Development of an Infection Assay for *Sporisorium reilianum*, the Head Smut Pathogen on Sorghum

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ABSTRACT

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The practical value of dry teliospores of *Sporisorium reilianum* as inoculum in head smut infection assays was determined from a series of studies using three different isolates of the pathogen. Overall germinability of teliospores ranged from 8.0 to 28.0% on culture media after 48 h of incubation. Teliospores germinated at slower rates in the vicinity of sorghum seedlings growing on germination paper, reaching maximum values of 11.5 to 13.0% after 120 h. These results suggest adaptation in the germination strategy of *S. reilianum*, probably modulated by the variable soil environment. Fifteen percent (15%) of the seedlings of a head smut susceptible maize hybrid inoculated with teliospores of *S. reilianum* at root protrusion openings of the mesocotyl tissues became infected, while seedlings similarly inoculated with sporidial suspensions remained uninfected. Three sorghum lines susceptible to head smut were inoculated in three separate experiments with dry teliospores of isolates from Corpus Christi and Taylor, Texas. Seeds were imbibed for 18 h, and the seedlings were planted in soil at 40% moisture content (wt/wt) and covered with a 1:50 (vol/vol) mixture of teliospores and autoclaved soil. Infection levels obtained in these experiments were 2.5 to 2.8 times higher than field infection levels on susceptible lines RT×7078 and B1. In this study, average infection levels of 65 to 79.5% and 84 to 87% for RT×7078 and B1, respectively, were obtained with Corpus Christi isolates of *S. reilianum*; whereas Taylor isolates infected 91.9 and 82.3% of the plants in these two lines. It is postulated that the increased infection efficiency observed with this inoculation technique results from uniform and higher levels of inoculum, timely delivered under stable soil conditions that provide the pathogen with an environment more conducive for infection.

Additional keywords: fungal inoculations, resistance, *Sorghum bicolor*

Host resistance in sorghum to *Sporisorium reilianum* (Kühn) Langdon & Fullerton is the only practical means for control of sorghum head smut. Identification of sources of resistance is a very slow process due to the difficulty in observing disease expression in late-maturing day-length-sensitive sorghums (3) and to a number of plant and environmental factors that can interfere with natural infection events (4).

Sorghum genotypes susceptible to head smut can escape disease due to the uneven distribution of infective propagules of *S. reilianum* in the soil or to environmental factors that prevent spore germination or seedling infection. Inoculation with sporidial suspensions has been considered more reliable than natural infection (14), but it bypasses at least one resistance mechanism and is sensitive to poor technical skills and genetic heterogeneity of the plant material.

Soil factors, as well as plant factors, appear to be major determinants of infection and development of head smut. Soil temperatures in the range of 25 to 30°C appear to be the most favorable for infection both in maize and sorghum (2,6,10). Several reports indicate that soil moisture content near 40% of water holding capacity is most conducive to head smut development in sorghum (6,14). These and other soil factors may play a major role in inoculum survival as well. Plant age appears to be important for the initiation of infection. Most studies indicate that sorghum is susceptible only for a short period during the seedling stage (13).

The chlorotic fleck as an indirect assessment of head smut in maize described by Foster (6) and Matyac and Kommedahl (11) was not useful in sorghum. An alternative seedling reaction screening technique was described by Craig and Frederiksen (3). This identifies resistance acting at or near infection courts, but its physiological basis is not understood, and its usefulness in routine screening of diverse genetic material (or early generations) is unknown.

Head smut resistance in sorghum results from the action of one or more different

host mechanisms. A factor or group of factors acting at the meristematic level, possibly controlled by race specific resistance genes, has been described by Craig and Frederiksen (3) and can be identified by inoculation with appropriate races of the pathogen. Another resistance mechanism appears to prevent infection under natural conditions by interfering with penetration or early colonization events. Host resistance of the nonmeristematic type is considered to be race-non-specific and effective across locations (3). It is a valuable form of resistance that can only be identified in genetic backgrounds carrying no meristematic resistance for the *S. reilianum* race under study; thus, few sorghum inbred lines are positively known to carry this type of resistance (15). Two important tasks must be carried out for the future identification of nonmeristematic factors determining sorghum resistance to the head smut pathogen: (i) finding sorghums carrying no meristematic factors for resistance, and (ii) reducing escape rates to a minimum.

