lesion length was significantly correlated with both infection efficiency ($r = 0.21$, $P = 0.002$) and sporulation per lesion ($r = 0.44$, $P = 0.0001$). Infection efficiency and sporulation per lesion were not correlated.

Specific resistance estimations. Jenns et al (7) proposed two methods to estimate the relative amounts of specific resistance in a set of host genotypes inoculated in all combinations with a set of pathogen genotypes. In both methods, the disease severity scores for each host line inoculated with a series of pathogen isolates were regressed against isolate virulence indexes. In the first method, the isolate virulence index (VIM) was calculated as the mean disease severity induced by that isolate over all host lines in the test. In sets of model genotypes, the slopes of the regression lines for host genotypes were correlated with their levels of isolate-specific resistance. In the second method, the isolate virulence index (VIS) was calculated as the disease severity induced by that isolate on a host line with minimal specific resistance (ie, a susceptible check).

TABLE 1. Analysis of variance of lesion length, percent infection efficiency,[a] and spores per lesion[b] obtained in three trials with isolates of *Bipolaris maydis* race O on corn lines at three temperatures[c]

Source	Lesion length		Infection efficiency (%)		Spores per lesion	
	df	Mean square	df	Mean square	df	Mean square
Trial (random)	2	0.397	2	9.288***	2	5.144***
Isolate (fixed)	3	21.751	3	0.020	3	1.812
Line (fixed)	5	71.589**	5	0.169	5	2.217
Temperature (fixed)	2	123.300***	2	0.020	2	12.419**
Isolate × Line	15	1.600	15	0.161	15	0.468
Isolate × Temperature	6	0.371	6	0.043	6	0.158
Line × Temperature	10	5.425***	10	0.096*	10	0.386
Isolate × Trial	6	8.828***	6	0.087	6	1.034***
Line × Trial	10	15.506***	10	0.175***	10	0.984***
Temperature × Trial	4	2.817**	4	0.034	4	1.054***
Isolate × Line × Trial	30	1.363**	30	0.147***	30	0.331
Isolate × Temperature × Trial	12	0.340	12	0.060	12	0.409*
Line × Temperature × Trial	20	1.276*	20	0.049	20	0.445**
Isolate × Line × Temperature	30	0.690	30	0.037	30	0.274
Error	59[d]	0.791	57	0.053	48	0.236

[a] Percent infection efficiency = [(mean number of lesions)/(mean number of spores)] × 100. Percent infection efficiency transformed to $\log_{10}$ (1 + percent infection efficiency) prior to analysis.

[b] Spores per lesion transformed to $\log_{10}$ (1 + spores per lesion) prior to analysis.

[c] Plants were grown at day/night temperatures of 30/26, 26/22, or 22/18 C for 1 wk before and after inoculation at 24 C.

[d] Differences in degrees of freedom for error are due to missing values. Asterisks *, **, and *** indicate effect significant at $P < 0.10$, $P < 0.05$, and $P < 0.01$, respectively.

In this case, the levels of isolate-specific resistance in the host lines were correlated with deviations from the regression of disease severity versus isolate virulence index.

Two rating systems were employed in estimating levels of specific resistance (7). Rating 1, the best estimator of numbers of genes for specific resistance in the model genotypes, was based on mean disease severity of the host line over all isolates, the mean square for deviations from the regression on VIS, and the slope of the regression on VIM. Rating 2, the best estimator of the proportion of genes that were isolate-specific, was based only on the deviation mean square and the slope for regressions on VIM and VIS, respectively.

Ratings 1 and 2 were calculated for the six corn lines in these tests for all three measures of disease severity in the temperature and illuminance level experiments. Ratings 1 and 2 and a simplified method, based on variance of disease severity of each line over all isolates in the test (5), were used to rank the inbred lines according to the amount of specific resistance they possessed (Tables 3,4).

