Comparison of Sorghum Seedling Reactions to *Sporisorium reilianum* in Relation to Sorghum Head Smut Resistance Classes

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ABSTRACT

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Seedlings of sorghum genotypes resistant and susceptible to *Sporisorium reilianum* were grown in peat pellets and inoculated by infesting vermiculite surrounding seedling epicotyls with 7- to 10-day-old teliospore cultures. Four days after inoculation, the seedlings were removed from the pellets, placed in test tubes containing water deep enough to completely submerge the first leaf, and incubated in darkness at 24 C for 5 days. After incubation, symptoms on the first leaf blade differentiated susceptible and resistant genotypes. Symptoms on the first leaf blades of susceptible genotypes were general chlorosis and brown spots. Leaf symptoms of resistant genotypes consisted of interveinal chlorosis and an absence of brown spots on the leaf blade. The inoculation and incubation procedures described in this study will identify types of head smut resistance that prevent the pathogen from reaching the apical meristem of the sorghum plant. This type of resistance appears to be effective against the variability in virulent biotypes of *S. reilianum* that has overcome the race-specific head smut resistance factors deployed in the past.

Additional keywords: horizontal resistance

Head smut of sorghum, caused by *Sporisorium reilianum* (Kühn) Langdon & Fullerton (syns. *Sphacelotheca reiliana* (Kühn) Clinton and *Sorosporium reilianum* (Kühn) McAlpine), is an important disease of sorghum (*Sorghum bicolor* (L.) Moench) in Africa, Asia, Australia, Europe, and North America (5). The disease became a serious problem in Texas in the 1950s (9,16). Resistant cultivars were developed to control the disease, but head smut remains a sporadically important disease in Texas because of the pathogen's ability to produce new races that are virulent to previously resistant sorghum cultivars (1,4,8,10).

Satisfactory control of this disease requires the development of sorghum hybrids with durable resistance. Improvement of head smut resistance in sorghum has been hampered by the lack of efficient methods for identification of genotypes resistant to *S. reilianum*. Currently, screening for head smut resistance is conducted in field nurseries using either natural infection or artificial inoculation (6,7). Several sources of resistance have been identified in this manner. However, field trials are expensive in terms of time and money and are restricted to the growing season. Identification of a resistant reaction reguires development of a panicle; this precludes the evaluation of day-length-sensitive sorghum cultivars (a major source of genetic diversity) that do not flower in Texas. In nurseries dependent on natural infection, repeated trials are required for reliable results because of environmental factors unfavorable to disease development (2) and the erratic distribution of inoculum. An alternative to natural infection is an inoculation technique in which seedlings are injected with sporidia of the pathogen by a hypodermic syringe (3). However, this procedure is laborious and requires a skilled operator to secure reliable results. In addition, this technique sometimes bypasses resistance factors that provide satisfactory levels of resistance to natural infection in the field and identifies only factors for resistance in or near the apical meristem that are expressed as incompatible host/pathogen interactions (9,17). In most cases, these factors have been race-specific and vulnerable to changes in virulence in the pathogen population (6,9).

A technique for the identification of non-race-specific resistance in sorghum at the seedling growth stage would alleviate most of the problems noted above. This paper reports a procedure for inducing symptoms of infection by *S. reilianum* in sorghum seedlings and compares the relationship of these symptoms to known head smut resistance.

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MATERIALS AND METHODS

Teliospore production. Seedlings of the sorghum hybrid NK1210 (Northrup King and Co., Minneapolis, MN), four plants per 8-L plastic pot, were grown in the greenhouse and inoculated at 3 wk of age with *S. reilianum* by hypodermic injection of sporidia using a previously described technique (3). At 5–6 wk after inoculation, sori of *S. reilianum* were produced on some of the inoculated plants. Each sorus was collected within 24 hr after the rupture of the peridium and dried in the greenhouse. The mean daily greenhouse temperatures during this period ranged from 24 to 28 C. After drying, the sorus was rubbed against a wire screen to dislodge the teliospores. Teliospores were placed in open petri dish halves and stored in a refrigerator at 10 ± 1 C.

