Criteria for Evaluation of Resistance to Maize Anthracnose

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ABSTRACT

Criteria were determined for the evaluation of susceptible, resistant, and hypersensitively resistant leaf reactions to *Colletotrichum graminicola* based on characteristics of individual lesions under greenhouse conditions. These lesion types are defined. Neither lesion size nor the extent of leaf tissue affected was judged an adequate independent measure of host reaction.

Three isolates of *C. graminicola* of different geographic origin were tested against each of 183 corn inbreds. The results demonstrated that differential resistance and susceptibility to *C. graminicola* isolates is common for individual corn genotypes, and suggest the natural occurrence of physiologic races of the pathogen.

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The increased frequency and broad geographic distribution of maize anthracnose in the United States suggests that this disease has the potential of becoming a limiting factor to corn production (5, 6, 7, 8, 9, 13, 14, 15). The anthracnose pathogen, *Colletotrichum graminicola* (Ces.) Wils., causes, either singly or in a variety of combinations, leaf, stalk, ear, root, and kernel infections on individual plants (1, 3, 4, 10, 13, 15). In addition, considerable variability in the pathogenicity of isolates of *C. graminicola* is apparent with respect to infection of corn inbreds and hybrids, and host range within the cereals and grasses (2, 8, 10, 11, 14).

Previous surveys of corn (*Zea mays* L.) for leaf resistance to anthracnose have been based on greenhouse data for lesion size (12) or extent of tissue damage (9, 14). Our early greenhouse inoculations suggested, however, that neither lesion size, percentage of leaf area covered, nor time of symptom expression after inoculation, represented an adequate test of the host reaction. To date, no standards for host symptom expression have been outlined for consistent evaluation of anthracnose leaf lesions with respect to resistance and/or susceptibility. This report concerns the description of leaf symptoms of maize inbreds infected with *C. graminicola* and proposes criteria for evaluation of resistance based on lesion type. The results of greenhouse inoculations of corn inbreds with three isolates of *C. graminicola* of different geographic origin are presented.

MATERIALS AND METHODS.—Isolates of *C. graminicola* obtained from diseased corn leaves from Mississippi (isolate M), Indiana (isolate B) and North Carolina (isolate NC) were maintained on oatmeal agar. Spore suspensions were prepared by flooding culture plates with sterile distilled water, scraping the culture surface, filtering the suspensions through cheesecloth, and adjusting the volume to a concentration of 7.5×10^5 spores/ml. One drop of Tween-80 per 100 ml inoculum was added as a wetting agent.

Seedlings were grown in the greenhouse (14-hour daylength, 24-28 C) and inoculated 14-15 days after planting by spraying the inoculum onto leaves with an atomizer pressurized at approximately 0.5 atmosphere (7 psi). Corn inbreds were randomly distributed in inoculation chambers to insure uniform inoculum coverage of replicates for randomized results. After inoculation, plants were maintained in a moisture chamber for a maximum of 18 hours. Disease ratings for lesion reaction type and extent of leaf coverage were recorded at 2, 3, 6, 7, 8, 12, and 15 days after inoculation. Symptoms appearing on the primary (plumular) leaf were not considered.

RESULTS.—Lesions of susceptible, resistant and hypersensitively resistant reaction types first appeared as chlorotic flecks within 24-48 hours after inoculation. The subsequent rate of lesion development depended upon the host and/or host-isolate combination. Generally, symptom expression was complete within 7 days after inoculation. However, inbreds varied with respect to time of full symptom development, extent of tissue affected (lesion coverage) and type of symptom expressed. As expected, lesion size depended in part on the number of infection sites within a given leaf area; the greater the density, the smaller each individual lesion. However, even isolated lesions (those separated by healthy leaf tissue) exhibited size variability. Irrespective of lesion size, the symptom- or reaction-type was consistent for each host-isolate combination.

