Effects of Temperature and Host Maturity on Lesion Development of Colletotrichum graminicola on Corn

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ABSTRACT

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Corn plants of 12 genotypes were inoculated with *Colletotrichum graminicola* in the four- to five-leaf stage and grown at 20, 24, 26, 28, 30, and 32 C in controlled environment rooms. The optimum temperature for lesion elongation was 30 C. Lesion elongation was linear with time. Significant differences in susceptibility were noted among the 12 genotypes. Plants of corn cultivar Gaspe Flint were grown in a controlled environment room and inoculated at 2, 3, 4, 5, 6, 7, and 8 weeks after planting. Plants shed pollen at 33 days and reached physiological maturity at 58 days. After 9 to 10 weeks, most plants were senescent. Plants inoculated at 5 and 6 weeks after planting developed fewer lesions per square centimeter of leaf area, and the lesions were smaller than those on plants inoculated either earlier or later in their development. The greatest numbers of lesions per square centimeter occurred on lower leaves of young plants and on the top leaves of plants 7 or 8 weeks old at inoculation. The relationship between lesion size and leaf position on the plants was nearly linear, with the largest lesions on the lowest leaves. These results suggest that the pattern of early summer leaf infections and late summer leaf and stalk infections in the field is related to changes in host susceptibility. Little or no disease was found in the field in midsummer.

Additional key word: anthracnose.

We observed moderate to severe lodging of corn (*Zea mays* L.) from stalk rot (caused by *Colletotrichum graminicola* (Ces.) G. W. Wils.) in several locations in North Carolina in 1972, 1973, and 1974. Warren et al. (3) reported an epidemic of *C. graminicola* on sweet corn in Indiana in 1972. According to Wheeler et al. (4) a new race of *C. graminicola* which is more virulent to corn than previous races, appeared in 1969 and was identified in at least five states in 1971. The increasing importance of this pathogen in the United States has stimulated interest in what had previously been considered a very minor disease of corn.

In North Carolina, the occurrence of *C. graminicola* infection of corn is similiar to that described by Dale (1) in Arkansas. The fungus causes leaf spots on young plants early in the growing season, and stalk rot and leaf spots on maturing plants near the end of the growing season. Between these times it is difficult to find the disease. Our investigations were undertaken to determine the effects of temperature and host development on the disease, and to determine the optimum conditions for screening breeding lines for resistance.

MATERIALS AND METHODS

Temperature.—Effects of temperature on elongation of *Colletotrichum graminicola* lesions were tested on 12 genotypes of corn. Plants were grown in 10 cm diameter plastic pots (two plants per pot) in a mixture of RediEarth (a commercial peat-vermiculite mixture made by W. R. Grace Co., Travelers Rest, S. C.) and No. 16 gravel (1:2, v/v). Nutrients were added as needed by irrigating the plants with a standard Phytotron nutrient solution containing: $Ca(NO_3)_2 \cdot 4\ H_2O$, 320 mg/liter; NH_4NO_3, 160 mg/liter; $Mg(NO_3)_2 \cdot 6\ H_2O$, 130 mg/liter; Na_2SO_4, 34 mg/liter; K_2SO_4, 30 mg/liter; $KH_2PO_4 \cdot 3\ H_2O$, 28 mg/liter; KH_2PO_4, 24 mg/liter; H_3BO_3, 1.4 mg/liter; $MoO_3 \cdot 2\ H_2O$, 0.01 mg/liter; Sequestrene 330 Fe (10% Fe), 50 mg/liter; Sequestrene cobalt (14% Co), 0.002 mg/liter; Hampene zinc (14.5% Zn), 0.09 mg/liter; Hampol manganese (9% Mn), 1.26 mg/liter; and Hampol copper (9% Cu) 0.06 mg/liter.

Experiments were conducted in the Phytotron of the Southeastern Plant Environment Laboratory, Raleigh, N. C. Plants were placed in the Phytotron greenhouse maintained at day/night temperatures of 30/26 C. One week after planting they were transferred to controlled environment rooms kept at 30/26 C. The rooms were equipped with a combination of cool-white fluorescent and incandescent lamps in an approximate ratio of 10:3 by wattage. Illumination was maintained at 40,000-50,000 lux 1 m below the lamps during the 9-hour day period and at 3,000-4,000 lux during a 3-hour dark interruption period at night.