One way to test the consistency of the rating methods is to compare the ranking of lines for degree of specificity of resistance in separate tests done at the same temperature and illuminance conditions. The temperature regime of 26/22 C and illuminance of 455 hlx was included in both the temperature and the illuminance studies. The correlation between the rankings assigned to the corn lines under the same conditions in the two studies was much higher than between the rankings under different conditions within the two experiments (Tables 3 and 4). The coefficients of correlation between the rankings assigned by lesion length in the two studies under the same conditions were 0.60 for the variance method, 0.89 for rating 1, and 0.94 for rating 2. By this criterion, ratings 1 and 2 gave highly reproducible results, whereas the variance method was less consistent.

Rating 1 gave the most consistent, and the variance method the least consistent, rankings for specific resistance among temperatures (Table 3) and illuminances (Table 4). Ratings 1 and 2 were very highly correlated. The variable that had the most consistent ranking among temperatures and illuminances was lesion length for ratings 1 and 2. The rankings were not very consistent among variables at the same temperature or illuminance level. For instance, in the 30/26 C temperature regime, lines 2 and

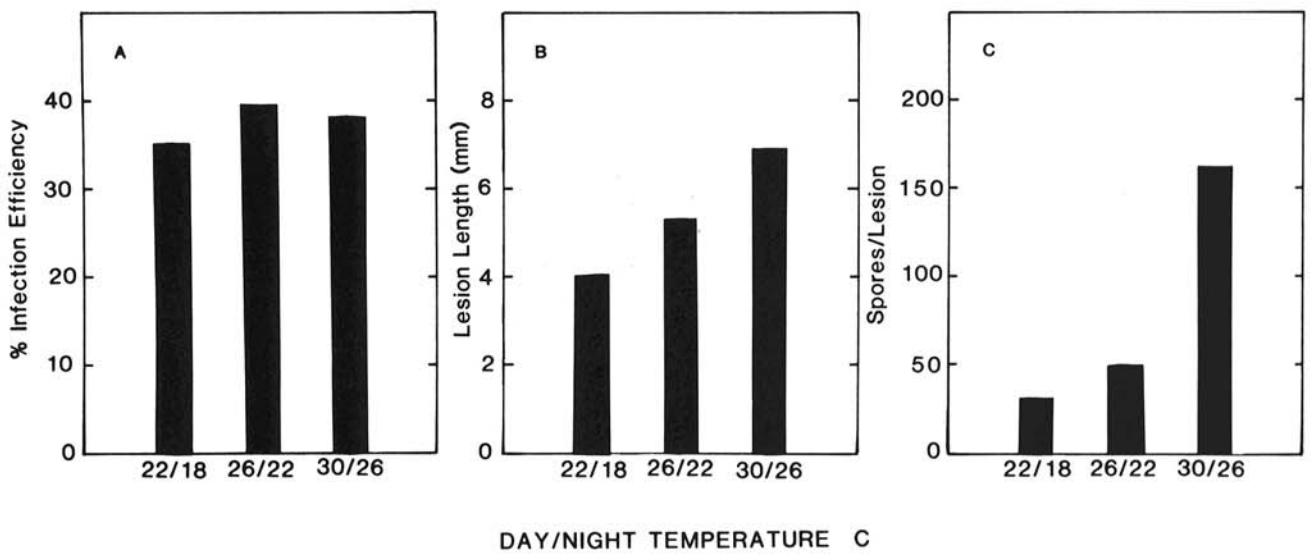

Fig. 2. Mean infection efficiency, lesion length, and spores per lesion produced by isolates of *Bipolaris maydis* race O on inbred lines of the open-pollinated corn cultivar Jarvis grown under three temperature regimes before and after inoculation.

