Resistance of Sorghum to *Colletotrichum graminicola*

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ABSTRACT

Ferreira, A. S., and Warren, H. L. 1982. Resistance of sorghum to *Colletotrichum graminicola*. Plant Disease 66:773-775.

Twenty-three sorghum (*Sorghum bicolor*) cultivars were evaluated in the greenhouse and at the Purdue Agronomy Farm for resistance to *Colletotrichum graminicola*. Seedling blight reactions in the greenhouse were significantly correlated with leaf blight reactions of adult plants in the field ($r = 0.87^{**}$, $P < 0.01$), although leaf anthracnose severity was usually higher in the field than in the greenhouse. None of the sorghum seedlings inoculated with *C. graminicola* 15 days after planting produced anthracnose symptoms; however, seedlings inoculated 25 or 35 days after planting in the greenhouse produced typical anthracnose lesions. Susceptibility or resistance to *C. graminicola* could be determined 25–35 days after planting at the four- to seven-leaf stages. The fungus sporulated in lesions on cultivars susceptible and resistant to *C. graminicola* but not in lesions on cultivars that were hypersensitive-resistant.

Anthracnose caused by *Colletotrichum graminicola* (Ces.) G. W. Wils. often damages sweet and grain sorghums (*Sorghum bicolor* (L.) Moench). The leaf blight, stalk rot, and head blight phases of *C. graminicola* can limit sorghum production in most arid regions (1,5,9,12), with grain yield reduced 50% or more on cultivars susceptible to *C. graminicola* under severe epidemics (3).

Sorghum cultivars have been evaluated for resistance to *C. graminicola* in the greenhouse and field (2,4–7); however, we found disease severity was generally less in the greenhouse than in the field. Adequate foliar anthracnose symptoms for evaluation of sorghum germ plasm for resistance to *C. graminicola* did not develop on young seedlings inoculated in the greenhouse. Although 2-wk-old sorghum seedlings inoculated with *C. graminicola* in the greenhouse had disease reaction similar to that of the resistant check, Harris and Sowell (5) found that cultivars highly susceptible to the pathogen inoculated in the greenhouse 15 days after planting could be identified but that field tests apparently were needed for further evaluation.

This study was conducted to evaluate sorghum cultivars at different stages of growth in the greenhouse for resistance to *C. graminicola*, to compare disease reaction on greenhouse- and field-inoculated plants, and to identify cultivars that have potential value in breeding programs. The effects of plant age on lesion development and period of incubation on sporulation of *C. graminicola* are also presented.

Contribution of Agricultural Research Service, U.S. Department of Agriculture, in cooperation with Purdue Agricultural Experiment Station, West Lafayette, IN.

Purdue Experiment Station Journal Series Paper 8463.

Accepted for publication 19 December 1981.

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MATERIALS AND METHODS

Laboratory procedures. Single-conidial isolates of *C. graminicola* obtained from naturally infected sorghum leaves from Texas, Mississippi, and Indiana were used in greenhouse and field studies. Pieces of diseased leaf tissue (dry) were surface disinfected for 2 min in 1% sodium hypochlorite containing 1 drop of Tween 80 (polyoxyethylene sorbitan) per 100 ml as a wetting agent. The leaf tissue was rinsed in sterile distilled water and placed in a moist chamber for 36 hr. Conidia were collected from the tissue and streaked on the surface of acidified potato-dextrose agar to obtain single-conidial cultures. Conidia from single-conidial colonies were transferred to oatmeal agar and grown for 7 days under constant, cool-white fluorescent lamps (3,600 lux) at 24 ± 2 C.

Conidial suspensions were prepared by flooding 7-day-old cultures of *C. graminicola* with distilled water, loosening the conidia with a rubber spatula, and straining the suspension through two layers of cheesecloth to separate hyphae. Equal quantities of the three isolate suspensions were combined into a single conidial suspension, and 1 drop of Tween 80 per 100 ml was added as a wetting agent.

Effect of seedling age on susceptibility to *C. graminicola*. Fifteen seeds each of 954114, 954164, and Br64, representing susceptible, resistant, and hypersensitive-resistant cultivars to *C. graminicola*, respectively, were planted in 15-cm plastic pots in the greenhouse. Ten days after planting, the seedlings were thinned to five plants per pot. These sorghum cultivars were planted three times at 10-day intervals, and inoculum (5×10^5 conidia per milliliter) was applied on the leaf surface of 15-, 25-, and 35-day-old seedlings with an atomizer at 0.5 atmosphere of pressure. Four replicates of each genotype were incubated in 100% relative humidity for 16 hr.