While the absence or presence of meristematic resistance factors can be determined in sorghum by injection with sporidial suspensions of an avirulent race of *S. reilianum* (4), the presence of resistance of the nonmeristematic type can only be inferred from field data for those lines lacking other resistance mechanisms. The development of an inoculation method with the characteristics of natural infection has been attempted in several studies (13,14). Developing infection assays for head smut in sorghum is further confounded by the additional difficulty of a less-than-adequate understanding of critical aspects of the infection process (16,19). An infection assay for head smut in sorghum grown in the greenhouse that overcomes the high escape rates found in field trials would be beneficial to a breeding program because screenings could be done anytime. In addition, the assay would be potentially useful for detailed studies of the early events in natural infection.

S. reilianum is a variable pathogen (7,17). Variation in virulence to sorghum allows the pathogen to overcome genetic resistance in the host of the meristematic type (13). In the absence of nonmeristematic resistance, various aspects of race-specific resistance could also be studied using a natural infection assay.

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Here we describe the development of an infection assay for use in the greenhouse. The main features of this technique are derived from studies by Metha (13), Wilson (18), and Matyac and Kommedahl (10) regarding methods of inoculation, teliospore germination, and effects of soil moisture content on head smut development. The applications of this infection assay for screening purposes as well as in studies pertaining to the infection process are discussed.

MATERIALS AND METHODS

Fungal inoculum. Sori from sorghum plants infected with head smut were collected from the Uniform Head Smut Nursery grown in Corpus Christi, Texas, and in a commercial sorghum field at Taylor, Texas, during the summer of 1994. The individually bagged sori were allowed to dry at room temperature for a week before the teliospores were passed through a fine metal sieve to separate them from plant debris. The harvested teliospores were placed in sterile plastic culture tubes and stored at 4°C. Because field isolates of *S. reilianum* normally carry some bacterial and fungal contaminants, two isolates of the fungus (CrCh-103 and Tayl-117) were increased in the greenhouse. Teliospores of the two isolates were washed in 70% ethanol, suspended in sterile distilled water, plated on potato dextrose agar (PDA), and incubated in the dark at 28°C for 48 to 72 h. Isolated sporidial colonies were transferred to new PDA plates and incubated for an additional 48 h, and a few colonies were transferred to flasks containing potato broth for increase on a rotary shaker for 4 days. Liquid media containing sporidia were injected into seedlings of the inbred line RT×7078, as described by Edmunds (4).

Head smut sori produced in inoculated plants in the greenhouse were collected, processed, and stored as described above. One maize head smut isolate of *S. reilianum* collected from Halfway, Texas, was used for teliospore and sporidial inoculations of maize plants. Sporidial inoculum was prepared as described above.

Plant material. A head smut susceptible maize hybrid (P81950, Pioneer HiBred International, Johnston, IA) was used for preliminary infection trials. Seed of head smut susceptible sorghum lines RT×7078 and SC241-12E were obtained from storage stocks of our laboratory. Seed of the susceptible line B1 was obtained from D. T. Rosenow (Texas A&M University Experiment Station, Lubbock, Texas).

Teliospore germination. To establish the levels at which the fungal isolates germinated under optimal conditions, teliospores were washed in 70% ethanol, suspended in sterile distilled water (50,000 to 80,000 per ml), and incubated at 28°C in the dark on glass slides coated with 1% sucrose, 2% agar medium (WSA) or potato

dextrose agar (PDA). Teliospores were also incubated in water sucrose 1% (WS). After 48 h, direct microscopic observations were made on five slides per treatment, counting the number of germinated teliospores per microscopic field at ×100 magnification. Three microscopic fields were observed in each slide, and the average germination was recorded as a percentage.

