Temperature Effects on Lesion Development and Sporulation After Infection by Races O and T of Bipolaris maydis

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ABSTRACT

Bipolaris maydis races O and T sporulated on corn leaf tissue at 15, 22.5, and 30 C. Spore production by *B. maydis* race T was sensitive to temperature, whereas production by race O was less sensitive. At 15 C, few race T spores were produced. The greatest number of spores was produced at 30 C for both races. However, race T produced five times more spores at 22.5 C, and two times more at 30 C than race O. More lesions formed, and expanded more rapidly at 30 C than at 22.5 or 15 C. Lesion size differed markedly between inbreds containing cms-T cytoplasm inoculated with race T. Differences in lesion size between inbreds containing normal cytoplasm inoculated with race O were smaller.

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Bipolaris maydis (Nisik.) Shoemaker (= *Helminthosporium maydis* Nisik. & Miyake) is an important foliar pathogen of corn (*Zea mays* L.) in the United States. A recent report (2) indicates that optimum temperatures differ for sporulation of races O and T of *B. maydis*. Hooker et al. (2) suggested that race O prevails in warmer regions, and race T is found in cooler regions. Cultural distinctions (5) and amount of sporulation at different temperatures have been studied in vitro (1). Effects of humidity on lesion number (7, 8) and sporulation (3) also were studied. However, the effect of temperature on in vivo sporulation, lesion expansion, and number of spores produced per mm^2 of diseased leaf area, have not been adequately studied. These data are needed to determine requirements for disease initiation, as well as to provide information for the development of disease forecasting programs.

In this study, temperature effect was evaluated to identify some of the factors that may control sporulation, lesion development, and spore numbers of *B. maydis* races O and T.

MATERIALS AND METHODS.—Five isolates each of races O and T of *B. maydis* were obtained from A. J. Ullstrup, Purdue University. For inoculum, conidia were collected from sporulating lesions on diseased leaf material that had been placed on moistened filter paper in petri plates. Single-spore isolates of the fungus were grown on lactose-casein hydrolysate medium (pH 6.5) at 24 C (9), and routinely reisolated from diseased leaf tissue, to preclude any possible loss of virulence from prolonged storage in vitro.

Inbred maize lines W64A, Oh43, and A239 with both normal and Texas male-sterile cytoplasm (cms-T) were used. Unless otherwise stated, five plants per pot were grown in the greenhouse and inoculated when they were 12-14 days old. Each treatment was replicated twice and the test was repeated at least five times.

Conidial suspensions used to prepare inocula were filtered through cheesecloth and adjusted to 50,000 spores per ml. One drop of Tween 80 per 100 ml of spore suspension was added as a wetting agent. The inoculum was sprayed onto the leaves with an atomizer attached to

TABLE 1. Initiation of sporulation by *Bipolaris maydis* races O and T on maize inbreds W64A and Oh43 at three different temperatures

	Sporulation initiation (days after inoculation)			
	Race O on normal cytoplasm		Race T on cms-T cytoplasm	
Temperature	W64A	Oh43	W64A	Oh43
15	7	7	6	6
22.5	4	5	3	3
30	6	6	3	3

an air-pressure line, at a dosage of about 0.75 ml per plant. The inoculated plants were immediately placed in a chamber containing moistened peat moss, which saturated the chamber with moist air. The chambers were made of plastic, and temperature and light readings were recorded from inside the chambers. The chambers were placed in rooms and programmed for a 16-hour photoperiod. Rooms with temperatures of 15, 22.5, and 30 C were used. The temperatures inside the chambers ranged from 1-2 C above that of the room. The light source consisted of 20-w cool-white fluorescent and 100-w incandescent lamps. Light in the controlled environmental chambers in microeinsteins per m^2 per second was measured with a Model L1-185 quantum meter [Lambda Electronics Corp., Melville, (L.I.), N.Y.]. The light readings ranged from 8500 ± 500 lux (125 to 140 microeinstein units).

Lesion development was observed daily, and the number of days required for sporulation was determined. Sporulation was determined by spore collection from lesions of intact plants immediately after they were removed from the growth chamber. Twenty lesions from the third and fourth basal leaves of each replicate were observed microscopically for conidiation in situ.