After 10 mo of storage, 0.2 g of teliospores was placed in 15 ml of sterile distilled water in a centrifuge tube, shaken into suspension, and precipitated by centrifuging at 500 *g* for 10 sec; the water was then decanted. The process was repeated three times. Washed teliospores were suspended in 15 ml of sterile distilled water, and 0.5 ml of the teliospore suspension (approximately 16 × 10^6 teliospores) was added to 50 ml of sucrose agar (3% sucrose, 0.25% agar, w/v, adjusted to pH 3.8 with lactic acid) in a 250-ml Erlenmeyer flask. The cultures were incubated on a rotary shaker operated at 100 rpm in a room where temperature ranged from 24 to 28 C. After 96 hr of incubation, culture samples were examined microscopically and 200 teliospores were observed to determine the germination percentage. The germination test was conducted three times; the mean percentage of spore germination in the trials was 38%.

Test plants. The reactions of sorghum genotypes selected for this study had been determined by several years of trials in Texas head smut nurseries using natural infection and artificial inoculation (R. A. Frederiksen, *unpublished*) (6–9). The selected genotypes represented four classes of reaction to *S. reilianum*

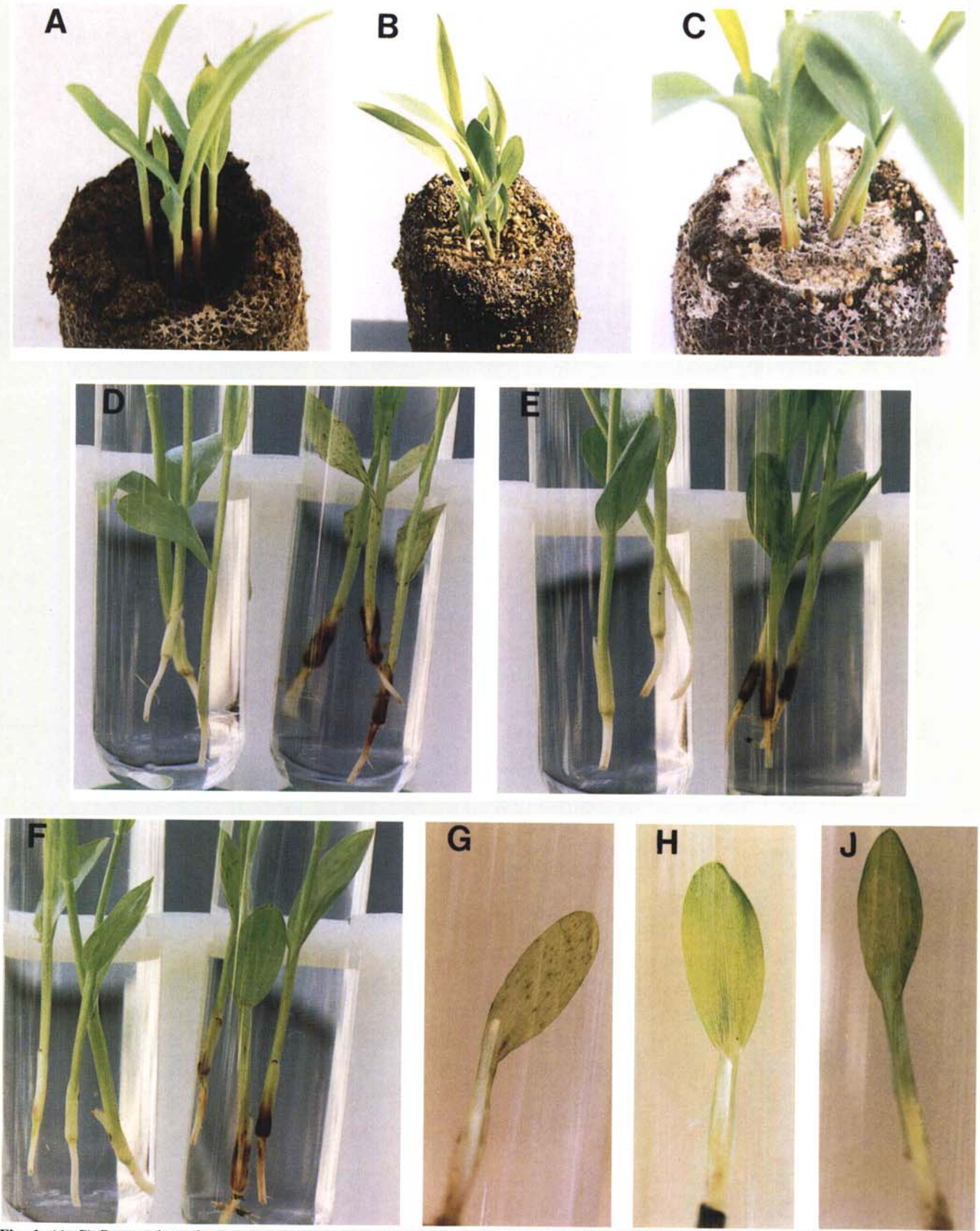

Fig. 1. (A–C) Preparation of sorghum seedlings for inoculation with *Sporisorium reilianum*: **(A)** Seedlings in peat pellet at first-leaf growth stage, **(B)** vermiculite added to pellet, and **(C)** mycelial growth on surface of vermiculite 3 days after inoculation. **(D–F)** Control (left) and inoculated (right) treatments after 4 days of incubation in water: **(D)** SC241-12E (reaction class S1, susceptible), **(E)** Tx430 (reaction class R1, resistant), and **(F)** TAM428, (reaction class R1, resistant). **(G, H, and J)** Symptoms on first leaf of inoculated plants after 5 days of incubation in water: **(G)** SC241-12E (reaction class S1, susceptible), **(H)** Tx430 (reaction class R1, resistant), and **(J)** TAM428 (reaction class R1, resistant).