Teliospore germination was also assessed in the presence of germinated sorghum seeds (RT×7078) growing on germination paper (Anchor Papers Ltd., West Lafayette, IN) or in sterile soil. At 24, 48, 72, and 120 h, mesocotyl tissues from three seedlings were washed, sectioned, and stained in giemsa or cotton blue before microscopic observations were made to detect the presence of germinated teliospores. Teliospore germination was defined in this study by the production of either promycelia or elongated hyphae.

Seed germination and seedling handling. Seeds of maize hybrid P81950 were surface-disinfected in 5% sodium hypochlorite for 2 min, rinsed in distilled water, and allowed to germinate on moistened germination paper at 25°C for 96 h. Seedlings were removed from the germination paper and immediately inoculated with either sporidial suspensions or dry teliospores of the maize isolate of *S. reilianum*.

Seeds of sorghum lines B1, RT×7078, and SC241-12E were surface-disinfected for 5 min in 5% sodium hypochlorite, rinsed in distilled water, and allowed to germinate on Anchor paper, in autoclaved vermiculite, or in autoclaved soil mix. Seed placed for germination on Anchor paper was removed after 18 h, when radicle protrusion had taken place and plumule length was 5 to 10 mm. Seedlings at this stage were used directly for infection assays. To assess teliospore germination on sorghum seedling tissues, autoclaved vermiculite or soil mix was mixed with dry teliospores of *S. reilianum* isolates CrCh103-1 or Tayl-117 before seed was planted. Alternatively, seed was placed on Anchor paper that was previously moistened and dusted with a 1:3 dry agar:teliospore mixture. Germination was allowed to proceed as previously described.

Maize infection assay. Germinated maize seed typically produces roots in the mesocotyl area within a few days. At the site of root protrusion, a natural wound is created that gives free access to the pericycle tissues of the developing seedling. Seeds of the susceptible maize hybrid P81950 were germinated on Anchor germination paper and removed after 96 h. Dry teliospores of a maize isolate of *S. reilianum* were suspended in 0.2% water agar to a concentration of 100,000 teliospores per ml, and then 100 µl of the suspension was applied to the uppermost root protrusion opening of 48 seedlings. Inoculated seedlings were left undisturbed on

top of moist paper towels for 1 h before transplanting them individually to plastic pots containing steamed soil mix. Plants were grown in the greenhouse to maturity to permit head smut symptoms to develop.

A second group of 44 seedlings was inoculated in a similar fashion with a sporidial suspension at a concentration of 10⁶ sporidia per ml. As control plants in this study, 39 seedlings were inoculated with 0.2% water agar before transplanting to soil.

Sorghum infection assays. Three infection assays were conducted using sorghum lines susceptible to head smut. Previous attempts to infect sorghum with sporidia as inoculum delivered externally to different parts of the seedlings have been unsuccessful (13,14). Therefore, dry teliospores were used in this study as the only type of inoculum of *S. reilianum*.

In the first assay, seed of two susceptible lines (RT×7078 and SC241-12E) were imbibed as described for 18 h before planting. Steamed soil mix was used to fill 18-cm-diameter plastic pots, and water was applied to maintain a water content around 40% (wt/wt). At the time of planting, three holes (5 cm deep, 2 cm wide) were opened in the soil with a plastic stick, and one seedling was placed at the bottom of each hole. To cover the seedlings, a 50:1 soil:teliospore mixture was prepared. Dry soil mix was passed through a sieve, the fine soil part was recovered, autoclaved, and allowed to cool before mixing with dry teliospores of a composite of field isolates 1 to 31 collected from a single field in Taylor, Texas (isolate mixture Tayl 131). After completely filling the holes to cover the seedlings with the soil:teliospore mixture, no water was applied for a 5-day period, but light watering was provided afterward. Plants were grown to maturity in the greenhouse, and the number of infected plants was recorded. A total of 140 and 115 seedlings were inoculated for RT×7078 and SC241-12E, while 50 and 40 seedlings, respectively, were noninoculated controls.