Plants were inoculated 14 days after planting when they were in the four- to five-leaf stage. Inoculum consisted of an aqueous suspension of 250,000 conidia/ml which was sprayed on the plants with a hand sprayer attached to an air pump. Conidial suspensions were prepared by flooding 2- to 3-week-old cultures of *C. graminicola* and scraping them to release the condia. Mycelial fragments were removed by filtering the suspensions through four layers of cheesecloth. The *C. graminicola* isolate used was obtained from a diseased stalk of Pioneer Brand 3369A corn collected near Wallace, North Carolina in 1973, and maintained on PDA slants at 4 C.

After inoculation, the plants were placed in a dark moist chamber at 28 C for 16 hours. They then were transferred to controlled environment rooms maintained at a constant temperature of 20, 24, 26, 28, 30, or 32 C. There were not enough rooms to run all treatments simultaneously, so the treatments were randomized with time. Each treatment consisted of four pots (two plants per pot) each of 12 corn genotypes. Seven days after inoculation, the five largest individual lesions on the fourth leaf (from the bottom) on each plant were measured. At 20 C the lesions were measured at 6, 10, and

TABLE 1. Effects of host genotype, temperature, and time on length of anthracnose lesions on leaves of corn plants inoculated in the four- to five-leaf stage

Host genotype	Mean lesion length (mm)[a]	Regression coefficients of lesion length on: Postinoculation temperature[b]	Days after inoculation[c]
NC2A12	1.4 v	0.15 ± .04	0.63 ± .11
SC276Q2	4.2 w	1.00 ± .24	0.66 ± .05
NCG5D15	4.9 wx	0.68 ± .20	0.70 ± .06
NC2A7	5.1 wx	1.00 ± .17	0.79 ± .08
H84	5.1 wx	0.59 ± .15	1.10 ± .05
NC7417	5.2 wx	0.92 ± .21	0.71 ± .05
Mo17	5.3 wx	0.53 ± .19	0.76 ± .03
B37 × H84	5.5 wxy	0.29 ± .12	0.88 ± .08
C103	5.7 wxy	0.84 ± .12	0.86 ± .09
NCG2HN	6.2 xy	0.60 ± .16	1.13 ± .08
B37	6.9 y	0.50 ± .17	1.38 ± .10
Pioneer Brand 3369A	7.7 z	0.52 ± .19	1.12 ± .10

[a]Means for postinoculation temperatures of 20, 24, 28, 30, and 32 C; means followed by the same letter do not differ significantly, $P = 0.05$, based on Duncan's multiple range test.
[b]Regression of lesion length on temperature over the range including 20, 24, 28, and 30 C.
[c]Data from 6, 10, and 14 days at 20 C.

