Physiological Races of *Colletotrichum graminicola* on Sorghum

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ABSTRACT

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Nine sorghum isolates of *Colletotrichum graminicola* from different geographical areas were tested for pathogenicity on six sorghum lines in the greenhouse and at the Purdue Agronomy Farm. Sorghum lines were evaluated for reaction type and disease severity. Similar results were obtained from both field and greenhouse studies, except leaf anthracnose severity was higher in the field than in the greenhouse. Sorghum lines IS4225 and IS8361 were susceptible, whereas Br64 and 954206 were resistant to all isolates. Based on the differential responses of sorghum lines 954130 and 954062, the isolates were grouped into three physiological races designated 1, 2, and 3. These findings suggest that a resistant sorghum cultivar in one region may succumb to leaf anthracnose in another region because of the prevalence of a different virulent race.

Additional key words: anthracnose, *Sorghum bicolor*

Sorghum anthracnose, caused by *Colletotrichum graminicola* (Ces.) Wils., infects leaves, stalks, peduncles, and heads (12,15). In the United States, the disease was first reported in Texas in 1911, although it had previously been known for some time in India (15). Since then, sorghum anthracnose has been reported in most areas where sorghum is grown, but it is more prevalent and important in warm, humid regions of the world (1,13–15). Anthracnose limits grain sorghum production in many areas of the world, with losses estimated to exceed 50% on susceptible cultivars under severe epidemics (7,8).

Harris and Johnson (6) have previously suggested the existence of races of *C. graminicola*, and some sorghum cultivars are reported to react differently to anthracnose in Texas, Mississippi, and Georgia (5) as well as in different countries (10).

Although the previous reports (5,6,10) have suggested the existence of races of this pathogen, no thorough study has been done to identify races. Therefore, the following study was undertaken to determine whether races of *C. graminicola* pathogenic to sorghum exist.

MATERIALS AND METHODS

Nine sorghum isolates of *C. graminicola* were obtained from four geographical areas (Table 1). Single-conidial cultures of the isolates were prepared and maintained on oatmeal agar under constant, cool-white fluorescent light (3,600 lux) at 23 ± 1 C (3).

The isolates were examined for pathogenicity on six sorghum (*Sorghum bicolor* (L.) Moench) lines (IS4225, 954130, Br64, IS8361, 954062, and 954206) from the Purdue Sorghum Collection under both greenhouse and field conditions. Br64 is a hybrid, whereas the other sorghum lines are inbreds. These sorghum lines were selected as a set of differentials because of their varying levels of resistance to a mixture of three isolates of *C. graminicola* (3).

We prepared conidial suspensions as inoculum by flooding 7-day-old cultures of each isolate of *C. graminicola* with distilled water, dislodging the conidia with a rubber spatula, and straining the suspension through two layers of cheesecloth to separate hyphae. One drop of Tween 80 (polyoxyethylene sorbitan monolaurate) per 100 ml of conidial suspension was added as a wetting agent.

Greenhouse study. Fifteen seeds of each of the six sorghum lines were planted in one of the six rows arranged in each rectangular wooden frame 55 cm long and 35 cm wide. The six sorghum lines were planted in a split-plot arrangement of a randomized complete block design, with the isolates randomized as whole plots and sorghum lines randomized as subplots. Ten days after planting, the seedlings were thinned to eight plants per row. The plants were grown in the greenhouse at 26 ± 2 C. Supplemental lighting for a 14-hr photoperiod was provided by cool-white fluorescent tubes positioned 30 cm above the plants. The experiment was conducted twice, and the treatments were replicated three times in each experiment. Plants of

Table 1. Sources of isolates of *Colletotrichum graminicola*

Isolate	Location	Source
2768	Florida	E. Johnson, Funk Seeds, Bloomington, IL
3230	Georgia	E. Johnson, Funk Seeds, Bloomington, IL
486	Georgia	E. Johnson, Funk Seeds, Bloomington, IL
74-1395	Georgia	D. R. Smith, Dekalb Ag Research, IL
CG-24	Georgia	R. A. Frederiksen, Texas A&M University
CG-1A	Puerto Rico	R. A. Frederiksen, Texas A&M University
CG-1B	Puerto Rico	R. A. Frederiksen, Texas A&M University
CG-1C	Puerto Rico	R. A. Frederiksen, Texas A&M University
CGS-20	Indiana	H. L. Warren, Purdue University

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the six sorghum lines in each wooden frame were inoculated with one isolate to avoid contamination. The inoculum for each isolate was adjusted to 7×10^5 and 1×10^6 conidia per milliliter in the first and second experiment, respectively. Inoculum was applied on the leaf surfaces of 35-day-old plants with a DeVilbiss atomizer pressurized at 0.5 atmosphere. Plants were incubated for 18 hr in the dark at 100% relative humidity. The wooden frames were then randomly distributed on the greenhouse bench.