TABLE 2. Analysis of variance of lesion length, percent infection efficiency,[a] and spores per lesion[b] obtained in three trials with isolates of *Bipolaris maydis* race O on corn lines at three illuminance levels[c]

	Lesion length		Infection efficiency (%)		Spores per lesion	
Source	df	Mean square	df	Mean square	df	Mean square
Trial (random)	2	30.015***	2	0.159	2	0.180***
Isolate (fixed)	3	39.286**	3	1.380***	3	0.009
Line (fixed)	5	98.179***	5	0.737***	5	0.125***
Illuminance (fixed)	2	90.152***	2	0.003	2	0.392**
Isolate × Line	15	1.205	15	0.093	15	0.015
Isolate × Illuminance	6	1.103	6	0.100	6	0.011
Line × Illuminance	10	2.969*	10	0.115	10	0.059***
Isolate × Trial	6	5.061***	6	0.065	6	0.027***
Line × Trial	10	2.392**	10	0.072	10	0.012
Illuminance × Trial	4	0.553	4	0.080	4	0.035***
Isolate × Line × Trial	30	1.321	30	0.066	30	0.010
Isolate × Illuminance × Trial	12	1.818	12	0.064	12	0.008
Line × Illuminance × Trial	20	1.414	20	0.152	20	0.011
Isolate × Line × Illuminance	30	1.026	30	0.064	30	0.014
Error	59[d]	1.100	60	0.105	39	0.008

[a] Percent infection efficiency = [(mean number of lesions)/(mean number of spores)] × 100. Percent infection efficiency transformed to $\log_{10}$ (1 + percent infection efficiency) prior to analysis.

[b] Spores per lesion transformed to $\log_{10}$ (1 + spores per lesion) prior to analysis.

[c] Plants were grown at daytime illuminances of 455, 288, or 114 hlx for 1 wk before and after inoculation.

[d] Differences in degrees of freedom for error are due to missing values. Asterisks *, **, and *** indicate effect significant at $P = 0.10$, $P = 0.05$, and $P = 0.01$, respectively.

68 ranked 3 and 6, 2 and 6, and 6 and 1, respectively, according to both ratings 1 and 2 for specificity of resistance in terms of lesion length, infection efficiency, and sporulation (Table 3). At 22/18 C, lines 2 and 68 ranked 1 and 2, 4 and 2, and 4 and 6, respectively, for specificity of these components of resistance.

DISCUSSION

Nelson and Tung (10) showed that the size of lesions induced by race T of *B. maydis* on a susceptible corn hybrid increased with postinoculation temperatures between 20 and 31 C. Warren (22) found that lesion size and spore production increased but that lesion number did not as postinoculation temperature was increased from 15 to 30 C when inbred corn lines were inoculated with isolates of *B. maydis* race O. The results of our studies, which involved comparisons of the effects of combined pre- and postinoculation temperatures, were similar.

Exposure to light reduces sporulation by *B. maydis* race O both on artificial media (4) and on intact corn plants (11). The reduction of in vitro sporulation appears to be a direct effect of light on the fungus. Part of the reduction in sporulation that we observed on corn plants grown at high illuminance levels could have been due to such a direct effect on the pathogen, but part of the effect must also have been indirect through the influence of light on host resistance. Sporulation per lesion was correlated with lesion size, which was also reduced by high illuminance levels. Lukens and Mullany (8) observed that the percentage of blighted area caused by *B. maydis* race T on susceptible corn in a field adjacent to a wooded area

