The Issue of Races of *Colletotrichum graminicola* Pathogenic to Corn

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ABSTRACT

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Contrary to a report by others, seven isolates of *Colletotrichum graminicola* were not distinguished as physiological races. Lesion type and size were determined on corn inbreds Mo940 and 33-16 7 days after inoculation. The inbred Mo940 was susceptible to each isolate and the inbred 33-16 was hypersensitively resistant to each isolate. Thus, the seven isolates were not distinguished as races on these corn inbreds.

Additional key words: corn anthracnose, *Zea mays* L.

Forgey et al (1) reported eight physiological races of *Colletotrichum graminicola* (Ces.) Wils. pathogenic to corn (*Zea mays* L.). Each corn inbred they studied exhibited low infection characterized by chlorotic flecks with some isolates and high infection characterized by necrotic lesions with other isolates. Their report of race differential isolates of *C. graminicola* was inconsistent with our experience with the corn anthracnose disease (2,3,5,6,8–13). In particular, Forgey et al (1) reported races that would elicit low and high infection types on the corn inbreds Mo940 and 33-16. We have used these inbreds as archetypes of susceptibility and hypersensitive resistance (2,8) and have always found Mo940 to be susceptibile and 33-16 to be hypersensitively resistant to isolates of *C. graminicola* pathogenic to corn. This pattern has been consistently observed for 68 isolates of *C. graminicola* studied in our laboratory since 1972.

The inbreds Mo940 and 33-16 have also been used to demonstrate that the physiologic basis of resistance to *C. graminicola* is the accumulation of toxic phenols by the host (2,3). Thus, the report (1) of fungal isolates that would elicit a resistant rather than susceptible response from Mo940 and a susceptible rather than hypersensitively resistant response from 33-16 was of special interest to us. Such isolates would be important tools for studying the triggering of resistance in corn. However, this report describes our studies with seven of the 10 isolates of *C. graminicola* used by Forgey et al (1) and demonstrates that these isolates failed to elicit differential symptoms on the same corn inbred and that races of the fungus were not present in the isolates studied.

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MATERIALS AND METHODS

Isolates of *C. graminicola* used by Forgey et al (1) were obtained from them. Seven of the 10 isolates were successfully cultured. These were the isolates they designated as cultures 1, 2, 3, 4, 6, 7, and 9. Isolates 1 and 6 were reported to elicit necrotic lesions on Mo940 whereas isolates 2, 3, 4, 7, and 9 were reported to cause only chlorotic flecks on Mo940. The inbred 33-16 was reported to respond to isolates 1, 2, and 6 with necrotic lesions and to respond to isolates 3, 4, 7, and 9 with chlorotic flecks. An isolate from our collection designated as 105 was selected for comparison in inoculation trials.

Corn cultivars used for inoculation were the inbred lines Mo940 and 33-16. Several types of inoculation procedures were used in order to duplicate the methods used by Forgey et al (1) and to test their isolates under the standard conditions used in our laboratory (8,9). Procedures regularly used by us are described below.

Plants were grown in the greenhouse, as previously described (8), with supplemental lighting for a 12-hr photoperiod from two Sylvania Cool White FR96T12/SW/VHO/135 fluorescent tubes positioned 30 cm above plants. Plants were inoculated 14 days after planting when four leaves had opened from the whorl.

Inoculum was obtained from cultures of each isolate after 14-day growth on oatmeal agar under constant fluorescent light (3,500 lux) at 24 C (2). Spores were suspended in sterile distilled water and were filtered through cheesecloth. Spore suspensions were then adjusted to 5×10^5 spores per milliliter. One drop of Tween 20 per 100 ml of suspension was added as a wetting agent.

Seedlings were inoculated by spraying the inoculum onto leaves with an atomizer pressurized at 0.5 atmosphere. Plants were incubated for 18 hr in the dark at 100% relative humidity. Inoculum of each of the isolates was applied to 25 plants of each inbred, and the experiment was repeated three times.