The susceptible lesion-type.—Lesions characteristic of susceptible host-pathogen combinations were typically a grey-green color on upper and lower leaf surfaces and exhibited concentric zones of enlargement. Irrespective of size, lesions were typically oval rather than circular or irregular in shape. The inbred Mo940 (Fig. 1, 2) was used as the standard for the susceptible lesion-type since it consistently exhibited a high degree of disease severity and the identical lesion type when inoculated with the M,

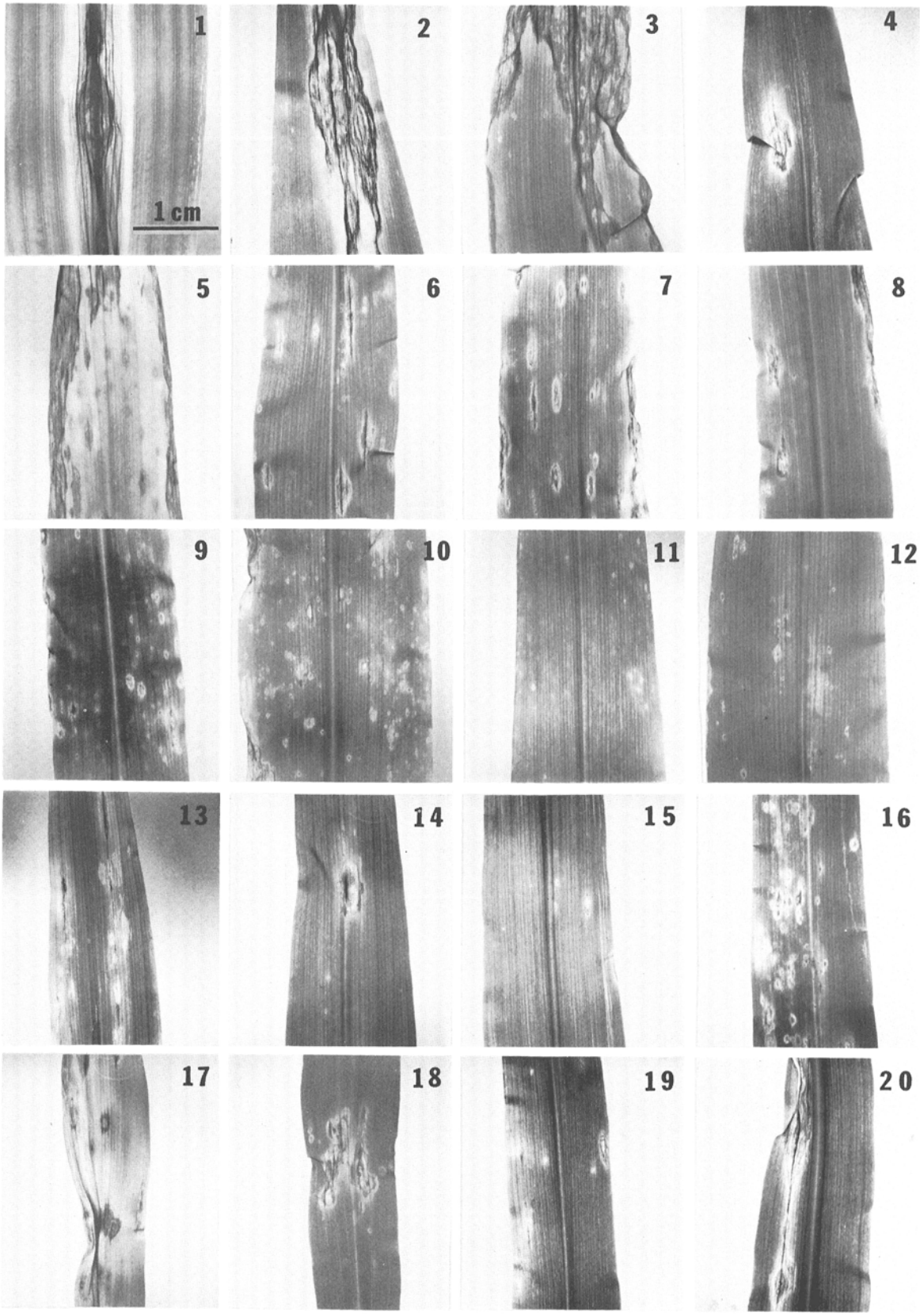

Fig. 1-20. Comparison of lesion types caused by isolates of *Colletotrichum graminicola* on corn inbreds under greenhouse conditions. Photographs taken 7 days after inoculation (21 days after planting). Inbreds in Fig. 1-12 were inoculated with isolate B of *C. graminicola*. **1** and **2**) Mo940, susceptible lesion type. **3**) Oh7b, susceptible lesion type. **4**) W37A, susceptible lesion type. **5**) I234, resistant lesion type. **6**) B37, resistant lesion type. **7**) H52, resistant lesion type. **8**) CI. 7, resistant lesion type. **9**) K159, resistant lesion type. **10**) Oh45b, resistant lesion type. **11**) CI82B, hypersensitive lesion type. **12**) 33-16, hypersensitive lesion type. **13**) CI85B inoculated with isolate M, susceptible lesion type. **14**) CI85B inoculated with isolate B, susceptible lesion type. **15**) CI85B inoculated with isolate NC, resistant lesion type. **16**) H22 inoculated with isolate M, resistant lesion type. **17**) H22 inoculated with isolate B, susceptible lesion type. **18**) H22 inoculated with isolate NC, susceptible lesion type. **19**) I159L1 inoculated with isolate B, resistant lesion type. **20**) I159L1 inoculated with isolate NC, susceptible lesion type.