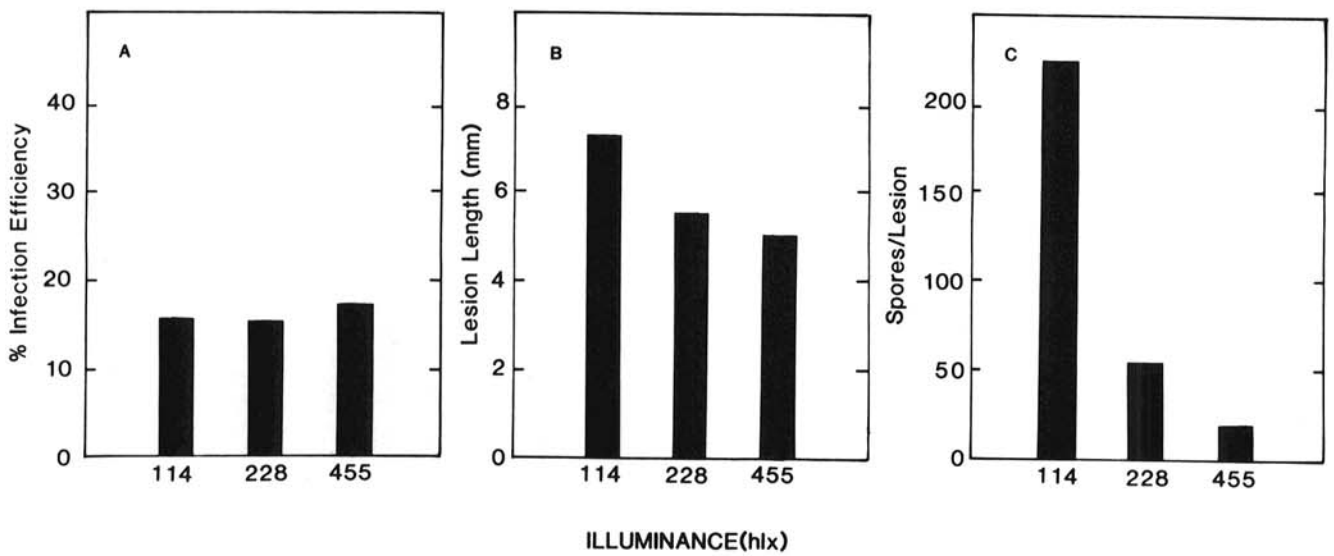

Fig. 3. Mean infection efficiency, lesion length, and spores per lesion produced by isolates of *Bipolaris maydis* race O on inbred lines of open-pollinated corn cultivar Jarvis grown under three levels of illuminance before and after inoculation.

TABLE 3. Ranking of corn lines according to their specific resistance to *Bipolaris maydis* race O in the temperature study

Temperature regime (day/night, C)	Line	Lesion length				Infection efficiency				Sporulation			
			Rank[a] by rating:				Rank[a] by rating:				Rank[a] by rating:		
		Mean	Var.[b]	1[c]	2[d]	Mean	Var.	1	2	Mean	Var.	1	2
30/26	2	4.7	3	3	3	39	5	2	2	47	6	6	6
	80	5.2	2	2	2	25	1	5	5	121	1	3	3
	316	6.2	1	1	1	32	4	1	1	155	2	5	5
	27	6.8	4	4	4	42	6	3	4	156	3	4	4
	349	7.8	6	5	5	44	2	4	3	173	5	2	2
	68	10.4	5	6	6	45	3	6	6	317	4	1	1
26/22	2	4.2	2	3	3	40	5	5	5	24	1	3	3
	80	4.3	3	2	2	40	1	3	3	25	2	4	4
	316	4.6	1	1	1	38	4	4	4	47	4	1	2
	27	5.9	5	5	5	23	6	6	6	88	6	6	6
	349	6.4	4	4	4	48	2	1	2	32	3	5	5
	68	7.3	6	6	6	50	3	2	1	88	5	2	1
22/18	2	3.8	2	1	1	31	2	4	4	30	4	4	4
	80	3.1	6	4	3	29	4	1	1	31	5	5	6
	316	3.5	5	3	4	33	5	5	5	27	6	1	1
	27	4.6	3	6	6	28	3	3	3	74	3	3	3
	349	4.5	4	5	5	42	6	6	6	17	1	2	2
	68	5.8	1	2	2	44	1	2	2	55	2	6	5

[a] Ranking scale: 1 = least specific resistance, and 6 = most specific resistance.

[b] Variance among isolates of lesion length, infection efficiency, or spores per lesion adjusted by subtraction of value for line with lowest variance among isolates.

[c] Rating 1 = [(Deviation mean square for regression on Virulence Index based on line with least specific resistance/mean deviation mean square) + line mean/overall mean] × 2 + slope of regression on Virulence Index based on the mean disease severity (see reference 7).

