Pathotypes of *Colletotrichum graminicola* and Seed Transmission of Sorghum Anthracnose

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ABSTRACT

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Of 12 isolates, eight pathotypes of *Colletotrichum graminicola* from four areas (central and southern Texas; Griffin, GA; and Isabela, Puerto Rico) were identified using eight differential grain sorghum cultivars in the greenhouse. The Georgia population possessed virulence toward the differential cultivar SC326-6. The same virulence was not detected in the Texas or the Puerto Rico populations. The Texas pathotypes infected the fewest number of differentials. Except for the reaction of SC326-6, isolates from Puerto Rico and Georgia reacted similarly. One Puerto Rico pathotype was tested for possible seed transmission. Seed without visible acervuli showed no seed transmission of *C. graminicola* under conditions favorable for development of this disease. In seed with acervuli, 23% of the seedlings exhibited necrotic lesions and new acervuli on seedling tissue after 1–2 wk of incubation on sterilized moist vermiculite in enclosed chambers. Anthracnose lesions were observed on mesocotyledonary sheaths, primary leaf laminae, and primary roots.

Sorghum anthracnose, caused by *Colletotrichum graminicola* (Ces.) Wils., is a disease of increasing importance. Development of commercial sorghum production in tropical and subtropical areas with high temperatures and frequent precipitation have greatly increased the amount and severity of anthracnose outbreaks. Variability in pathogenicity has been reported to exist (1,3,6). Genetic variation in the pathogen population may complicate anthracnose control as resistant cultivars effective in one geographical area may become diseased in other production areas. Preliminary research has shown that there is a potential for seed transmission of sorghum anthracnose (7). Because the pathogen may be seed-transmitted and pathogenic variation exists, special precautions in seed production and movement might be warranted. The purpose of our studies was 1) to explore the pathogenic variability of *C. graminicola* on a wide geographical range, 2) to compare natural populations with agricultural and research station populations, and 3) to confirm the seed-transmission potential.

MATERIALS AND METHODS

Pathotype determination. Test cultures of *C. graminicola* were isolated from grain sorghum (*Sorghum bicolor* (L.) Moench) and johnsongrass (*S. halepense* (L.) Pers.). Each isolate was identified by a code identifying its host, geographic origin, and year of collection (e.g., isolate JGCTSP85 was isolated from johnsongrass near Catspring, TX, in 1985) (Table 1).

Single-spore isolates were grown on autoclaved leaf pieces suspended on water agar. Leaves of the grain sorghum cultivar BTX398 (Martin) or johnsongrass were used, depending on the original host of the isolate. The isolates were grown at 26 C and under constant light (Gro and Sho plant light, GE) in a Precision Dual Program Illuminated Incubator. The isolates were maintained on silica gel (8) or in sterile water (2). For inoculations, conidia were harvested by flooding the plates with 10 ml of distilled water and the resulting suspension was adjusted to 1×10^6 conidia/ml. Tween 20 (1/1,000 ml) was added to the spore suspension as a dispersant. The control was distilled water with Tween 20.

The sorghum differentials were grain sorghum inbred cultivars, a subset of the International Sorghum Anthracnose Virulence Nursery (5). The experimental design was a split plot where cultivars were whole plots, and the 12 isolates and one control were subplots. Due to space limitation in the greenhouse, whole plots were divided into blocks over time. Only two differential hosts could be used at one time so that all isolates could be compared within the same environmental conditions. Four replications of three plants per replication were grown using a 14-hr photoperiod. Thirty days after planting germinated seed, plants were inoculated by applying 30 ml of spore suspension to all premoistened plants in the subplot. Inoculum was applied with an airbrush sprayer powered by an in-house air supply (at approximately 60 psi of pressure). The plants were then covered with plastic bags and placed in a dark Percival dew deposition chamber for 24 hr with a wall temperature of 15 C and a water temperature of 40 C, resulting in an air temperature of 26 C. The plastic bags were used to prevent cross-contamination and to promote favorable moisture deposition during incubation. The bags were perforated to provide aeration. After 24 hr, the plants were removed from the dew chamber and returned to the greenhouse.