In a second experiment, two sorghum lines susceptible to head smut were used (RT×7078 and B1), and the fungal inoculum was bulked dry teliospores from eight isolates (CrCh-101 through -108) collected from an experimental plot at Corpus Christi. This isolate mixture was labeled CrCh-109. Soil preparation, planting, inoculum mixture preparation, and plant handling were as described above. A total of 590 and 380 seedlings of RT×7078 and B1, respectively, were inoculated, while 130 and 115 were used as noninoculated controls.

In a third experiment, sorghum lines RT×7078 and B1 were inoculated as described in the two previous experiments, but two *S. reilianum* isolates were used (CrCh103-1, obtained in the greenhouse, and Tayl-131, a mixture of field isolates

described above). Other assay conditions remained unmodified as described above.

RESULTS

Teliospore and seed germination.

Germination of teliospores of *S. reilianum* was assessed on culture media to estimate the overall germinability of the propagules used in the infection assays. Surface disinfection of teliospores with 70% ethanol is applied, before field samples of the head smut fungus are germinated on culture media, to reduce the proliferation of bacterial and fungal contaminants. In this study, teliospores germinated in the range of 8 to 28% within 48 h of incubation in the dark at 28°C (Table 1). Although some germinating teliospores were observed as early as 18 h, first recordings of germination began at 24 h. The use of sucrose as the sole carbon source, over a more complete nutrient medium such as PDA, favored teliospore germination for the three isolates tested.

Germination of teliospores of *S. reilianum* obtained from plants grown in the greenhouse was also assessed in the presence of sorghum seedlings. Initial attempts to evaluate germination either in soil samples or in vermiculite samples taken from the vicinity of mesocotyl tissues proved to be extremely difficult, although a few teliospores were found germinated. All observations referred to herein relate to germination of those teliospores that were placed around germinating sorghum seeds on Anchor paper and incubated at 28°C.

Statistical analysis of teliospore germination (Table 2) indicated highly significant differences among media, isolates, and their interactions. Germination of teliospores was considerably slower on paper supporting sorghum seedling growth, compared with germination on media containing higher concentrations of simple sugars. The mean germination levels for the two isolates were 2.9, 5.8, 9.9, and 12.0% at 24, 48, 72, and 120 h of incubation. Both head smut isolates (Tayl-117 and CrCh-103-1) had similar germination rates (27% on WSA medium and 20% on WS), which were significantly higher than those achieved by the field isolate mixture Tayl-131 ($P < 0.01$).

Under the conditions of this study, maximum germination levels were observed on artificial media for two isolates that originated from plants grown in the greenhouse (Tayl-117 and CrCh-103-1) compared with the field isolate mixture, Tayl-131. These high rates (26 to 28% after 48 h in WSA) were not observed for the same isolates when incubated with germinated seed on high-cellulose paper even after 120 h.

Germination of sorghum seed was tested on Anchor germination paper, sterile vermiculite, and soil. Within 18 h, nearly 70% of the seed exhibited radicle protrusion (data not shown) regardless of the presence or absence of teliospores in the germination substratum. The initiation of seed germination coincided with the initiation of teliospore germination near seedling tissues.

Maize infection assay. When inoculated with teliospores of *S. reilianum* at the root protrusion sites, 14.5% of the maize seedlings of the susceptible hybrid P81950 became infected and developed symptoms of head smut at maturity; whereas seedlings inoculated with a low volume of sporidial suspension remained uninfected. Symptoms of head smut in maize typically develop in the ear and occasionally in the male inflorescence. In this assay, ears were replaced by sori in all infected plants; additionally, some male inflorescences exhibited some phyllody or the development of small sori replacing individual florets. The proportion of infected plants (14.5%) was considerably lower than infection levels observed in maize in other studies (10,12).