The pots were then randomly placed on a greenhouse bench at 22 ± 2 C and symptoms recorded at 2-day intervals for 14 days. Disease severity (percentage of leaf area covered with lesions) was estimated using an index of 1–5 for the leaves that were fully exposed to the inoculum. The scale for estimating anthracnose leaf blight severity was: 1 = lesion absent, 2 = lesions covering 25% of inoculated leaves, 3 = lesions covering 50% of inoculated leaves, 4 = lesions covering 75% of inoculated leaves, and 5 = lesions covering 100% of inoculated leaves or plants dead.

Greenhouse studies. In another study, 23 sorghum cultivars from the Purdue Sorghum Collection were assayed for susceptibility or resistance to *C. graminicola* in the greenhouse. The same inoculation procedures were followed as above; however, plants were inoculated 35 days after planting, and the percentage of leaf area damaged was estimated 8 days after inoculation.

Field studies. The same 23 sorghum cultivars were planted in a split-plot arrangement of a randomized complete block design, with the inoculation treatments as whole plots and genotypes randomized as subplots. The experiment, which was replicated three times, consisted of four rows 80 cm apart and 20 m long with plants spaced 6–10 cm apart. Plants were inoculated with a conidial suspension, as described above, at 42 and 50 days after planting. There was a range of maturity among the introductions, and it was impossible to inoculate each cultivar at the same physiologic stage of maturity (1 wk prior to heading). Therefore, two dates were chosen for inoculating the early and late maturing cultivars.

Grain inoculum was prepared by pipetting 5 ml of a conidial suspension onto 50 g of sterilized sorghum grain in 1-L flasks. The inoculated grain was incubated for 21 days under constant, cool-white fluorescent lamps (3,600 lux)

at 24 ± 2 C. Approximately 1 g of infected sorghum grain was placed in the leaf whorl of plants in two of the four rows 52 or 65 days after planting. Sorghum grain provided a source of inoculum over a longer period of time and simulated natural secondary infection. Approximately 5 ml of a *C. graminicola* spore suspension was placed in the whorl using a pressurized sprayer 2 days following inoculation with infected sorghum grain. The plants were rated 98 and 114 days after planting using the greenhouse rating scale.

Leaf tissue from field-inoculated plants was excised, surface disinfected for 2 min in 1% sodium hypochlorite plus 1 drop Tween 80 per 100 ml of water, rinsed in distilled water, and incubated at 100% relative humidity for 48 or 80 hr. Leaf sections with lesions were observed under the microscope at ×200 magnification for acervuli and conidia of *C. graminicola*.

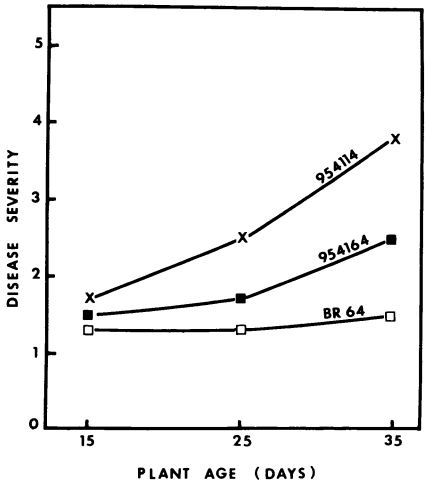

Fig. 1. Relationship of plant age to infection by *Colletotrichum graminicola* of three sorghum cultivars differing in degrees of resistance. Cultivar 954114 is susceptible, 954164 is resistant, and Br64 is hypersensitive-resistant.

Production of conidia. The number of conidia per square centimeter of leaf tissue from greenhouse-inoculated plants was determined by placing 10 leaf sections from three 35-day-old plants of cultivars Br64, 954164, and 954114 in moist chambers and incubating them at 22 C under a 16-hr light period (3,600 lux). After 48 or 80 hr of incubation, a 2-mm^2 section of leaf tissue from a lesion was placed in 2 ml of 0.1% water agar containing 0.01% Tween 80. The leaf tissue was minced in a 14-ml vial and agitated to remove conidia, and the conidial concentration was determined using a hemacytometer. The size of the lesion was determined by measuring it directly with a polar planimeter. After determination of the size of lesion, the number of conidia per square millimeter per lesion was calculated. Ten sections from diseased leaf tissue per plant and at least three plants per treatment were counted.