In the greenhouse, dividing grids were used to separate plants inoculated with each isolate. Disease reactions were scored 7 days after inoculation using a continuous 1–5 rating scale, where 1 indicated an unblemished leaf, greater than 1 but less than 3 had hypersensitive lesions without acervuli, and greater than 3 had varying amounts of tissue damage due to acervular eruption. Ratings of

Table 1. Collection of isolates of *Colletotrichum graminicola*

Code	Host	Site	Year	Habitat
JGCTSP85	Johnsongrass	Catspring, TX	1985	Natural
JGTELF85	Johnsongrass	Telferner, TX	1985	Natural
JGPLAV85	Johnsongrass	Port Lavaca, TX	1985	Natural
JGBB85	Johnsongrass	College Station, TX	1985	Research station
JGBB86	Johnsongrass	College Station, TX	1986	Research station
TOPOG85	Sorghum, Topaz	Orange Grove, TX	1985	Agricultural
TOPOG86	Sorghum, Topaz	Orange Grove, TX	1986	Agricultural
TX430BB85	Sorghum, TX430	College Station, TX	1985	Research station
TAM428PR86	Sorghum, TAM428	Isabela, Puerto Rico	1986	Research station
TAM428G85	Sorghum, TAM428	Griffin, GA	1985	Research station
QL3PR86	Sorghum, QL3(IND)	Isabela, Puerto Rico	1986	Research station
SC326G85	Sorghum, SC326-6	Griffin, GA	1985	Research station

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1–2.5 were considered resistant, 2.5–3 were moderately susceptible, and 3–5 were susceptible.

Seed transmission study. During the fall of 1986, field plantings of the sorghum cultivar QL3(IND) in Isabela, Puerto Rico, developed typical anthracnose symptoms on leaves, panicles, and grain. This cultivar had shown a resistant reaction in the three previous seasons. The susceptible reaction was considered to be caused by a new pathotype of *C. graminicola*. To determine if anthracnose was effectively seed-transmitted, seed from the infected QL3(IND) was collected. Clean threshed seed were examined under the binocular stereoscope at 7–40× and separated based on the presence or absence of acervuli. The experimental replicates were six 38-L glass aquariums containing approximately 10 cm of moist vermiculite, sterilized for 1 hr at 22 C. A single seed type was planted in each chamber. Two hundred seeds were planted in each of the six chambers. Three chambers had asymptomatic seed and three had seed with visible acervuli. Chambers were sealed with a plastic film wrap to prevent contamination and desiccation and were allowed to incubate at 26–28 C. The experiment was arranged in a complete block design on the laboratory benches. Counts of germination were performed at 7 and 14 days after planting. Seedlings with lesions were examined using whole mounts under the binocular microscope and confirmatory observations under the compound microscope to verify development and transmission of *C. graminicola* in seedlings. The sealed chambers were never watered during the experiment. Data were taken on frequency of seedling infection, percent seed germination, and seedling fresh weight.

RESULTS

The reactions of the eight differential cultivars to the isolates of *C. graminicola* are summarized in Table 2. Of 12 isolates tested on eight differential cultivars, eight pathotypes were discerned. Among the eight Texas isolates tested, four pathotypes were detected.

The isolates TOPOG85 and TOPOG86 were collected 2 consecutive years on the commercial sorghum hybrid Topaz near Orange Grove, in southern Texas. These isolates appeared similar to the johnsongrass isolates from Port Lavaca and central Texas, except for their inability to infect QL3(IND). The TOPOG isolates are the least virulent of all tested isolates and were morphologically distinct having shorter, more falcate conidia, less conidial matrix mucilage, and short kinked setae. These constitute the first Texas pathotype.

Two isolates (JGBB85 and JGBB86) were collected in consecutive years from johnsongrass in the Texas A&M University Research Station in the central Texas region. These isolates were avirulent on all differentials, except BTX398 (Martin), although they had marginal ability to cause lesions on QL3(IND). Another johnsongrass isolate (JGPLAV85) collected from Port Lavaca, 150 miles to the south, had the same virulence pattern on the eight sorghum cultivars as the isolates from the Brazos Bottoms research station. These three johnsongrass isolates (two from central and one from southern Texas) constitute a second Texas pathotype.

Two johnsongrass isolates from Catspring and Telferner in southern Texas characterized a third Texas pathotype, with virulence on both BTX398 (Martin) and BTX378 (Redlan).

The most virulent pathotype in Texas came from the Texas A&M research station in the central Texas region where it was isolated from the sorghum cultivar TX430. The isolate TX430BB85 was virulent on three differential cultivars, and constituted the fourth Texas pathotype.

Two sorghum isolates taken from the Tropical Agriculture Research Station farm at Isabela, Puerto Rico, produced similar reactions on all host differentials, except SC414-12E. On this basis, they may be separated into two pathotypes.

Two isolates were obtained from sorghum plants grown at the Bedsoe research farm at Griffin, GA. These isolates were of different pathotypes, as shown by interactive responses of two host differentials, SC414-12E and BTX378. Isolate TAM428G85 was virulent on BTX378, but not on SC414-12E. Conversely, the isolate SC326G85 was virulent on SC414-12E, but not on BTX378. These pathotypes were the most virulent of all tested, causing susceptible reactions on seven of eight differentials. Notably, this Georgia population possesses virulence on SC326-6, a sorghum cultivar known for its high level of anthracnose resistance.