For determining the number of spores per lesion, a 2-mm^2 section of leaf tissue from a lesion was placed in 2 ml of water containing 0.1% Tween 80. The leaf section was minced in a 14-ml (4-dram) vial and agitated, to remove conidia from the leaf surface. Spore concentration was determined with a hemacytometer. After determination of the size of the lesion, the number of spores per mm^2 per lesion was calculated. Ten sections from diseased leaf tissue per plant were counted, and an average of three lesions per plant was studied. This method was more rapid than and as accurate as the direct microscopic examination of preliminary studies.

Twenty-five lesions from the third basal leaves in each replication were measured 2, 4, and 6 days after inoculation. These lesions were marked with India ink for future identification. Measured directly with a polar planimeter, lesion area was directly proportional to length and width (mm^2). To study the rate of lesion formation, spore concentrations of 10,000, 20,000, and 30,000 spores per ml were used. The method of inoculation of maize seedlings was the same as described above. Lesions on the third and fourth leaf of each plant were counted 72 hours after inoculation.

RESULTS.—*Days required for sporulation of races O and T.*—On either normal or cms-T cytoplasm at the various temperatures, the number of days required for initial sporulation of races O and T was about equal (Table 1). However, there were differences between and within races. At 22.5 and 30 C, race T sporulated within 3 days after inoculation, and at 15 C, within 6 days. Race O on normal cytoplasm required a longer period for

TABLE 2. Lesion size on normal cytoplasm and cms-T cytoplasm corn inbreds infected with *Bipolaris maydis* races O and T at three temperatures

		Lesion size (mm^2)					
		Race O on normal cytoplasm			Race T on cms-T cytoplasm		
Inbred line	Days after inoculation	15C	22.5C	30C	15C	22.5C	30C
W64A	2	0.7[a]	1.3	2.6	0.8	3.9	10.4
	4	3.9	6.5	10.4	5.2	20.2	28.6
Oh43	2	0.5	1.2	2.1	0.7	2.6	5.2
	4	3.3	5.2	9.3	5.2	18.4	23.4
A239	2	0.5	2.6	2.6	0.7	2.2	5.2
	4	3.2	5.8	10.4	3.4	12.8	18.2

[a]Each value is the means of 20 lesions, four lesions on the third and fourth leaf from each of five infected plants.

TABLE 3. Number of spores produced per square millimeter of lesion at three temperatures by *Bipolaris maydis* races O and T on three maize inbreds 2 days after spore formation

Temperature	Race O			Race T		
(C)	W64A	Oh43	A239	W64A	Oh43	A239
15	38 c	40 c[yz]	40 c	4 b	0 a	6 b
22.5	68 d	88 d	60 d	389 e	460 f	381 e
30	508 g	523 g	516 g	874 h	998 i	989 i

[y]Each figure is the average of ten 1-mm^2 sections from 10 different lesions. The test was repeated three times.
[z]Values followed by a common letter do not differ significantly, $P = 0.05$, according to Duncan's multiple-range test.