RESULTS

Relationship of seedling age and susceptibility to *C. graminicola*. Sorghum cultivars inoculated in the greenhouse at three different growth stages reacted differently to *C. graminicola* (Fig. 1). Of the three growth stages tested, the six- to seven-leaf stage (35 days old) was most susceptible to *C. graminicola*; 100% of the susceptible cultivars developed typical, susceptible-type *C. graminicola* lesions. Lesions characteristic of susceptible host-pathogen combinations were circular-to-elliptic spots 2–6 mm in diameter. The spots were well defined and, depending on the cultivar affected, were red, tan, orange, or bluish black on the upper and lower leaf surfaces, with concentric zones of enlargement. Under conditions of high humidity, many spots exhibited a blackish growth that was part of the acervulus.

Small necrotic spots (hypersensitive reaction) developed on leaves of the cultivar Br64, which is resistant to *C. graminicola*. Seedlings of the cultivar 954114, which is susceptible to *C. graminicola*, and the cultivar 954164, which is moderately resistant, were inoculated at the four- to five-leaf stage (25 days after planting); both developed necrotic flecks and susceptible-type lesions. The older leaves tended to have more susceptible-type lesions and fewer necrotic flecks than the younger leaves. Lesions were mature 10 days after inoculation, although the rate of lesion development varied depending upon the cultivar. Small, oval, chlorotic flecks (less than 1 mm in diameter) were observed on all cultivars 3 days after inoculation at the three-leaf stage (15-day-old plants). The chlorotic flecks did not enlarge, and many disappeared within 10 days after inoculation.

Evaluation of cultivars in the field and greenhouse. Disease severity ratings of leaf blight were usually higher in the field than in the greenhouse (Table 1). Field and greenhouse ratings for anthracnose leaf blight were based on the percentage of leaf area damaged. The disease severity index of 23 sorghum cultivars grown in the field and greenhouse ranged from 1.2 to 5.0 and 1.3 to 4.0, respectively. The correlation coefficient for sorghum cultivars evaluated for foliar anthracnose in the greenhouse and field ($r = 0.87$) was highly significant at the 0.01% probability level. These evaluations were made on plants inoculated 52 or 64 days after planting in the field and 35 days after planting in the greenhouse, respectively. Of the 23 cultivars evaluated under both conditions, 12 were susceptible, 4 were resistant, and 7 were hypersensitive-resistant to *C. graminicola*.

A variety of lesion types were observed. The lesion type, color, size, and number of conidia per square centimeter of leaf tissue varied among cultivars. However, disease reaction types of *C. graminicola* for each cultivar were similar, regardless of whether they were compared in the field or greenhouse. The seven cultivars exhibiting the hypersensitive-resistant reaction types did not produce conidia under field conditions, and conidia failed to form in lesions on excised leaves from greenhouse- or field-inoculated plants when placed in moist chambers. The remaining cultivars, resistant and susceptible to *C. graminicola*, produced typical susceptible-type lesions with

Table 1. Disease severity and sporulation response of sorghum genotypes to foliar infection by *Colletotrichum graminicola* in the field and greenhouse

Sorghum genotypes[a]		Disease severity index		Sporulation[b] (conidia/mm^2)
		Greenhouse	Field	
154225	S	4.0[c]	5.0	10^6
954114	S	3.5	4.8	10^6
R5671	S	3.5	4.7	10^6
158361	S	3.8	4.5	10^6
952127	S	3.5	4.5	10^6
954063	S	2.3	4.3	10^2
157822	S	3.1	4.2	10^6
932296	S	2.5	4.2	10^2
932075	S	3.4	4.0	10^6
954255	S	3.2	4.0	10^6
954062	S	2.7	3.5	10^6
954104	R	1.8	3.3	10^2
954130	S	3.2	2.7	10^4
157579	R	2.3	2.7	10^4
956036	R	2.2	2.5	10^2
932027	HR	1.5	2.5	0
954164	R	2.2	2.3	10^2
152319	HR	2.0	2.3	0
932062	HR	1.7	2.3	0
159198	HR	1.9	2.0	0
159569	HR	1.5	1.8	0
954206	HR	1.9	1.3	0
Br64	HR	1.3	1.2	0

[a] S = susceptible, R = resistant, and HR = hypersensitive reaction.
[b] Sporulation observed on plants in the field.
[c] 1 = lesion absent and 5 = leaves covered 100% with lesions or plant dead.