[d] Rating 2 = (Deviation mean square for regression on Virulence Index based on line with least specific resistance/mean deviation mean square) + slope of regression on Virulence Index based on the mean disease severity (see reference 7).

decreased as the distance from the edge of the woods increased. Nelson and Tung (10,11) found that susceptible corn plants placed in the dark after inoculation with race T developed larger lesions (10) with more sporulation per unit area (11) than plants incubated in the light. They suggested, however, that shading per se was unlikely to have caused the increased sporulation and attributed it instead to the higher relative humidity or other factors associated with the dark conditions (11). We found that both lesion length and sporulation increased as illuminance decreased, even when relative humidity and temperature were constant.

The statistically significant effects of the line × temperature interaction for lesion length and line × illuminance interaction for sporulation suggests that there are different resistance mechanisms among lines. The different mechanisms could account for the differences in sensitivity of the resistance of different lines to changes in temperature or illuminance. This is supported by the relatively large effects of the line × temperature interaction for infection efficiency and the line × illuminance interaction for lesion length. In contrast, the effects of isolate × temperature and isolate × illuminance interactions were not significant for any variable.

Under the conditions of controlled temperature and illuminance employed in these experiments, we found no significant ($P<0.05$) interactions between corn lines and isolates of *B. maydis* except for sporulation at the lowest illuminance. It has been suggested (5,13) that detection of a host cultivar × pathogen isolate interaction significant at $P<0.05$ is a very conservative test for specificity of resistance. Thus, our results do not necessarily imply the absence of any line × isolate specificity for the lines and isolates that we tested.

Our results differ from those that we obtained in similar experiments in less controlled environments in the greenhouse (6). In those earlier experiments, 10 lines of corn were inoculated in all combinations with 10 isolates of *B. maydis*, and the lengths of resulting lesions were compared. Although the combined analysis of variance for data over all six trials of that experiment showed no significant line × isolate interaction, the interaction effect was statistically significant for every analysis of variance for individual trials. In the present work, when data for each temperature regime in each trial were analyzed separately, only two of nine analyses for lesion length and infection efficiency and only one of nine analyses for sporulation showed a significant isolate × line interaction effect. Separate analyses for each illuminance regime in each trial showed significant isolate × line interaction effects in only one of nine analyses for lesion length and two of nine analyses for sporulation. Combined analyses of variance over the three trials at each temperature or illuminance regime revealed a significant isolate × line interaction only for sporulation in the 114-hlx illuminance regime.

The discrepancy between results of the earlier greenhouse experiment and the current growth chamber studies suggests that the relatively high level of isolate × line specificity detected in individual greenhouse trials may have been partly due to artifacts produced by environmental variables that were not well controlled in the greenhouse. Another possibility is that the use of 10 lines and 10 isolates in the greenhouse experiment compared with only six lines and four isolates in growth chambers made the detection of isolate × line interactions more likely for individual greenhouse trials than for the growth chamber trials. All six corn lines and two of the four isolates of *B. maydis* that were used in the growth chamber studies were chosen from among those that had been used in the earlier greenhouse experiment. Furthermore, the other two isolates used in the growth chamber studies were progeny of conidial isolates used in the greenhouse experiment crossed with isolate Hm 80. Therefore, the actual levels of isolate specificity of the resistance of corn lines should have been similar in the two sets of experiments.

The isolate × line × trial interaction effect was significant in the greenhouse trials (6) and also in the temperature study in the phytotron for lesion length and percentage infection efficiency. This suggests that the isolate × line interaction is affected by factors that vary among trials other than those that were controlled.

The analysis of variance has been shown to be an insensitive test for detecting isolate specificity in quantitative resistance (5,13). Therefore, a conservative approach in breeding for resistance would be to allow for the possibility of pathogen adaptation to polygenic resistance even when an analyis of variance shows no statistically significant pathogen isolate × host cultivar interaction. The methods of Jenns et al (7) and Jenns and Leonard (5) can be used to predict which of a series of cultivars may have quantitative

TABLE 4. Ranking of corn lines according to their specific resistance to *Bipolaris maydis* race O in the illuminance study