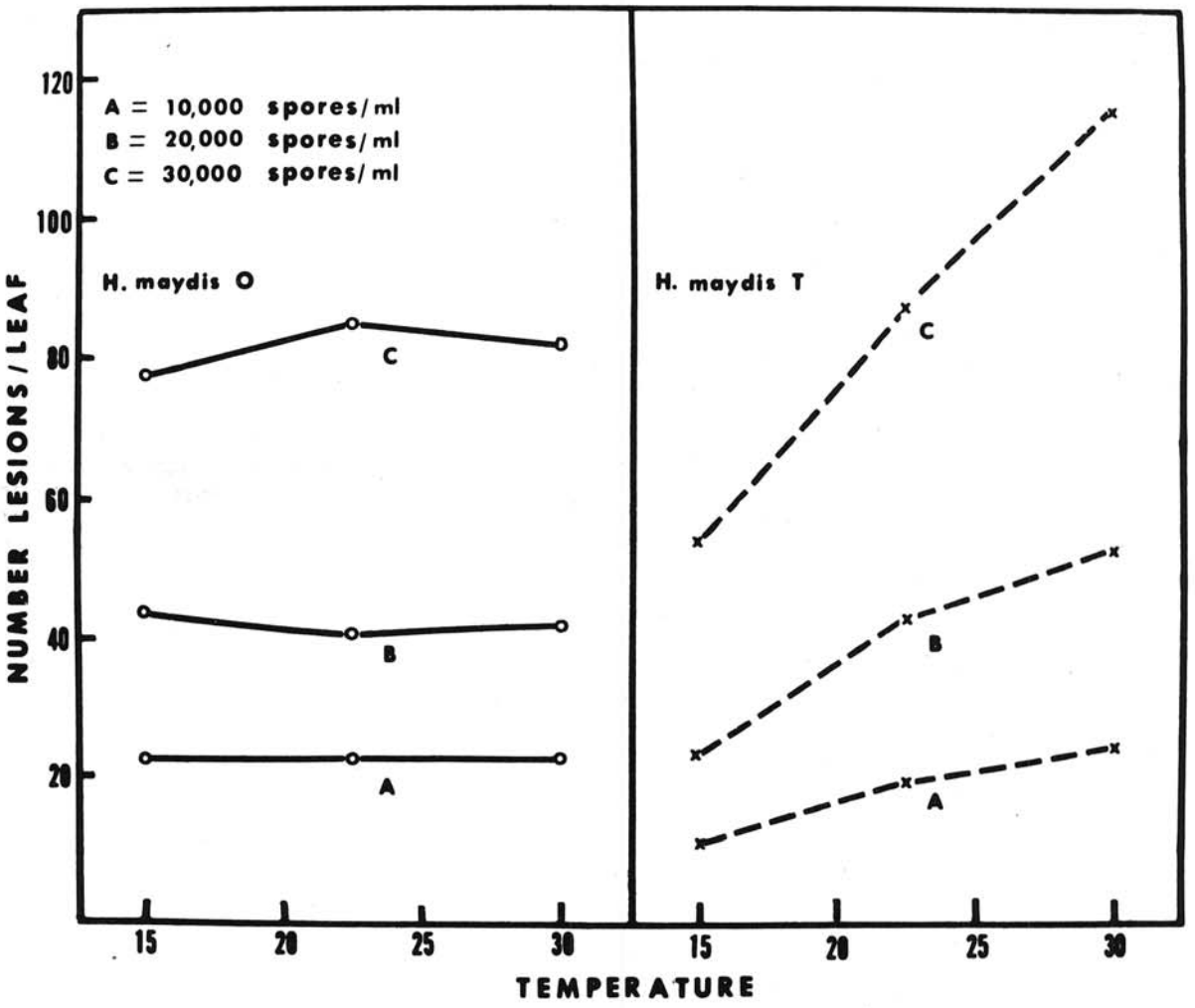

Fig. 1. Average number of lesions per leaf 72 hours after inoculation of 14-day-old maize seedlings with *Bipolaris maydis* races O and T. After inoculation, the plants were placed in growth chambers set at 15, 22.5, and 30 C.

sporulation at each temperature than race T. The optimum temperature was 22.5 C for sporulation in situ of both races on normal and cms-T cytoplasm. These data agree with those of other workers who measured sporulation on detached leaves (3).

Effect of temperature on lesion size.—Lesion expansion appeared to be sensitive to temperature. Initially, inbreds containing cms-T cytoplasm produced larger lesions than their normal-cytoplasm counterparts (Table 2). However, in these trials, when lesions were allowed to develop for more than 6 days they coalesced, and no difference between races or inbreds could be determined at 22.5 or 30 C. Lesion expansion was restricted at 15 C up to 12 days after inoculation. Lesion size increased with increase in incubation time. Seedlings with cms-T cytoplasm which were inoculated with the race T pathogen and incubated at 30 C were often killed within 12 days.

Lesions developed more rapidly at 30 C than at 22 or 15 C. For example, lesion measurements on W64A, cms-T cytoplasm, were 5.2, 20.2, and 28.6 mm^2 at 15, 22.5, and 30 C, respectively, 4 days after inoculation (Table 2). Lesion size differed markedly between inbreds containing cms-T cytoplasm. Lim and Hooker (4) reported similar results. They found no significant difference among normal cytoplasm inbreds inoculated with race T. In this study, this relationship was also observed when normal cytoplasm inbreds were inoculated with race O.