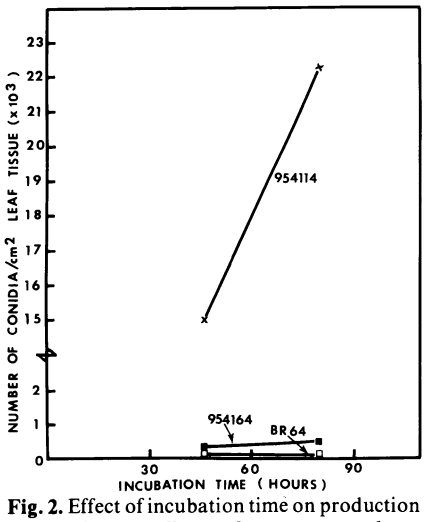

Fig. 2. Effect of incubation time on production of conidia by *Colletotrichum graminicola* on three sorghum cultivars differing in resistance to the pathogen. Cultivar 954114 is susceptible, 954164 is resistant, and Br64 is hypersensitive-resistant.

numerous conidia; however, the moderately resistant cultivars produced fewer lesions and fewer conidia per lesion.

Number of conidia produced per square centimeter. Incubation time affected the number of conidia produced on the cultivars used in this test, with the number of conidia per square centimeter of leaf tissue increasing with an increase in incubation time (Fig. 2). The fungus sporulated in lesions on sorghum cultivars 954114 and 954164 after the inoculated leaves were detached and incubated in a moist chamber for 48 and 80 hr. Lesions examined 80 hr after incubation produced the greatest number of conidia as compared with the 48-hr incubation period. The number of conidia produced in lesions on 954164, the cultivar resistant to *C. graminicola*, was intermediate between 954114, the susceptible cultivar, and Br64, the hypersensitive-resistant hybrid, which did not produce conidia in lesions under any of the test conditions. There were 10–20 times more conidia produced on the susceptible cultivars to *C. graminicola* than on the resistant cultivars.

DISCUSSION

Sorghum cultivars were evaluated in the greenhouse for resistance to *C. graminicola*. The essential requirements for a successful test are to have plants 25 or more days old, a temperature of 22 C or above, and a virulent sorghum isolate. The problem encountered with assaying young seedlings may reflect levels of hydrogen cyanide (HCN) produced in young seedlings that may be toxic to many organisms (8,10,11). The presence of HCN in sorghum plants may be important in resistance to pathogens, especially in young seedlings. Our initial efforts to establish pathogenicity of *C. graminicola* in 15-day-old seedlings were not successful. However, plants inoculated 25 and 35 days after planting developed typical symptoms of *C. graminicola*.

Myers and Fry (11) showed that the protective function of HCN in sorghum seedlings against potential pathogens was reduced as the plant leaf aged; however, after 28 days, the HCN-potential (HCN-p) was below the toxic level. This correlates well with our greenhouse studies, where the disease severity index was highest when seedlings were inoculated 35 days after planting. Thus, it appears that plant age is a critical factor for lesion development in sorghum seedlings. It is assumed that infection occurred after the HCN-p decreased below the toxic level as the leaf matured, but the HCN-p was not determined in these studies.

Variation in susceptibility of cultivars between the field and greenhouse based on the percentage of leaf area damaged was slight, but the percentage of leaf area covered was greater in the field than in the greenhouse. However, reaction types (susceptibility or resistance) were similar under both field and greenhouse conditions, and the correlation coefficient was highly significant. The high correlation coefficiency of field and greenhouse results also suggests that resistance of sorghum to *C. graminicola* can be determined under greenhouse conditions. Disease severity was compared on plants inoculated 52 and 65 or 35 days after planting in the field and greenhouse, respectively. The difference in plant age at the time of inoculation, and secondary infection under field conditions, may account for the higher disease severity rating observed in the field when compared with the greenhouse studies.

The number of conidia and type of disease reaction (Fig. 2, Table 1) were influenced by genotypes. On susceptible cultivars, the number of *C. graminicola* conidia per square centimeter was 10–20 times greater than on resistant cultivars, and no sporulation occurred in hypersensitive-resistant lesions. Coupled with larger lesions and more conidia produced per square centimeter, the inoculum potential of *C. graminicola* appears greater from susceptible lesions than from resistant ones. These results offer a basis for initial greenhouse evaluation of cultivars and should aid in breeding programs for resistance to *C. graminicola*.

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