Microscopic examination of maize seedlings at the site of inoculation with sporidia revealed the localized proliferation of short hyphae, presumably originating from sporidia that might have germinated and fused. However, this did not result in head smut development. Sterile plants (characterized by the failure to form seed) were observed among the noninoculated plants (6.0%), as well as among those inoculated with teliospores (68.0%) or sporidial suspension (15%). The higher sterility rates observed in plants inoculated with teliospores suggest the possibility that higher infection rates actually occurred

with this inoculation procedure. Unfortunately, this trial was unreplicated, which prevented statistical comparisons. Since typical head smut symptoms did not develop and microscopic confirmation of infection was not made, no conclusions could be drawn without further investigations.

Sorghum infection assays. Three sorghum inbred lines susceptible to head smut were used in the infection assays described in this study. Seed of these lines were previously analyzed for the presence of fungal contaminants, and a treatment with sodium hypochlorite was applied before exposing the seeds to high moisture for imbibition. A high level of seed germination was observed 18 h after the initiation of imbibition. Inoculation of seedlings at this early stage of development resulted in high percentages of head smut infection in all assays conducted and for the three susceptible lines RT×7078, SC241-12E, and B1 (Table 3).

The susceptible inbred line SC241-12E, which supported high levels of infection in field trials at several locations in Texas, exhibited lower infection levels (39.5%) than RT×7078 when inoculated with teliospores collected from one sorghum field at Taylor, Texas. Interestingly, sori developed only on very few infected plants, whereas sterile panicles with varying degrees of phyllody were found in every infected plant of this particular sorghum line. Prominent sori developed in place of the panicle in most infected plants on the other two lines used in this study.

The isolates of *S. reilianum* used in the infection assays described here originated from two distinct populations of the pathogen. The overall infection levels achieved with these isolates on the susceptible line B1 had little variation among assays or between isolates (82.3 and 85.9% for Taylor and Corpus Christi isolates, respectively). The infection levels induced by Taylor isolates on RT×7078 were higher (average 91.8%) than those induced by the Corpus Christi isolates (average 82.3%), and these values are considerably higher than the infection levels obtained in field trials with this line.

Table 1. Germination of teliospores from three isolates of *Sporisorium reilianum* on synthetic media and in the presence of sorghum seedling tissues

Isolate	Teliospore germination (%)						
	PDA	WSA	WS	M24	M48	M72	M120
Taylor 131	12 a ^y	10 b	16 b				
Taylor 117	10 ab	28 a	20 a	3 (4) ^z	6 (7)	12 (10)	13 (11)
CrCh103-1	8 b	26 a	20 a	3 (1)	6 (3)	8 (6)	12 (8)

^y Teliospore germination was determined by the proportion of teliospores with a promycelium after 48 h of incubation in the dark at 28°C on potato dextrose agar (PDA), water sucrose agar (WSA), water sucrose (WS), or on mesocotyl surfaces (M) at 24, 48, 72, and 120 h. Values followed by the same letter do not differ statistically according to Duncan's multiple range test.

^z Teliospore germination on Anchor paper in the absence of sorghum seedlings.

Table 2. Analysis of variance for teliospore germination of *Sporisorium reilianum* on artificial media

Source	df	SS	MS
Main plot	14	1,164	
Blocks	4	54.9	13.7 NS ^y
Medium (M)	2	1,053.3	526.5** ^z
E (a)	8	55.8	7.0
Subplots	44	2,436	
Isolates (I)	2	373.3	186.6**
M × I	4	693.7	173.4**
E (b)	38	205	5.4

^y Not significant.

^z Significance at $P = 0.01$.