Number of spores produced per square millimeter.—Temperature affected the number of spores produced on the corn inbreds used in this test. Lesions from both normal and cms-T cytoplasm examined 2 days after sporulation at 15 C produced fewer spores per square millimeter than at 22.5 or 30 C (Table 3). Race T produced an average of three spores/mm^2 at 15 C, compared to 40 spores for race O. The greatest number of spores was produced at 30 C for both races, although race T produced about two times as many spores as race O. At 22.5 C, race T produced more than five times as many spores as race O. There were no significant differences in number of spores produced per square millimeter on inbreds containing normal cytoplasm inoculated with

race O, but there were significant differences between inbreds inoculated with race T. Significant differences on inbreds existed between temperatures for both races.

Effect of inoculum concentration on lesion development.—The effect of inoculum concentration on number of lesions was studied at constant temperatures of 15, 22.5, and 30 C in saturated moist air in chambers. Lesion-development response to all three temperatures was about equal for race O (Fig. 1). An average of 23 lesions per leaf developed at each temperature when plants were inoculated with 10,000 spores per ml. However, the number of lesions at inoculum concentrations of 20,000 and 30,000 at all temperatures varied slightly. This variation is within range of experimental error. With all concentrations, *B. maydis* race O caused more lesions to develop than did race T. However, at 22.5 and 30 C, more lesions developed with race T, except at 22.5 C at the low inoculum concentration.

The effect of inoculum concentration on lesion development varied in relation to temperature for race T. At 15 C, the fewest number of lesions developed, and at 30 C, the most did. The relationship between inoculum concentration and number of lesions per leaf is practically linear for all temperatures.

DISCUSSION.—Sporulation by *B. maydis* is a complex physiological process. Temperature influenced rate of lesion development, lesion size, and formation of spores. Spore formation of race O isolates appeared to be less sensitive to temperature between 15 and 30 C than that of race T. With race T, the number of spores per square millimeter of diseased tissue was five times greater at 22.5 C than that with race O. Both races produced the greatest number of spores at 30 C. However, race T produced about twice as many as race O. At 15 C, *B. maydis* race T produced an average of three spores per square millimeter of diseased tissue (Table 3). This result indicated that race T has a higher temperature requirement for optimum sporulation, and in part may explain the distribution pattern and seasonal occurrence of spores (6, 11) and their relationship to the spread of southern corn leaf blight epidemics from the southern to the northern regions of the USA (6).

When inoculated with *B. maydis* race T, all inbreds containing cms-T cytoplasm produced larger lesions than their normal counterparts. Coupled with larger lesions and more spores produced per square millimeter, the inoculum potential of race T can be predicted to be much greater than that of race O. This becomes significant, if these data are put into disease-forecasting programs such as EPIMAY (10). Lim and Hooker (4) found significant differences in lesion-size among inbreds containing cms-T cytoplasm. Also, when inbreds containing cms-T and normal cytoplasm were inoculated with *B. maydis* race T, inbreds containing cms-T cytoplasm produced larger lesions than their normal counterparts. In this study, when normal and cms-T cytoplasm corn plants were inoculated with races O and T, respectively, lesions on cms-T cytoplasm plants were larger.

Lesion development on plants inoculated with various concentrations of inoculum was studied with the aim of determining the effect of temperature on lesion numbers. However, at 15 C, 72 hours after inoculation, number of lesions counted is more precisely a count of penetration of leaf tissue by *B. maydis* race T. The evidence that fewer lesions developed at 15 C than at 22.5 or 30 C suggests that spore germination with later penetration and lesion development is temperature-sensitive for *B. maydis* race T.

The low incidence of lesions at low temperatures suggests than environmental conditions were not highly favorable to disease development. Nelson and Tung (7, 8) showed that where environments are favorable for race T, significantly more infections occurred. However, at 15 C, most race O lesions yielded very few conidia, did not expand rapidly, and sporulation was delayed.

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