The Georgia and Puerto Rico populations appeared to contain most of the same virulence characteristics, with the exception of the capability to infect SC326-6. Isolate TX430BB85 from Texas had similar virulence factors as expressed in the Georgia and Puerto Rico populations. These isolates infected TAM428, TX2536, and SC328C. However, TX430BB85 did not show virulence on QL3(IND), SC414-12E, or BTX378.

In the seed transmission study, after 6 days of incubation, typical lesions of anthracnose were found on seedlings from seed with visible acervuli before their sowing in an enclosed laboratory chamber. No typical anthracnose lesions were found on plantlets from seed without acervuli. Lesions were first and mostly found on the mesocotyledonary sheath. They were sunken, diamond-shaped cankers having acervuli typical of *C. graminicola*. Lesions were also found on primary leaf lamina and primary roots. Overall, 23% of emerged seedlings derived from seed with acervuli had anthracnose lesions. Germination of the seed with acervuli of *C. graminicola* averaged 20.1%. Seed without signs of the fungus averaged 53.2% germination.

DISCUSSION

It is theorized (9) that individuals with unnecessary virulence should be at a selective disadvantage when competing with individuals containing minimum necessary virulence to infect a host. From the isolates selected from sorghum at various geographically isolated research stations, assuming no migration among stations, it appears that individuals in the pathogen population are accumulating virulence genes. These complex isolates were selected under the experimental conditions where hundreds of genotypically distinct sorghums were planted side by side. They were much more complex than those of natural and agricultural populations. On the other hand,

Table 2. Reaction of eight sorghum cultivars to 12 isolates of *Colletotrichum graminicola* in the greenhouse, in order of increasing virulence

	Pathotype 1		Pathotype 2			Pathotype 3		Pathotype 4	Pathotype 5	Pathotype 6	Pathotype 7	Pathotype 8
Host	TOPOG85	TOPOG86	JGPLAV85	JGBB85	JGBB86	JGCTSP85	JGTELF85	TX430BB85	TAM428PR86	TAM428G85	QL3PR86	SC326G85
SC326-6	R[a]	R	R	R	R	R	R	R	R	R	S	S
SC414-12E	R	R	R	R	R	R	R	R	R	S	R	S
BTX378	R	R	R	R	R	S	S	R	M	M	S	R
TAM428	R	R	R	R	R	R	R	S	S	S	S	S
TX2536	R	R	R	R	R	R	R	S	S	S	S	S
SC328C	R	R	R	R	R	R	R	S	S	S	S	S
QL3(IND)	R	R	M	M	M	R	R	R	S	S	S	S
BTX398	S	S	S	S	S	S	S	M	S	S	S	S

[a] R = resistant, S = susceptible, M = moderately susceptible.

isolates collected from johnsongrass within the research station were as simple as the isolates collected from wild swarms of johnsongrass. These observations support Vanderplank's theory of stabilizing selection (9). It appears that the individuals within the population that possess additional virulence capabilities were not selected on the wild grass.

It is noteworthy that isolates selected randomly from roadside johnsongrass over a 150-mile linear distance were either the same pathotype or had minimal differences in virulence characteristics.

Until it is known whether complex isolates persist in a population and whether they are a threat outside of research stations, care should be exercised. Seed transmission of *C. graminicola* is possible when care is not taken to eliminate seed showing anthracnose acervuli. Risk of introducing virulent isolates of sorghum anthracnose can be minimized by selection of clean seed for transport. Furthermore, the possibility of chance introduction would be eliminated if the clean seed is treated with a systemic fungicide effective against *C. graminicola*, such as benomyl, in addition to the normal topical treatment with captan (4).

Further research should be conducted to determine if complex isolates would be selected against in natural and/or agricultural systems. The isolates of anthracnose from johnsongrass that infect sorghum and cause little damage might be manipulated as a useful biological control agent by possibly preempting more virulent isolates by niche possession.

There is a need for researchers who are studying the variability of *C. graminicola* to agree on a standard set of host-differential cultivars. The selected cultivars must exhibit low genotype × environment variance if they are to be adequate indicators of pathogen variability. Low host genotype × environment interaction appears in the study by Ali and Warren (1), in which plants at different growth stages produce the same reaction type in the field and greenhouse. Until there is some concordance among researchers it will not be possible to combine data to analyze population structures of this pathogen around the world.

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