TABLE 1. Comparison of corn inbreds for lesion type and disease severity to three different isolates of *Colletotrichum graminicola* under greenhouse conditions (14-hour daylength, 24-28 C)[a]

Corn inbred	Host response and disease severity per *C. graminicola* isolate[b]		
	M	B	NC
A	S,5.0[c]	S,3.0	S,4.5
Arg	R,1.0	R,1.0	R,0.5
A15	S,4.0	S,4.5	S,4.0
A71	S,3.0	S,2.0	S,4.0
A73	S,2.5	S,2.0	S,2.0
A239	S,2.5	S,2.5	S,2.5
A295	S,3.0	S,3.0	S,3.0
A298	S,1.5	S,1.0	S,2.0
A545	S,2.5	S,2.5	S,3.0
A554	S,2.0	S,3.5	S,3.5
A619	S,3.0	S,3.0	S,3.0
A632	R,1.5	R,1.5	R,1.5
B2	S,2.0	S,2.0	S,2.0
B8	S,3.5	S,3.0	S,3.0
B10	S,2.5	S,2.0	S,2.5
B14	S,4.0	S,2.0	S,2.0
B14A	S,4.0	S,5.0	S,5.0
B37 (Cornell)	R,1.5	R,2.0	R,2.0
B37	R,1.0	R,1.0	R,0.5
B38	S,2.0	S,2.0	S,1.0
B40	S,2.0	S,2.0	S,2.0
B41	R,2.0	R,2.0	R,2.0
B46	R,1.5	R,1.5	R,1.0
C103	S,3.5	S,3.5	S,3.0
C123	R,1.5	R,2.0	R,2.0
C153	R,1.0	R,1.5	R,0.5
CH3	S,4.5	S,4.5	S,4.5
CI. 7	R,1.5	R,1.5	R,1.5
CI. 15	S,5.0	S,3.0	S,3.5
CI. 28A	S,2.0	S,1.5	S,2.0
CI. 38B	R,1.5	R,1.5	R,1.0
CI. 44	S,5.0	S,3.5	S,3.5
CI. 64N	R,2.5	R,3.0	R,2.0
CI. 66	S,4.5	S,3.5	S,5.0
CI. 74	R,3.0	R,3.0	R,1.0
CI. 82B	H,1.0	H,1.0	H,1.0
CI. 84A	S,2.0	S,2.5	S,2.0
CI. 90A	R,1.0	R,1.5	R,0.5
CI. 91B	S,3.0	S,1.5	S,2.5
CZ4	R,1.5	R,2.0	R,0.5
G8	S,2.5	S,4.0	S,2.5
GE440	S,4.0	S,3.0	S,3.5
H5	S,1.5	S,1.5	S,1.5
H14	R,1.5	R,0.5	R,1.5
H21	S,3.5	S,3.5	S,3.0
H23	S,1.5	S,1.5	S,1.5
H25	S,4.5	S,3.5	S,3.5
H49	S,4.0	S,3.5	S,4.5
H50	S,1.0	S,1.0	S,1.0
H51	R,1.5	R,1.5	R,1.5
H52	R,0.5	R,0.5	R,0.5
H53	S,4.0	S,4.0	S,4.0
H54	S,2.5	S,1.5	S,2.5
H55	S,5.0	S,3.0	S,4.5
H56	R,1.5	R,1.5	R,1.0
H58	S,1.5	S,1.5	S,2.0
H59	S,4.0	S,4.5	S,4.0
H60	S,2.0	S,2.5	S,2.5
H61	S,3.5	S,1.5	S,3.5
H65	S,1.0	S,1.0	S,1.0
H71	R,3.0	R,1.0	R,3.0