based on susceptibility to natural infection, susceptibility to artificial inoculation, and differential reaction to Texas races of the pathogen (7) referred to as R1, R2, R3, and S1.

Horizontal (not race-specific) resistance to natural infection and horizontal susceptibility to inoculation with hypodermic syringe is designated R1. Presumably, the resistance factors involved inhibit the progress of the pathogen through the exterior plant tissues surrounding the apical meristem. R1 entries for this study were TAM428 and Tx430 (5–8) (Table 1).

Vertical (race-specific) resistance R2 is characterized by resistance to some races of *S. reilianum* and susceptibility to others, with the same response to natural infection as to artificial inoculation. Apparently, the resistance factors are expressed in the apical meristem as an incompatible host/pathogen interaction and do not provide the horizontal resistance to natural infection postulated for the R1 reaction class. The R2 entries for this study were SA281, SC170-6-17, and Tx414 (5–8,10) (Table 2).

Horizontal resistance to natural infection and inoculation is designated R3. As demonstrated by the resistant response to artificial inoculation, resistance factors in the apical meristem presumably produce an incompatible host/pathogen interaction. In the absence of races that were virulent to the resistance factors in the apical meristem, it was not possible in the field nursery trials to determine the presence or absence of R1-type resistance. The R3 entries were FC6601 and Lahoma sudangrass (7–10) (Table 1).

Horizontal susceptibility to natural infection and inoculation is designated S1. Presumably, none of the factors that confer resistance are present. The S1 entries were SC241-12E and Tx7078 (6,7).