DISCUSSION

Several aspects associated with the infection of sorghum seedlings by *S. reilianum* remain unexplained. The fate of teliospores released to the ground at the end of the sorghum growing season is only partially understood. Yet teliospores represent the only means that the pathogen has to survive in the soil for extended periods of time (12) and probably the only means for infection of the host, because many studies have failed to connect the sporidial phase to any role in pathogenesis under natural environments (7). In this study, we investigated some aspects of teliospore germination of the sorghum head smut fungus as it relates to the infection process. Our results agree with those of Al-Sohaily (1), who found maximum germination rates around 15%. The overall teliospore germinability of the three isolates used varied from 8 to 28%, as measured after 48 h of incubation on culture media. Higher values could conceivably be observed with longer incubation times.

One hypothesis explaining the teliospore germination pattern in *S. reilianum* is the occurrence of variable degrees of dormancy in any teliospore population. Exposure to a germination-conducive soil environment in the absence of host, either prolonged or cyclic, would not result in elimination of the pathogen population.

Germination of teliospores of *S. reilianum* in the soil or in the presence of sorghum seedlings has been reported by Al-Sohaily (1) and Wilson (18). Both authors indicated a very low frequency of germination and no evidence of sporidia being formed. Teliospore germination on tissues of sorghum seedlings growing in autoclaved soil proved very difficult to assess in this study, partly because germination was very low. Germination of teliospores alone and in the vicinity of sorghum seedlings growing on high cellulose paper was found to be slower and of lower magnitude than germination in culture media. Elongated hyphae rather than promycelium and sporidia were often seen on mesocotyl surfaces.

A complementary hypothesis to explain the teliospore germination strategy of *S. reilianum* can be postulated. Besides dormancy, which is a constitutive attribute that ensures survival in the changing soil environment, teliospores of the head smut fungus may have two distinct germination programs. A sporidial germination program could be envisioned as a component of an ancestral infective system, responsive to high moisture and readily available nutrients. This opportunistic system would have lost pathogenic relevance in the head smut pathogen of sorghum due in part to the adaptation of the host to dry, warm environments. With the experimental evidence available today, a minor role for this system in pathogenicity and recombination under some specific sets of soil environments could not be discounted. A second program requires infective hyphae as the characteristic feature that would allow the fungus to be pathogenic in dry environments where the host exists. Conceivably, this program would be responsive to factors associated with the sorghum rhizosphere through the activation of pathogenic determinants that require the diploid or dikaryotic stage to gain infective competence.

Maize plants inoculated with teliospores at root protrusion sites of the upper mesocotyl developed head smut symptoms, whereas those inoculated with sporidia did not. While these results do not rule out the formation of sporidia during the infection of maize plants, they suggest one possible entry point for the fungus with free access to the outer pericycle and just below the apical meristem. Direct penetration of maize mesocotyls by infective hyphae of *S. reilianum* was reported by Farag (5), but no additional evidence has been published.

More durable strategies for control of sorghum head smut will rely on discovery and use of host resistance (3). To that end, we need to elucidate the nature of critical infection events, the genetic basis for resistance, and the genetics of virulence in the pathogen. Studies of early infection events have not been successful due pri-

marily to the very low chance for microscopic detection of germination-penetration directly on host tissues, but the possibility of direct penetration has been suggested by Wilson (18).

Our present understanding of the genetics of head smut resistance in sorghum stems from analysis of data from repeated field trials. A long-standing proposition to discover sources of resistance, define genetic control, and identify resistance genes in segregating populations to accelerate breeding programs is the development of faster and more accurate screening techniques. One such technique was described by Edmunds (4) and identifies resistance factors at meristematic regions in any genetic background. A second technique with the features of natural infection has been sought for a long time (3,9,13,14), but the variable results obtained clearly suggest that infection of sorghum by *S. reilianum* is sensitive to changes in multiple factors of the rhizosphere.