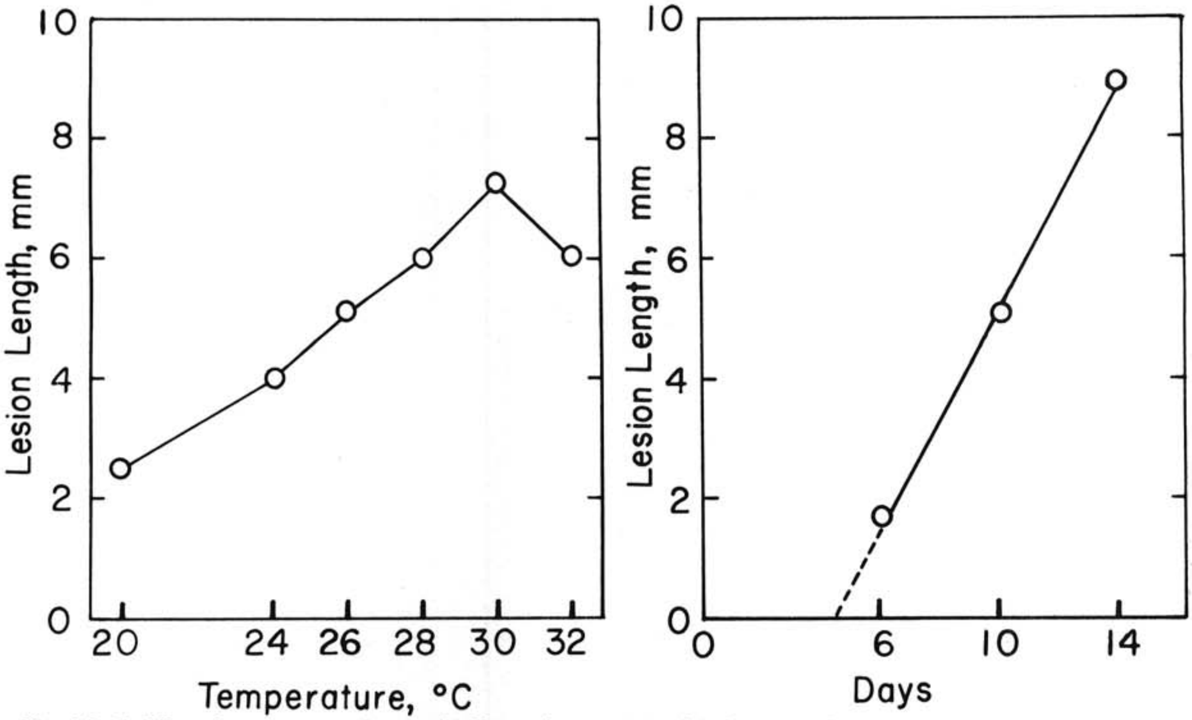

Fig. 1-2. 1) Effect of temperature on length of *Colletotrichum graminicola* lesions on the fourth leaves of corn plants inoculated in the four- to five-leaf stage. Data are means from lesions on 12 different corn genotypes 7 days after inoculation. **2)** Increase of lesion length on the fourth leaves of corn plants inoculated with *Colletotrichum graminicola* in the four- to five-leaf stage and grown at 20 C after inoculation. Data are means from lesions on 12 different corn genotypes.

14 days, and the lesion length for 7 days was calculated by interpolation. At higher temperatures, lesions were measured only at 7 days, because they elongated and coalesced rapidly. Individual lesions could not always be distinguished after more than 7 days.

Host development.—The effects of plant development on anthracnose infection were studied with a double cross hybrid derived from four partly inbred lines of the very early corn cultivar Gaspe Flint. Mature plants are only about 1 m tall and can be handled easily in the controlled environment rooms. Under the conditions of this study, Gaspe Flint plants shed pollen in 33 days and reached physiological maturity (distinguished by black layer formation in the grain) in 58 days. Mature plants had 10 or 11 leaf nodes with a single ear at the fifth leaf node.

Gaspe Flint plants were grown in the soil mix in 15-cm diameter plastic pots (one plant per pot). They were grown, before and after inoculation, in a controlled environment room at day/night temperatures of 30/26. Light was supplied for a 9-hour day period and a 3-hour dark interruption period as described above.

A set of pots was planted each week for 7 weeks; each set consisted of nine plants in 15-cm diameter pots except for the youngest set which had 16 plants in 10-cm

TABLE 2. Numbers of anthracnose lesions per square centimeter on leaves of Gaspe Flint corn plants that were 2 to 8 weeks old at inoculation

Leaf position[a]	Lesions/cm² on plants inoculated at indicated age (weeks)						
	2	3	4	5	6	7	8
3	0.45	1.48	...	...	...	...	...
4	0.38	0.53	0.70	0.08	0.21	...	...
5	0.20	0.46	0.51	0.08	0.29	0.42	...
6	0.04	0.39	0.26	0.04	0.25	0.14	0.15
7	...	0.17	0.16	0.02	0.11	0.08	0.24
8	...	0.13	0.13	0.01	0.08	0.09	0.19
9	...	0.04	0.11	0.00	0.03	0.10	0.19
10	...	0.00	0.18	0.00	0.04	0.08	0.43
11	...	...	0.04	0.01	0.04	0.55	0.62

[a]Leaves numbered in sequence from the lowest to the highest position on the plants.