After the 18-hr incubation, leaf surfaces were allowed to dry and the pots were randomly distributed on the greenhouse bench. Host reaction type (8) and lesion size were determined 7 days after inoculation. Lesion size was determined by photographing leaves illuminated from behind, preparing enlargements, and measuring the lesion area (mm^2) with a polar planimeter. The size of five lesions from the fourth leaf of each of four plants was determined for each inbred inoculated with each *C. graminicola* isolate. Results of lesion size determinations were statistically analyzed by Duncan's multiple range test.

Forgey et al (1) prepared inoculum by flooding cultures with sterile distilled water and bringing the spores into suspension by scraping the culture surface. They used this concentrated

Table 1. Anthracnose lesion type and size elicited by isolates of *Colletotrichum graminicola* on corn inbreds Mo940 and 33-16

C. graminicola isolate	Mo940		33-16	
	Lesion size[x] (mm^2)	Lesion type[y]	Lesion size (mm^2)	Lesion type
6	9.04 a	S	0.09 a[z]	HR
7	8.85 ab	S	0.11 a	HR
4	7.05 abc	S	0.08 a	HR
9	6.60 abc	S	0.10 a	HR
3	6.45 abc	S	0.10 a	HR
2	6.28 abc	S	0.11 a	HR
105	6.12 bc	S	0.12 a	HR
1	5.91 c	S	0.11 a	HR

[x] Values followed by the same letter in the column do not differ significantly according to Duncan's multiple range test ($P = 0.05$).

[y] S = susceptible lesion, HR = hypersensitively resistant lesion.

[z] Measurements were for the necrotic tissue in the HR lesion and did not include the zone of chlorosis.

spore suspension "without dilution" and inoculated by "placing approximately 1 ml of inoculum in the whorl of each plant." We duplicated this procedure with plants grown with and without supplemental lighting in the greenhouse.

Because light affects anthracnose disease development and lesion size (2,9,14), we also inoculated plants of each inbred with each isolate using high (37,600 lux) and low (9,600 lux) light intensities in growth chambers maintained at 22.5 C.

RESULTS

All methods of inoculation resulted in the symptom development previously reported by us (2,8). Minor differences in aggressiveness of the isolates were observed, but they had no influence on expression of host reaction type. Each *C. graminicola* isolate elicited the susceptible reaction on inbred Mo940 and the hypersensitively resistant reaction on inbred 33-16 (Table 1). Thus no differential symptom expression occurred. These symptoms are shown in Fig. 1.

When lesion sizes on inbred 33-16 were measured by polar planimetry, we found that no statistically significant differences could be distinguished on the basis of the isolate used as inoculum (Table 1). Some differences in mean lesion size caused by the different isolates were evident on the susceptible inbred Mo940. These differences may be associated with differences in aggressiveness of the isolates. As expected, the lesions on the hypersensitively resistant inbred 33-16 were significantly smaller than those on the susceptible inbred Mo940.

Use of the inoculation technique (1) wherein inoculum was pipetted into the whorl resulted in the development of extensive areas of tissue necrosis as a response to each isolate on both inbred lines. This necrosis was the result of lesion coalescence. However, lesions characteristic of the susceptible type and the hypersensitively resistant type developed where lesions were not coalesced. All isolates induced a susceptible response on inbred Mo940 and a hypersensitively resistant response on inbred 33-16. As expected, the extent of lesion development and tissue necrosis was greater for plants maintained without supplemental lighting than for those maintained with supplemental lighting, and supplemental lighting had no effect on host reaction in response to any of the *C. graminicola* isolates (2,9).