All plants of each of the six sorghum lines were evaluated for reaction type and disease severity on leaves 12 days after inoculation. Disease severity (percentage of leaf area covered with lesions) was estimated with an index scale of 1–5 for the leaves that were fully exposed to the inoculum as established by Ferreira and Warren (3).

Field study. The six sorghum lines used in greenhouse experiments were planted in a split-plot arrangement of a randomized complete block design, with the isolates randomized as whole plots and sorghum lines randomized as subplots. This design was chosen to avoid contamination from the different isolates during inoculation. The isolate treatments were separated by four rows of the resistant sorghum hybrid Br64 to prevent interplot contamination. Field rows were 0.75 m apart and 5.2 m long, with plants spaced 5–10 cm apart. To detect external inoculum and interplot contamination, a control treatment was included in which the plants were sprayed only with water. The experiment was conducted in 1983 and 1984 at the Purdue Agronomy Farm, and the treatments were replicated three times each year.

Inoculum was prepared as previously described for the greenhouse experiments. About 5 ml of conidial suspension was applied into the plant whorl with a pressurized sprayer. In both years, plants were inoculated late in the afternoon to minimize rapid drying of leaves by high temperatures and to ensure infection. In 1983, the plants were inoculated 55 and 62 days after planting, with the inoculum concentrations for each isolate adjusted to 5×10^6 and 3×10^6 conidia per milliliter, respectively. Plants of the six sorghum lines were evaluated as whole plots for reaction type and disease severity on leaves 28, 46, and 53 days after the initial inoculation. In 1984, the plants were inoculated 64 and 71 days after planting, with inoculum concentrations adjusted to 1×10^6 and 2×10^6 conidia per milliliter, respectively. Plants of the six sorghum lines were evaluated as whole plots for reaction type and disease severity on leaves 23, 37, and 51 days after the initial inoculation. The scale of 1–5 for estimating leaf anthracnose severity was the same as that used by Ferreira and Warren (3). Disease severity ratings presented in Table 2 were the mean of the initial ratings in 1983 and 1984.

RESULTS

Interactions among the various pathogen-host combinations were characterized by either a susceptible or a hypersensitively resistant reaction (Fig. 1). Lesions characteristic of susceptible host-pathogen combinations were circular-elliptical spots up to 5 mm in diameter. Lesions appeared on both upper and lower leaf surfaces. The lesions varied from tan to orange-red to black depending on the sorghum genotype. Black specks, which are part of the acervulus, were observed at the centers of lesions. The hypersensitively resistant reaction was characterized by small necrotic spots, and the fungus failed to sporulate in this type of lesion both in the greenhouse and field.

In the field, no disease symptoms were observed in the plots sprayed with water. This indicated that there was no interference from either external inoculum or interplot contamination.

The nine isolates of *C. graminicola* varied in their pathogenic capabilities on the six sorghum lines (Tables 2 and 3). Sorghum lines Br64 and 954206 were hypersensitively resistant to all isolates, whereas IS4225 and IS8361 were

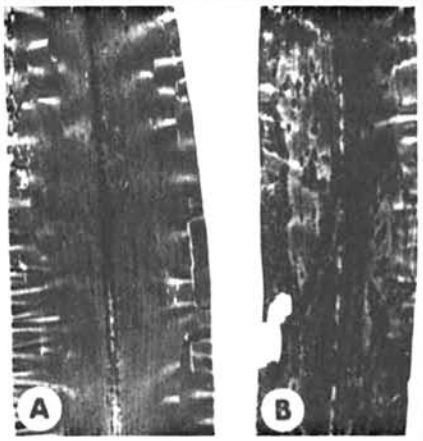

Fig. 1. Symptoms of sorghum leaf anthracnose: **(A)** hypersensitive, resistant reaction of sorghum and **(B)** susceptible reaction.