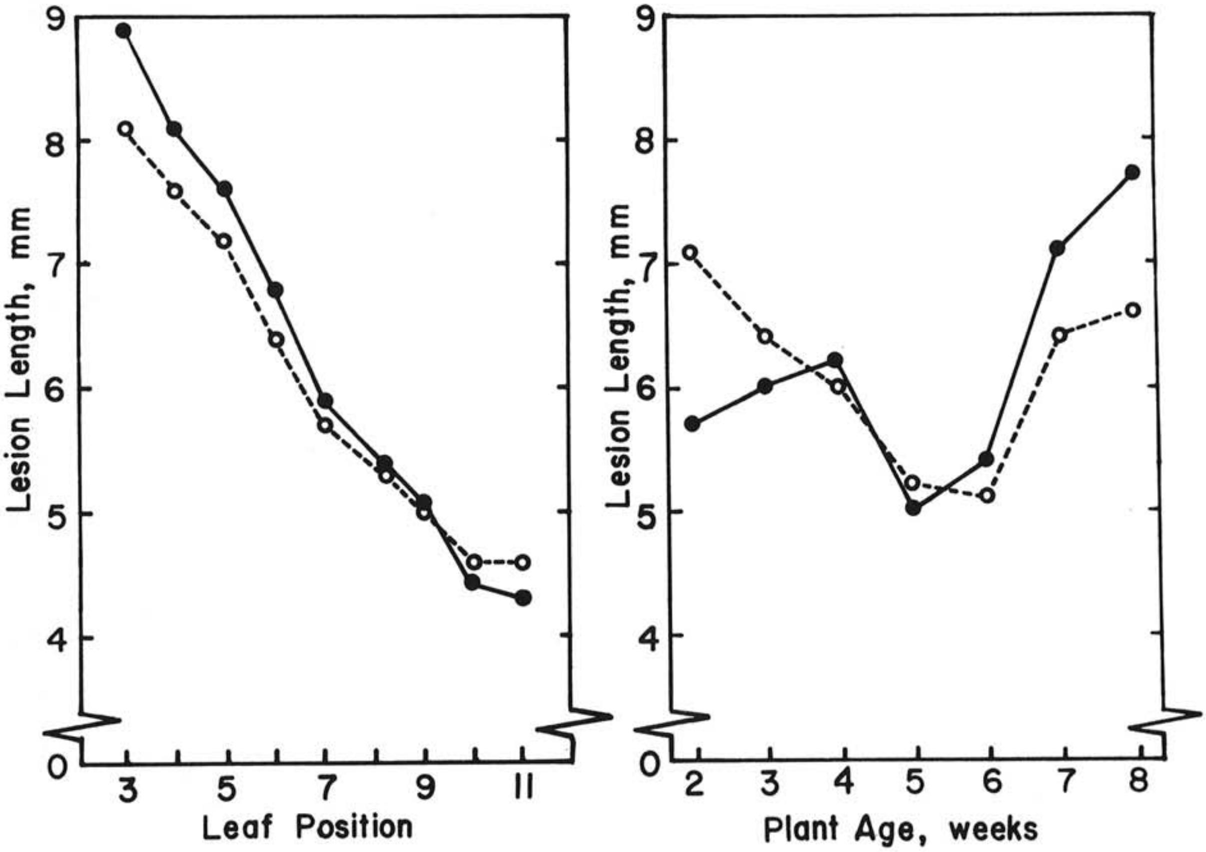

Fig. 3-4. 3) Effect of leaf position on length of *Colletotrichum graminicola* lesions on corn cultivar Gaspe Flint. Data are derived from plants inoculated 2, 3, 4, 5, 6, 7, and 8 weeks after planting. Solid lines represent data adjusted by multiple regression to remove plant age effects. Broken lines are for unadjusted means. **4)** Effect of plant age on length of *Colletotrichum graminicola* lesions on corn cultivar Gaspe Flint. Solid lines represent data adjusted to remove leaf position effects. Broken lines are for unadjusted means.

diameter pots. When the youngest plants were 2 weeks old, all of the plants of the seven sets were inoculated at the same time by spraying them with an aqueous suspension of 250,000 conidia/ml as described above. Inoculated plants were incubated in a dark moist chamber at 28 C for 16 hours, and then returned to the room in which they had been grown before inoculation.

The numbers of lesions that developed on each living leaf of each plant were counted, and the five largest lesions on each leaf were measured. It was not possible to measure all of the lesions in one day, so lesions were measured on six sets of plants 7 days after inoculation, and on three of these sets plus the three remaining sets 9 days after inoculation. These data were summarized to give an average lesion length for each leaf position for each plant age. Numbers of lesions per leaf were converted to lesions per square centimeter based on leaf area calculated as length × width × a correction factor of 0.702 as determined by Williams et al. (5).

RESULTS

Temperature.—Lesions of *C. graminicola* elongated most rapidly at 30 C (Fig. 1). At 7 days after inoculation, lesions at 30 C were more than three times longer than those at 20 C and nearly twice as long as those at 24 C. At 26 C and above, the lesions were first visible on the third day, but at 20 C they did not appear until 5 days after inoculation. Lesion elongation was linear with time at 20 C (Fig. 2). We did not measure lesions after 7 days at temperatures above 20 C.