Table 1. (continued)

Corn inbred	Host response and disease severity per *C. graminicola* isolate[b]		
	M	B	NC
H72	S,2.0	S,1.0	S,3.0
H73	R,3.0	R,3.0	R,3.0
H74	S,2.0	S,2.0	S,1.5
H84	R,2.0	R,2.0	R,1.5
H87	S,4.5	S,2.5	S,3.5
H88	R,2.0	R,2.0	R,2.0
H89	S,1.5	S,2.0	S,1.5
H90	R,2.5	R,0.5	R,1.0
H91	R,2.0	R,1.0	R,1.0
H92	R,2.0	R,0.5	R,2.0
H93	R,3.0	R,3.0	R,2.5
H95	S,2.0	S,2.0	S,1.0
HY	R,2.5	R,2.5	R,2.5
Il53	S,2.5	S,1.0	S,2.5
I234	R,1.5	R,1.0	R,1.0
K4	S,2.5	S,2.0	S,2.5
K6	S,2.0	S,2.0	S,1.5
K41	R,3.0	R,2.0	R,3.0
K44	S,2.0	S,2.0	S,1.5
K61	S,2.0	S,1.0	S,1.5
K61-1	R,1.5	R,1.0	R,1.5
K61-2	S,2.0	S,1.5	S,1.0
K64	R,3.5	R,3.0	R,3.5
K148	R,2.0	R,2.5	R,2.5
K159	R,0.5	R,0.5	R,0.5
K166	S,2.5	S,2.0	S,3.0
K175	S,4.0	S,4.5	S,4.5
K237	S,2.5	S,2.5	S,3.0
Ky27	S,3.5	S,2.0	S,3.5
Ky201	S,1.5	S,2.0	S,2.5
Ky207	S,2.0	S,2.0	S,1.5
Ky36-11	S,2.5	S,2.5	S,5.0
Ky S	S,2.0	S,2.0	S,2.0
L	S,2.5	S,2.5	S,2.5
LF	S,2.0	S,1.5	S,2.0
L97	S,2.5	S,2.0	S,2.0
L289	R,1.0	R,1.0	R,1.0
L304A	R,1.5	R,1.0	R,1.0
L317	S,1.5	S,2.0	S,1.5
M431	S,2.0	S,2.0	S,2.0
Mo5	S,2.5	S,2.5	S,3.0
Mo17	S,3.5	S,3.5	S,3.5
Mo21A	S,1.5	S,1.5	S,1.5
Mo940	S,5.0	S,5.0	S,4.5
MoG	S,3.0	S,3.5	S,3.5
MS116	S,3.5	S,3.5	S,3.5
MS128	S,3.5	S,3.5	S,3.0
MS206	S,2.0	S,3.5	S,3.0
MS1334	S,3.0	S,3.5	S,3.0
N6	S,2.0	S,2.0	S,2.0
N25	S,3.0	S,3.0	S,2.0
N28	S,2.5	S,2.0	S,2.5
NC34	S,3.0	S,3.0	S,3.0
NC37	S,2.0	S,2.5	S,2.0
NYF	R,2.0	R,1.5	R,1.0
Oh4C	R,0.5	R,0.5	R,0.5
Oh07	S,4.0	S,3.0	S,4.0
Oh7b	S,3.5	S,4.0	S,4.0
Oh28	S,3.5	S,3.0	S,3.0
Oh40b	S,2.5	S,2.0	S,2.0
Oh43	R,2.0	R,2.0	R,2.0
Oh45	S,2.0	S,2.5	S,2.0
Oh45b	R,1.5	R,2.0	R,1.5
Oh51	S,2.5	S,2.5	S,2.0

(continued)

Table 1. (continued)