Pregerminated seeds of the sorghum test entries were planted in 5-cm peat pellets. The hydrated peat pellets were prepared by dibbling holes approximately 3.8 cm deep and 1.25 cm in diameter in the center of each. The sorghum seeds were dusted with a trace of captan fungicide (75 WP), germinated on moist paper, and planted shortly after germination. Six germinated seed per pellet were dropped in the planting hole. The pellets were squeezed gently to close the planting hole, and a depression approximately 1.5 cm deep and 2.5 cm in diameter was pressed into the top of the peat pellet.

The peat pellets were placed in an environment chamber operated at 30 ± 1 C and 80–90% RH with continuous illumination by 12 110-W fluorescent lamps. With rare exceptions, the seedlings emerged within the perimeter of the depression in the top of the peat pellet. Any seedlings that emerged outside the depression were cut out of the pellet.

Inoculation. Inoculum for the seedling trials was produced by culturing teliospores for 7–10 days, as described above for the teliospore germination tests. The teliospore cultures were examined microscopically after 7 days of incubation; the cultures contained germinated teliospores, ungerminated teliospores, sporidia, sporidia at various stages of fusion, mycelium presumably from sporidial unions, and secondary sporidia (12). Inoculum not used immediately was stored at 5 ± 0.5 C; inoculum stored for 3 wk remained infective.

The sorghum seedlings (12–24 plants per trial entry) were inoculated at the first-leaf stage of growth (auricle of the first leaf visible above the top of the epicotyl and auricle of the second leaf not exposed) (Fig. 1A). Before inoculum was applied, the depression at the top of each peat pellet was filled with grade 3 vermiculite (Fig. 1B), and enough distilled water was added to the trays to fully hydrate the peat pellets and the layer of vermiculite surrounding the seedlings in the depression. After hydration of the pellets, surplus water was poured out of the trays. Dry powdered agar was mixed with the inoculum suspension at the rate of 0.1 g of agar per 100 ml of suspension to increase viscosity.

Inoculum (5 ml in 2.5-ml doses, with enough time between doses to prevent excessive runoff) was placed on the surface of the vermiculite around the epicotyls of the seedlings. Hydration of the peat pellets and vermiculite coupled with the viscosity of the suspension ensured that much of the inoculum remained in the upper part of the vermiculite layer and near the epicotyls of the seedlings. Sucrose agar was applied to the controls.

The trays of inoculated plants were returned to an environmental chamber operated at 24 ± 0.5 C, continuous light (570 $\mu E \cdot m^{-2} \cdot s^{-1}$), and 80–85% RH. The seedlings were kept in the chamber for 4 days after inoculation, and distilled water was added to the trays as needed to prevent the infested vermiculite layer from drying. At 4 days after inoculation, the seedlings were removed from the peat pellets by breaking the pellets open. The plants were sprayed gently with water to remove debris, and the stems were separated from the lower parts of the plant by cutting through the mesocotyl approximately 5 mm below the base of the stem. The shoots were placed in test tubes (25 × 150 mm), four to six shoots per tube. Sufficient distilled water was added to the test tube to immerse the first leaf blade and the base of the second leaf blade (Fig. 1D–F). Care was taken to keep part of the second leaf blade above water. The tendency of the stems to float upward as water was added was minimized by holding the test tube at a 45° angle. The test tubes were covered with plastic caps and placed in darkness at 24 ± 0.5 C. The plants were examined for symptoms on the immersed portions with a ×2 hand lens at daily intervals for 5 days. The test of each sorghum genotype in the trial was repeated three or more times.

Table 1. Disease incidence in sorghum cultivars with horizontal reactions to natural infection by *Sporisorium reilianum* after natural infection or hypodermic inoculation

Cultivar	Head smut (%)	
	Natural infection[a]	Hypodermic inoculation[b]
TAM428	1	44.0
Tx430	1	52.0
FC6601	1	0.2
Lahoma sudangrass	0	0.0
Tx7078	30	72.0
SC241-12E	63	80.0

[a]Mean incidence in eight annual field trials.
[b]Mean incidence in five annual field trials.