Light regime (Daytime/ interrupted night [hlx])	Line	Lesion length				Infection efficiency				Sporulation			
			Rank[a] by rating:				Rank[a] by rating:				Rank[a] by rating:		
		Mean	Var.[b]	1[c]	2[d]	Mean	Var.	1	2	Mean	Var.	1	2
455/41	2	3.2	2	3	3	6	4	5	5	11	1	1	2
	80	4.0	6	1	2	13	3	3	3	10	5	3	4
	316	5.3	1	2	1	22	5	4	4	18	3	2	1
	27	5.0	3	4	4	27	1	1	1	9	4	4	3
	349	6.2	4	5	5	17	2	2	2	17	2	5	5
	68	6.5	5	6	6	15	6	6	6	45	6	6	6
228/20	2	3.0	3	2	2	12	2	2	2	7	3	1	1
	80	4.6	1	1	1	14	1	1	1	37	5	5	4
	316	6.0	6	5	5	14	5	5	5	122	6	6	6
	27	4.6	4	3	3	22	4	4	4	86	1	2	2
	349	6.6	2	4	4	16	6	6	6	33	2	3	5
	68	8.0	5	6	6	13	3	3	3	90	4	4	3
114/10	2	4.3	2	1	2	11	6	1	1	63	1	1	1
	80	6.1	5	3	3	11	1	2	2	54	2	4	5
	316	7.1	1	2	1	16	3	4	4	157	6	6	6
	27	6.9	3	4	5	22	5	6	6	122	4	3	3
	349	8.1	6	6	6	15	4	5	5	349	3	2	2
	68	10.3	4	5	4	17	2	3	3	604	5	5	4

[a] Ranking scale: 1 = least specific resistance, and 6 = most specific resistance.

[b] Variance among isolates of lesion length, infection efficiency, or spores per lesion adjusted by subtraction of value for line with lowest variance among isolates.

[c] Rating 1 = [(Deviation mean square for regression on Virulence Index based on line with least specific resistance/mean deviation mean square) + line mean/overall mean] × 2 + slope of regression on Virulence Index based on the mean disease severity (see reference 7).

[d] Rating 2 = (Deviation mean square for regression on Virulence Index based on line with least specific resistance/mean deviation mean square) + slope of regression on Virulence Index based on the mean disease severity (see reference 7).

resistance that is most vulnerable to pathogen adaptation. When we used those methods for the corn lines tested in the growth chambers, we found that rating 2 gave the most consistent results for repeated experiments under the same environmental conditions, although rankings obtained with rating 1 were highly correlated with those of rating 2.

The rankings of lines for estimated levels of isolate specificity of resistance varied considerably for different components of resistance. This might be expected for two reasons. First, since different genes may differentially influence different components of resistance, it is possible that those components might exhibit different types of specificity. Second, the estimates of relative specificity of resistance with respect to infection efficiency are likely to be less reliable than those for the other two components of resistance, because there was very little variation among lines or isolates in measurements of infection efficiency. The methods of estimating specificity should be most accurate when there is a relatively large range of resistance among lines and virulence among isolates (7). When rankings for specificity of resistance vary with different components of resistance, it is probably safest to use the ranking for the component that combines the most reliable estimates and greatest importance in the epidemiology of the disease.

Rankings for specificity of resistance among lines also varied with different temperature and illuminance levels. This may be another reflection of the differential sensitivities of the resistance of different lines to changes in temperature and light that were demonstrated by the significant line × temperature and line × illuminance effects in the analyses of variance in these studies. Since the greatest hazard from a breakdown in the effectiveness of resistance would be in the environments that are most conducive to disease in field situations, the specificity of resistance of cultivars should be rated in those environments. Thus, ratings of specificity in the 30/26 temperature regime and the 455 and 228 hlx illuminance regimes are most relevant to the possibility of adaptation of *B. maydis* race O to the quantitative resistance of corn lines. By this reasoning, the resistance of line 80 would be preferred over that of line 316. The resistance of line 80 might also be preferred over that of line 2 if lesion length is regarded as the most reliable indicator of resistance.

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