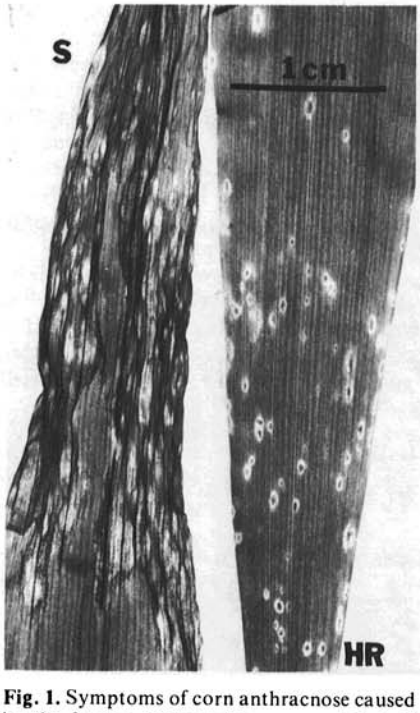

Fig. 1. Symptoms of corn anthracnose caused by the fungus *Colletotrichum graminicola* 7 days after inoculation. The leaf on the left (**S**) is from the susceptible inbred Mo940 and the leaf on the right (**HR**) is from the hypersensitively resistant inbred 33-16. The leaves were photographed with light shining through them in order to demonstrate the zone of chlorosis that surrounds necrotic tissue in the hypersensitively resistant lesion.

DISCUSSION

Forgey et al (1) characterized low infection as chlorotic flecks and high infection as necrotic lesions. They reported that different isolates of *C. graminicola* elicited the differential symptoms of chlorotic flecks or necrotic lesions on the same inbred line. Our results with seven of their isolates do not confirm their conclusions. Rather, we found that the isolates failed to show evidence of race differentiation. Although these isolates and others we have tested fail to exhibit characteristics of races, there is no reason to believe that races of *C. graminicola* would not occur in nature.

As we described previously (8), all lesions that develop on corn in response to *C. graminicola* are first observed as chlorotic flecks 24–48 hr after inoculation. Lesion formation is generally complete within 7 days. Lesions characteristic of the susceptible host response are oval, often exhibit concentric zones of lesion enlargement, and are a grey-green color on both leaf surfaces. The lesions on the hypersensitively resistant host typically do not enlarge beyond the size of the original chlorotic fleck. The lesion center forms a depressed area of necrotic tissue, and this is surrounded by a faint zone of chlorosis that is readily apparent when viewed by transmitted light (Fig. 1).

The rate of lesion development beyond the initial chlorotic fleck is largely a function of environment and host maturity. Leonard and Thompson (4) demonstrated that lesion elongation is affected by temperature and host maturity. Wheeler et al (14), Hammerschmidt and Nicholson (2), and Schall et al (9) demonstrated that growing inoculated plants in low light intensity increases disease severity. This occurs through an increase in lesion size (2). Conversely, high light intensity results in smaller lesions (2). It is important to note that although light affects lesion size, the type of host reaction does not change (2,9). Thus a susceptible, resistant, or hypersensitively resistant plant still exhibits the characteristic susceptible, resistant, or hypersensitively resistant lesion.

The extent of lesion development and tissue necrosis is also a function of the number of spores with which the plant is inoculated. Thus, when numerous lesions coalesce, extensive necrosis occurs. Moreover, necrosis would result regardless of whether the host response were one of susceptibility or hypersensitive resistance. Extensive tissue necrosis resulting from high spore concentrations has also been demonstrated for the hypersensitive response of apple leaves to *Venturia inaequalis* (7). Shahnaz and Nicholson (*unpublished data*) have calculated that a culture of a typical *C. graminicola* isolate grown in a 9-cm petri dish on oatmeal agar for 14 days in the light contains at least 600 million spores. Forgey et al (1) state that they used inoculum from a single culture plate without dilution and that the inoculum was not standardized because they "were interested in infection type and not the amount of infection." Our results demonstrated that inoculating into the whorl results in extensive tissue necrosis. This may explain the high infection type listed by Forgey et al (1) for the hypersensitively resistant inbred 33-16. We cannot explain their observations that the susceptible inbred Mo940 exhibited chlorotic flecks in response to some *C. graminicola* isolates and necrotic lesions in response to others.

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