Table 2. Relationship of symptoms induced in sorghum seedlings by *Sporisorium reilianum* to head smut reaction class

Sorghum genotype	Reaction class[a]	No. of inoculated seedlings[b]	No. of trials	Symptoms[c]	
				S (%)	R (%)
Tx430	R1	61	5	2	98
TAM428	R1	43	5	5	95
SA281	R2	46	3	96	4
Tx414	R2	36	3	97	3
SC170-6-17	R2	73	5	59	41
Lahoma sudangrass	R3	40	3	0	100
FC6601	R3	41	3	98	2
SC241-12E	S1	102	6	97	3
Tx7078	S1	68	5	89	11

[a]R1 = factors conferring horizontal resistance to races of *S. reilianum* present only in nonmeristematic tissue; R2 = factors conferring vertical resistance to avirulent races present only in meristematic tissue; R3 = factors conferring horizontal resistance to races present in meristematic tissue (condition of nonmeristematic tissue unknown); S1 = no factors for resistance in meristematic or nonmeristematic tissues.
[b]Total number of seedlings inoculated in three or more trials.
[c]S (%) = percentage of inoculated seedlings with brown spots on first leaf blade, R (%) = percentage of inoculated seedlings that did not develop brown spots on first leaf blade; means derived from aggregates of data of three or more trials.

RESULTS

Within 3 days after inoculation, a thin layer of gray mycelium appeared on the surface of the vermiculite in the peat pellets that had been infested with *S. reilianum* (Fig. 1C). In the control (sucrose agar) treatments, visible fungal growth was infrequent and differed from that in the inoculated treatments. When the seedlings were removed from the peat pellets, moderate to severe damage to the epicotyls was observed on the inoculated seedlings of all trial entries except Lahoma sudangrass. Epicotyl symptoms consisted of a few small tan lesions in Lahoma sudangrass but ranged from large red or black necrotic areas to death in the other genotypes (Fig. 1D–F). Most control seedlings had no symptoms of epicotyl damage (Fig. 1D–F), although a few showed black or brown discoloration of the extreme tip of the epicotyl. When observed with a ×2 hand lens after 2 days of incubation in darkness, 90–100% of the inoculated seedlings of each trial entry showed abundant mycelium on the epicotyls, whereas less than 1% of the seedlings in the control treatments had visible mycelial growth on the epicotyls.

Small, microscopically visible brown spots were observed on the first leaf blade of some inoculated seedlings of SC241-12E after 3 days of incubation in the dark. After 5 days of incubation, brown spots on the first leaf blade were characteristic of S1 reaction class entries SC241-12E (Fig. 1D and G) and Tx7078; R2 reaction class entries SA281, SC241, and Tx414; and R3 reaction class entry FC6601 (Table 2). R2 entry SC170-6-17 differed from other R2 entries by slower development of leaf spots and lower frequency of brown spot development within the 5-day incubation period (Table 2). Brown spots were never observed on the leaf blades of R3 class entry Lahoma sudangrass and were seen only rarely on inoculated plants of R1 class entries TAM428 and Tx430 (Fig. 1E, F, H, and J; Table 2).

A loss of green pigmentation in the first leaf blade of inoculated seedlings was observed after 4–5 days of incubation. In cultivars characterized by brown spots on the leaf blade, translucent areas of cleared tissue developed around the spots and enlarged rapidly. Loss of pigmentation was so severe in SC241-12E that leaves appeared nearly transparent (Fig. 1G). In cultivars that did not develop brown spots on the leaves of inoculated seedlings, water-soaked blotches on the leaves were followed by progressive clearing of interveinal tissue but retention of veinal tissue color (Fig. 1H and J). Seedlings of the control treatments (Fig. 1D–F) showed no loss of pigmentation at the conclusion of the incubation period.