Table 2. Reaction types and disease severity ratings of six sorghum lines to nine isolates of *Colletotrichum graminicola* in the field

	Race 1					Race 2			Race 3
Sorghum line	Florida 2768	Georgia 486	Georgia 74-1395	Georgia CG-24	Indiana CGS-20	Georgia 3230	Puerto Rico CG-1B	Puerto Rico CG-1C	Puerto Rico CG-1A
IS4225	S[a] (2.2)[b]	S (2.3)	S (2.2)	S (2.0)	S (2.1)	S (3.3)	S (2.7)	S (3.2)	S (3.3)
IS8361	S (2.8)	S (2.8)	S (2.0)	S (2.8)	S (2.2)	S (4.5)	S (4.0)	S (4.2)	S (3.0)
954130	HR (1.5)	HR (1.3)	HR (1.5)	HR (1.2)	HR (1.5)	S (4.0)	S (3.2)	S (4.1)	HR (1.7)
954062	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	S (2.3)
Br64	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)
954206	HR (1.5)	HR (1.3)	HR (1.5)	HR (1.3)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)

[a] Reaction types: HR = hypersensitive type resistance and S = susceptible.
[b] Numbers in parentheses indicate disease severity rating based on a scale of 1–5, where 1 = lesions absent and 5 = lesions covering 100% of leaf area.

Table 3. Reaction types and disease severity ratings of six sorghum lines to nine isolates of *Colletotrichum graminicola* in the greenhouse

	Race 1					Race 2			Race 3
Sorghum line	Florida 2768	Georgia 486	Georgia 74-1395	Georgia CG-24	Indiana CGS-20	Georgia 3230	Puerto Rico CG-1B	Puerto Rico CG-1C	Puerto Rico CG-1A
IS4225	S[a] (2.0)[b]	S (2.0)	S (2.0)	S (2.0)	S (2.0)	S (2.3)	S (2.3)	S (2.3)	S (2.0)
IS8361	S (2.5)	S (2.3)	S (2.0)	S (2.0)	S (2.2)	S (2.7)	S (2.2)	S (2.5)	S (2.0)
954130	HR (1.5)	HR (1.5)	HR (1.3)	HR (1.5)	HR (1.5)	S (3.7)	S (2.8)	S (3.2)	HR (1.5)
954062	HR (1.5)	HR (1.5)	HR (1.3)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	S (2.3)
Br64	HR (1.5)	HR (1.5)	HR (1.3)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)
954206	HR (1.3)	HR (1.3)	HR (1.3)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)	HR (1.5)

[a] Reaction types: HR = hypersensitive type resistance and S = susceptible.
[b] Numbers in parentheses indicate disease severity rating based on a scale of 1–5, where 1 = lesions absent and 5 = lesions covering 100% of leaf area.

susceptible to all isolates. Based on the differential reactions of sorghum lines 954130 and 954062, the nine isolates of *C. graminicola* were grouped into three physiological races designated 1, 2, and 3. Race 1 elicited a resistant reaction on both 954062 and 954130, race 2 elicited a resistant reaction on 954062 and a susceptible reaction on 954130, and race 3 elicited a resistant reaction on 954130 and a susceptible reaction on 954062. Although the reaction types (susceptibility or hypersensitive resistance) exhibited by the six sorghum lines to *C. graminicola* isolates were similar in both field and greenhouse, disease severity was more intense in the field.

DISCUSSION

Based on the reaction type elicited by the isolates of *C. graminicola* on leaves of sorghum lines 954130 and 954062, the isolates were grouped into three physiological races (1, 2, and 3). These findings are supported by previous observations suggesting the existence of races of *C. graminicola* pathogenic to sorghum (5,6,10). Forgey et al (4) reported eight physiological races from 10 isolates of *C. graminicola* pathogenic to corn. In a separate study, seven of their 10 isolates were not distinguished as physiological races (11). Regardless of this controversy, it is reasonable to believe that races of *C. graminicola* could occur in nature (11). Previous reports indicated that corn isolates of *C. graminicola* were pathogenic to corn but not to sorghum and that isolates from sorghum did not infect corn (2,9).

The association of *C. graminicola* with sorghum for a long time may explain the appearance of races of this pathogen on sorghum. The reaction types (susceptibility or hypersensitive resistance) exhibited by sorghum lines to the isolates of *C. graminicola* used in these studies were similar under both field and greenhouse conditions. The difference in plant age at the time of inoculation and secondary infection under field conditions may account for higher disease severity observed in the field than in the greenhouse. Similar results were reported previously (3).

These findings demonstrate the presence of physiological races within populations of *C. graminicola* pathogenic to sorghum. Additional research, with a broader spectrum of sorghum genotypes and pathogen isolates from wide geographical regions, will provide data needed to formulate a nomenclatural scheme suited to the needs of sorghum pathologists and breeders. There is an urgent need for studies on the inheritance of resistance so that near-isogenic lines can be developed to serve as differentials for race identification.

These results suggest that sorghum cultivars should be thoroughly screened against all possible races prevalent in an area before being released for large-scale production.

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