Under natural conditions, wet soil favors fast germination of seed and little or no infection. In dry soil, teliospores germinate to form elongated hyphae in synchrony with the pace of seed germination, which increases the chances for infection. To test this hypothesis, we planted seed at an early stage of germination (18 h of imbibition) in soil at 40% moisture content (wt/wt) and covered the seed with dry teliospores mixed with soil. Disease incidence obtained in four separate experiments was 2.5 to 2.8 times higher than field values for the susceptible lines RT×7078 and B1 (36 and 40% average incidence in field experimental plots).

Disease incidence on line SC241-12E under field conditions ranged from 50 to 80%. In this study, only 39.5% of the inoculated plants developed head smut symptoms. Interestingly, most of these plants exhibited sterile panicles and phyllody, but the typical sorus was rarely formed. Under field conditions, the two head smut susceptible lines, RT×7078 and B1, averaged 36 and 40% smutted plants, respectively, at the Texas A&M University Research and Extension Center at Corpus Christi. In this study, average infection levels of 65 to 79.5% and 84 to 87.8% for RT×7078 and B1, respectively, were obtained with the Corpus Christi isolates, while the Taylor isolates infected 91.9 and 82.3% of the plants in these two lines. It is postulated that this increased infection efficiency observed with the inoculation technique described here relative to field infection values results from higher levels of inoculum in the appropriate infection court and the provision of more stable conditions conducive to infection.

Three major components of this infection assay are emphasized: inoculum, host development stage, and soil moisture. Disease escape in field trials is explained by the lack of uniformity in the distribution of

Table 3. Infection of sorghum plants by *Sporisorium reilianum* following inoculation of seedlings pregerminated for 18 h with dry teliospores

Experiment	Line	Inoculated ^y		Noninoculated	
		Tested	Infected ^z	Tested	Infected
1	RT×7078	135	123 (91.1)	45	0
	SC241-12E	109	43 (39.5)	35	0
2	RT×7078	565	357 (65.2)	125	0
	B1	370	310 (84.0)	108	0
3.1	RT×7078	112	89 (79.5)	42	0
	B1	98	86 (87.8)	31	0
3.2	RT×7078	95	88 (92.6)	25	0
	B1	102	84 (82.3)	34	0

^y Inoculations done with dry teliospores mixed with autoclaved soil (1:50 vol/vol). The following isolates were used: composite of field isolates from Taylor and Tayl-131 (experiment 1 and 3.2); composite of field isolates from Corpus Christi, CrCh-109 (experiment 2), and CrCh-103-1 (experiment 3.1).

^z Numbers in parentheses indicate percentage.

inoculum (14). The application of a homogeneous mixture of dry teliospores and fine soil above seedlings eliminates this problem. Several studies have demonstrated the susceptibility of sorghum during early development to infection by *S. reilianum* (8,13), but the precise developmental stages associated with susceptibility remained ambiguous due to limited control over soil or plant factors important for infection. Uniformity in the stage of seedling development at the time of exposure to the head smut fungus appears to be important for the efficiency of infection. The favorable influence of low soil moisture content on head smut development in maize and sorghum was documented by others (2,6,10). Soil moisture content in the present study was maintained at approximately 40% for the first 4 weeks, then increased to avoid water stress that would prevent panicle emergence.

Reduced moisture content in the soil could influence infection by a number of different mechanisms, including alteration of O₂ balance, turgor of host root tissues, the rate of cell division in roots, or enhanced adhesion of soil particles that contain teliospores to host tissues. All of these possibilities, however, remain to be investigated.

Some of the requirements for implementation of this infection assay are a source of inoculum, proper storage of dry teliospores, use of clean seeds to ensure adequate stand, and regulation of soil moisture. The assay described here offers

an opportunity to study infection events as they take place under natural conditions. While faster techniques would be ideal for head smut screening purposes in large populations, this assay is a valuable tool to detect nonmeristematic resistance in genetic backgrounds devoid of meristematic resistance factors.

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