With the exception of SC170-6-17, each tested cultivar was nearly homogeneous for the type of symptom induced in the inoculated seedlings (Table 2). The symptoms of inoculated sorghum cultivars in which nonmeristematic resistance factors were present (R1 reaction class) (Table 1) consisted of interveinal leaf clearing (Fig. 1H and J). In contrast, the symptoms expressed by sorghum cultivars in which nonmeristematic resistance factors were absent (R2 and S1 reaction classes) (Tables 1 and 2) were more severe chlorosis of veinal and interveinal tissues and brown spots on the leaf blades (Fig. 1G). Symptoms similar to those observed on the first leaf sometimes were seen on the submerged portion of the second leaf blade, but occurrence was erratic.

DISCUSSION

The germination rate of teliospores used in this study was appreciably higher than that observed previously (R. A. Frederiksen, *unpublished*; 15). A probable cause for poor teliospore germination in past studies was the colonization of the sori by fungi and bacteria (15). The procedure described for producing teliospores in this study minimized damage to the sori by other fungi, and the low pH (3.8) of the media inhibited the development of any bacteria to infest the teliospores. These measures used against deleterious organisms probably accounted for the relatively high percentage of germination. Pai and Pan (14) reported that storage conditions had a significant effect on teliospore germination. In their studies, teliospores of *S. reilianum* stored for 30 days on moist paper at 30–35 C germinated at the rate of 60–90% when placed in a sucrose solution. In contrast, teliospores stored for 30 days in a cool, dry environment had a germination rate of 10%. After 3 mo of storage at 30–35 C in a moist atmosphere, teliospore germination decreased, falling to less than 10% after 6 mo of storage (14). In the current study, storage of teliospores for 10 mo at 10 ± 2 C had no drastic effect on germination. Teliospores germinated at the rate of 46% before storage and 38% after 10 mo of storage.

The means by which this pathogen invades the sorghum seedling after inoculation is unknown. We theorize that the pathogen progresses through the epicotyl, invades the leaf sheath, progresses up the leaf sheath, and enters the leaf blade. Halisky and Petersen (11) conducted a histological study of sudangrass with head smut and found mycelium of the pathogen in the phloem and xylem. Presumably, these vascular tissues served as a means for the pathogen to proceed from the leaf sheath to the leaf blade. Alternatively, the mycelium observed on the immersed epicotyls could produce secondary sporidia that germinated and invaded the leaf blades. Natural (13) reported that sporidia of *S. reilianum* placed on sorghum leaf blades invaded the leaves.

The processes responsible for the symptoms induced by immersing the inoculated sorghum seedlings are not known. Apparently, the immersion of the leaves in water and the absence of light favor the colonization of host tissue by the pathogen in both resistant and susceptible genotypes, although the effect of this leaf colonization appears to be more severe in susceptible genotypes (Fig. 1G). Presumably, the factors that determine symptom expression on the leaves are the same as those responsible for resistance to progress of the pathogen through the exterior tissues of the sorghum plant to the meristematic region of the stem. The difference between the two R3 entries FC6601 and Lahoma sudangrass in symptom type (Table 2) was particularly interesting because these cultivars did not differ in reaction to natural infection or artificial inoculation in field trials (Table 1). The results of the earlier artificial inoculation trials demonstrated that both cultivars possessed meristematic factors for resistance. The results of this study led to the conclusion that Lahoma sudangrass also possessed nonmeristematic factors for resistance and that FC6601 did not (Table 2).

Inoculation and incubation procedures described above will identify types of head smut resistance that prevent *S. reilianum* from reaching the apical meristem of the sorghum plant. This type of resistance appears to be effective against the variability for virulent biotypes in the fungus that has overcome the race-specific head smut resistance factors deployed in the past.

The genotypes tested in this study are a very small sample of the sorghum population, and there may be sorghum genotypes susceptible to *S. reilianum* that would not be detected by this technique. However, stringent field trials of sorghum selections identified as resistant by the seedling test would detect any anomalies. Given this safeguard, the seedling reaction test for head smut resistance is an efficient, economical replacement for field trials.

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