Corn inbred	Host response and disease severity per *C. graminicola* isolate[b]		
	M	B	NC
Oh51A	S,2.5	S,2.5	S,2.5
Oh514	S,2.0	S,2.0	S,2.0
Oh545	S,3.0	S,3.0	S,3.0
Os420	S,3.0	S,3.0	S,3.0
Os426	S,2.0	S,4.0	S,2.5
P8	S,2.0	S,3.0	S,3.0
P39A	S,2.5	S,4.0	S,2.5
P39-5	S,1.5	S,2.5	S,2.0
P51B	S,3.5	S,3.5	S,3.0
Pr	R,0.5	R,0.5	R,0.5
Pr1	R,0.5	R,0.5	R,0.5
R4	R,1.5	R,2.0	R,1.5
R30	S,2.5	S,2.5	S,2.5
R39	R,1.0	R,0.5	R,0.5
R53	S,2.5	S,2.5	S,2.5
R61	R,1.5	R,1.5	R,1.0
R71	S,2.5	S,3.0	S,2.5
R109B	R,2.5	R,2.0	R,2.5
SynB254	S,1.5	S,1.5	S,1.5
SynC	S,1.5	S,2.0	S,1.0
SynD304-11	S,3.5	S,4.5	S,3.5
Tr	S,2.5	S,3.0	S,2.5
Va35	S,3.0	S,3.0	S,3.0
W20	R,2.5	R,2.5	R,2.0
W22	S,3.0	S,2.5	S,2.0
W23	S,2.0	S,2.0	S,2.0
W26	S,3.0	S,2.5	S,2.5
W32	S,3.0	S,3.0	S,3.0
W64A	S,3.0	S,3.0	S,3.0
W182B	S,2.5	S,2.5	S,2.5
W182D	S,3.0	S,2.5	S,2.5
WF9	R,1.0	R,2.0	R,2.0
4Co82	R,1.0	R,1.0	R,1.0
33-16	H,0.5	H,0.5	H,0.5
38-11	H,1.0	H,0.5	H,0.5
81-1	S,3.5	S,4.0	S,4.0
90	R,1.5	R,1.5	R,0.5
106	R,1.0	R,1.0	R,1.5
166	S,3.0	S,3.5	S,3.0
187-2	R,2.5	R,1.5	R,2.0
245	S,3.5	S,4.0	S,3.0
461-3	S,3.5	S,4.0	S,2.5
540	S,3.5	S,4.0	S,3.0

[a]Plants were inoculated 14-15 days after planting and maintained in a moisture chamber for up to 18 hours.

[b]*Colletotrichum graminicola* isolates were from Mississippi (M), Indiana (B) and North Carolina (NC). Spore suspensions for inoculations were 7.5×10^5 spores/ml for each isolate.

[c]Data represent evaluations of host reaction type and disease severity 7 days after inoculation. Individual lesion types were classified as susceptible (S), resistant (R) and hypersensitively resistant (H). Disease severity was recorded on a 0.0-5.0 scale with 5.0 representing 100% coverage and plant death.

B, and NC isolates. Figures 3, 13, 14, 17, 18, and 20 demonstrate similar susceptible lesions. Some chlorosis surrounded lesion margins in most host genotypes. Those inbreds for which extensive marginal chlorosis was a lesion characteristic did not exhibit the typical grey-green color except at early stages of lesion development. In such cases, no inhibition of lesion enlargement was observed, and lesions tended to be irregular in shape. The response of Inbred W37A is an example of this susceptible reaction type (Fig. 4).

Resistant and hypersensitively resistant lesion types.—A variety of resistant lesion types was observed (Fig. 5-12). However, lesions typically had tan-to-brown centers, were often surrounded either by a chlorotic or bright yellow-orange zone of discoloration, and were circular to irregular in shape. Lesions designated characteristic of hypersensitive resistance typically did not enlarge beyond the size of the original chlorotic fleck (Fig. 11, 12). These lesions eventually became depressed areas of necrotic tissue and were not surrounded by areas of discoloration.

Table 1 presents a summary of greenhouse ratings for anthracnose based on lesion types. The extent of tissue coverage by lesions (disease severity) is presented on a 0 - 5 rating scale with a rating of 5 representing 100% coverage and death of leaf tissue. Fifteen seedlings (five per 15-cm diameter pot) were inoculated with each isolate of the pathogen for each experimental replicate. The experiment was repeated twice for susceptible lines and a minimum of three times for lines exhibiting resistance. The susceptible inbred Mo940 was included in each trial. Host reaction type to a given *C. graminicola* isolate did not vary within or between replications. Variation in disease severity occurred by a maximum factor of one indexing unit, except for inbreds exhibiting hypersensitive resistance where no variation was observed.