One genotype, NC2A12, was highly resistant; the mean lesion length over all temperatures was 1.4 mm (Table 1). The most susceptible genotype was Pioneer Brand 3369A with a mean lesion length of 7.7 mm. This hybrid was chosen because it has been widely grown in North Carolina, and has been damaged by *C. graminicola* in commercial production plantings.

Corn genotypes differed in the effects of temperature on their responses to anthracnose infection. Regression coefficients for the effect of increasing temperature from 20 to 30 C on lesion length ranged from 0.15 for NC2A12 to 1.00 for SC276Q2 and NC2A7 (Table 1). The resistant NC2A12 had small lesions regardless of temperature. The most susceptible genotypes, Pioneer Brand 3369A and B37, had relatively low regression coefficients, which indicates that they tended to be susceptible at all temperatures. Pioneer Brand 3369A and B37 were also among the four genotypes with the greatest rates of lesion elongation at 20 C (Table 1).

Host development.—On Gaspe Flint plants inoculated at 2-8 weeks after planting, lesion length was affected by two factors. Analysis of variance showed that the effects of leaf position and plant age were significant ($P = 0.01$), but interaction of leaf position × plant age was not significant ($P = 0.05$). The relationship between lesion length and leaf position was nearly linear; the largest lesions developed on the lowest leaves (Fig. 3). The data in Fig. 3 were adjusted by multiple regression to show expected lesion lengths if leaves at all positions were of the same age. Lesion length generally increased with plant age at inoculation (Fig. 4). However, there was a distinct decrease in susceptibility at 5 weeks which was about the time of pollination. The data in Fig. 4 were adjusted by multiple regression to show expected lesion lengths if leaves of all ages occurred at the same position on the plants. Lesions on 2-week-old plants were larger than the adjusted data indicate because the lesions occurred mainly on the more susceptible lower leaves. Lesions on 7- and 8-week-old plants were slightly smaller than the adjusted data indicate because these plants lacked the most susceptible lower leaves.

Analysis of variance indicated significant (1% level) effects of leaf position, plant age, and leaf position × plant age interaction on numbers of lesions per square centimeter of leaf. In general, upper leaves tended to have fewer lesions per square centimeter, but on plants 7 or 8 weeks old at inoculation, the top one or two leaves were highly susceptible to infection (Table 2). During the first 4 weeks after planting, susceptibility to infection increased with age. However, plants inoculated at 5 weeks after planting (pollination stage) developed very few lesions. After the fifth week, susceptibility increased, especially on the top two leaves. We could not separate leaf position effects from plant age effects on lesions per square centimeter, because of the highly significant interaction between the two parameters.

DISCUSSION

Krüger (2) reported that the optimum temperature for growth on artificial medium by *C. graminicola* from corn in South Africa was 27 C and that growth at 31 C was only slightly less than optimal. Dale (1) obtained similiar results with an isolate of *C. graminicola* from corn in Arkansas. On artificial media, Dale's isolate grew best at 28 C and slightly slower at 32 C. The optimum temperature of 30 C for lesion elongation with our isolate suggests that it is similiar to those of Krüger (2) and Dale (1), and that the effect of temperature on growth of *C. graminicola* in the host is similiar to its effect on growth of the fungus on artificial media. Preliminary experiments with our isolate indicate that the optimum temperature for establishment of infection during incubation in the moist chamber is also 30 C (Naylor and Leonard, *unpublished*).

With cultivar Gaspe Flint corn plants, there were two periods of high susceptibility to anthracnose. Young plants developed many large lesions because the first leaves formed were highly susceptible to infection and lesion elongation. Plants nearing maturity developed many lesions on the top leaves because the top leaves became much more susceptible to infection as they aged. In addition, lesions on other leaves tended to be slightly longer on plants at physiological maturity than on plants of intermediate age. Plants of cultivar Gaspe Flint were most resistant at the time of pollination when both numbers of lesions formed and lesion elongation were minimal.

If commercial hybrids react as did cultivar Gaspe Flint, they should develop large lesions early in the summer on lower leaves of young plants, and smaller lesions on the top few leaves late in the summer as the plants begin to mature. This is the pattern that we see in North Carolina even though midsummer temperatures usually are nearer the optimum for lesion development than are early summer temperatures. Our results suggest that the seasonal occurrence of anthracnose symptoms in the field depends largely on changes in susceptibility as the corn plants develop.

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