Differential resistance and susceptibility to Colletotrichum graminicola.—The majority of corn

TABLE 2. Differential resistance and susceptibility of corn inbreds to three isolates of *Colletotrichum graminicola* under greenhouse conditions (14-hour daylength, 24-28 C)[a]

Corn inbred	Host response and disease severity per *C. graminicola* isolate[b]		
	M	B	NC
A297	S,3.5[c]	R,2.0	S,3.5
CI85B	S,4.0	S,3.0	R,3.5
H22	R,3.0	S,3.0	S,3.5
H42	S,3.0	S,3.0	R,2.0
H57	S,3.5	R,2.5	R,3.0
H75	S,3.5	R,2.5	S,3.5
H94	S,3.5	S,3.0	R,3.0
H96	S,3.0	R,1.0	S,3.0
I159L1	S,3.0	R,2.5	S,2.0
K63	S,2.0	S,3.0	R,1.5
Ky126	S,2.5	R,1.0	S,1.0
M14	S,2.0	S,1.0	R,1.5
P14	S,2.5	R,2.0	R,2.5
SynA	S,1.5	S,0.5	R,0.5
W37A	R,1.5	S,2.5	R,1.0

[a]Plants were inoculated 14-15 days after planting and maintained in a moisture chamber for up to 18 hours.

[b]*Colletotrichum graminicola* isolates were from Mississippi (M), Indiana (B) and North Carolina (NC). Spore suspensions for inoculations were 7.5×10^5 spores/ml for each isolate.

[c]Data represent evaluations of host reaction type and disease severity made at 7 days after inoculation. Lesion types were classified as susceptible (S), resistant (R) and hypersensitively resistant (H). Disease severity was recorded on a 0.0-5.0 scale with 5.0 representing 100% coverage and plant death.

genotypes exhibited consistent resistance or susceptibility to each of the *C. graminicola* isolates tested (Table 1). However, some genotypes exhibited differential resistance and susceptibility to the *C. graminicola* isolates (Table 2). Figures 13 to 20 show differential responses of the inbreds CI85B, H22, and I159L1. Hypersensitive resistance was not observed in any differential host responses.

DISCUSSION.—Three isolates of *C. graminicola*, differing in geographic origin, elicited a variety of symptoms on leaves of the corn inbreds tested. Neither lesion size nor extent of tissue coverage was an adequate measure of resistance to anthracnose under greenhouse conditions. However, the *type* of lesion formed was found to fit a pattern for susceptible, resistant and hypersensitively resistant host responses.

A susceptible lesion was typically characterized by a grey-green color on upper and lower leaf surfaces, oval shape, and the presence of concentric zones of lesion enlargement. Some host lines tended to exhibit areas of chlorosis surrounding lesions and often did not retain the typical grey-green lesion color. Resistant lesion types were circular to irregular, with tan to brown centers and were often surrounded by a zone of chlorotic to bright yellow-orange discoloration. Lesions characteristic of hypersensitive host resistance rarely enlarged beyond the chlorotic fleck stage. Necrosis of the fleck areas occurred eventually, especially where a number of lesions were in close proximity.

The host genotype apparently had little effect on the type of lesion developed in compatible host-pathogen combinations. However, the host genotype appeared to affect disease severity as indicated by variation in the extent of lesion coverage in hosts with susceptible lesion types (Table 1). The variety of lesion types observed in resistant host-pathogen combinations suggest that symptoms were more dependent on host genotypes than on susceptible host-pathogen combinations.

The differential response of some host inbreds to the *C. graminicola* isolates (Table 2) suggests physiological race differences among the isolates. Confirmation of isolates as races, however, requires study of both host and pathogen with a defined set of differential host cultivars.

This study describes what we consider to be characteristic symptoms of susceptible, resistant, and hypersensitively resistant corn seedlings with *C. graminicola* in the greenhouse. Although greenhouse data can not be assumed to correspond with field inoculation data, the results should offer a basis for initial genotype screening in breeding programs for anthracnose resistance.

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