Reproduction of *Fusarium moniliforme* Basal Stalk Rot and Root Rot of Grain Sorghum in the Greenhouse

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ABSTRACT

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Basal stalk rot and root rot of grain sorghum (*Sorghum bicolor*) were reproduced in plants grown in the greenhouse in *Fusarium moniliforme*-infested soil at optimal soil moisture until flowering, then subjected to a gradual development of severe moisture stress between flowering and the middough stage, followed by rewetting. Stalk rot did not develop and root rot was not severe in plants grown to maturity at optimal soil moisture although many of these plants were infected by *F. moniliforme*. Stalk and root rot developed in the majority of stressed plants grown in soil initially uninfested but contaminated by *F. moniliforme* after planting.

Systematic surveys indicate that *Fusarium moniliforme* Sheldon is the fungus most commonly associated with basal stalk rot and root rot of grain sorghum (*Sorghum bicolor* (L.) Moench) in New South Wales, Australia (15). The fungus was isolated consistently from plants affected by stalk rot and from a small proportion of symptomless plants. *Macrophomina phaseolina* (Tassi) Goid. and *Nigrospora sphaerica* (Sacc.) Mason, which have also been associated with stalk rot of sorghum (8,13), were isolated regularly from diseased plants during these surveys. Results indicate that in New South Wales, however, these two fungi are secondary pathogens that normally colonize the stalk after it has been infected by *F. moniliforme* (15). Basal stalk rot was usually associated with severe root rot. *F. moniliforme* and *Periconia circinata* (Mangin) Sacc. were the most common fungi isolated from diseased roots and both have been reported as root rot pathogens of sorghum (2,7,9,15).

During these surveys, we collected many plants affected by basal stalk rot and root rot from which *F. moniliforme* was the only pathogen isolated. The experiment reported in this paper was designed to reproduce the symptoms common to such plants.

The typical field symptoms of basal stalk rot and root rot associated with infection by *F. moniliforme* have been described (15,17). Symptoms usually become obvious 2–3 wk after anthesis. Diseased plants initially wilt, and within a few days, the leaves and stalks develop a bleached and dry appearance. The base of the stalk becomes soft and spongy and a purple discoloration develops internally. Subsequently, the purple discoloration fades as the pith tissue disintegrates, leaving the vascular strands loose inside the outer cylinder of the stalk, a condition Tullis (17) described as "shredded."

Hyphae, conidia, and conidiophores of *F. moniliforme* may be present on the vascular strands, which are buff to salmon in color. Lodging usually occurs at the fourth node, about 5–10 cm from the crown, any time after the stalk tissue becomes soft and spongy. The stalk is most susceptible to lodging, however, after the pith has disintegrated. Root rot usually develops simultaneously with basal stalk rot. Initially, the nodal (crown) roots become soft and deep brown and the inner tissues disintegrate, leaving a dry hollow shell that lacks all structural integrity. The symptoms described are common in at least some crops in most seasons in New South Wales. Under very dry conditions, however, soft spongy stalks desiccate rapidly, becoming hard and constricted before the pith disintegrates (15). This condition was also observed in Texas by Tullis (17). Although these stalks are quite brittle, they are less suceptible to lodging than stalks with typical symptoms of basal stalk rot.

There have been no studies on the mode of infection and colonization of sorghum by *F. moniliforme*. Regarding basal stalk rot, Tullis (17) suggested that the fungus enters the stalk at or near ground level through wounds or infected roots. He also concluded that the fungus initially colonized the stalk via the vascular bundles, from which it spread outward into the pith. Presumably, *F. moniliforme* could also infect the stalk directly from airborne inocula or rain-splashed inocula from soil. The fungus is known to persist in soil in infested crop residues (1,10), to be dispersed in the atmosphere (1,12), and to be seedborne (2).

F. moniliforme has been associated with basal stalk rot of sorghum in eastern Australia and the United States for many years (2,15,16). There is little experimental evidence, however, that it is the primary cause of the disease. Although there have been several reports that incidence of basal stalk rot is associated with dry conditions (2,17), there have been no reports of experimental studies on the role of environmental factors in disease development.

Tullis (17) reported that *F. moniliforme* caused stalk rot in several sorghum cultivars in field and greenhouse trials using his numbers one and three methods of inoculation, respectively, but made no reference to soil-moisture regimes. Although he assessed the extent of purple discoloration and the spread of fungus in inoculated stalks, he made no mention of pith disintegration, which is typical of basal stalk rot, in these trials. We were unable to reproduce typical basal stalk rot using similar inoculation procedures in two *F. moniliforme* stalk rot-susceptible sorghum hybrids grown under optimum soil-moisture regimes in Australia. The open-pollinated varieties used by Tullis (17) were not available.

Tullis (17) concluded that the incidence of basal stalk rot in the field in Texas was correlated with hot dry weather conditions. Our field observations indicate that when basal stalk rot and root rot occur, they are generally found in crops that develop under optimal or near-optimal conditions between planting and flowering but are then subjected to moisture stress. We have not observed severe basal stalk rot and root rot in dryland or irrigated sorghum plants grown with adequate soil moisture from planting until maturity. The finding that ecofallow reduced the incidence of basal stalk rot in field trials in Nebraska (4) provides additional, though indirect, experimental evidence that moisture stress may increase the incidence of this disease.

It has been postulated that severe stalk rot occurs more often in plants that have a photosynthate sink that is large relative to their capacity for photosynthesis

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and are subjected to prolonged moisture stress during the grain-filling period (2,3). Furthermore, Dodd (3) has suggested that stalk rot in sorghum is largely the result of environmental stresses and that fungi, which have been regarded as the primary causes of stalk rot, are of secondary importance.

It has been shown experimentally in sorghum that the development of charcoal rot caused by *M. phaseolina* is dependent on moisture stress after anthesis (8,11). This disease is similar to basal stalk rot and the two are often associated with each other (6,17).

This paper is a report on the reproduction of basal stalk rot and root rot in sorghum in the greenhouse using *F. moniliforme* and the role of moisture stress in disease development.

MATERIALS AND METHODS

Media and inoculum preparation. Carnation leaf-piece agar (CLA) (5) was used for isolating *F. moniliforme* from sorghum tissue. This medium consists of 2% water agar containing carnation leaf pieces sterilized by γ-irradiation and is suitable for isolating all fungi recognized as stalk and root rot pathogens of sorghum (14,15). A selective medium for *Fusarium* spp., peptone-pentachloronitrobenzene (PCNB) agar (PPA) (14), was used for isolating *F. moniliforme* from soil by the dilution-plate technique (14). A chaff-grain medium (CGM) was used to prepare inoculum for addition to soil (14). This medium consists of 50 g of oat (*Avena sativa* L.) chaff and 10 g of crushed oat grain. The chaff and grain are mixed thoroughly, soaked in water for 24 hr at 5 C, drained, and then autoclaved. The sterile medium is then transferred to a γ-irradiated polyethylene bag, which is sealed around a large cotton-wool plug. Each bag of CGM was inoculated with a 50-ml spore suspension of *F. moniliforme* using a culture grown on CLA from a germinated single conidium. The inoculated CGM was incubated in a growth room with an alternating temperature regime, 25 C day/20 C night, and a 12-hr photoperiod (14). The bag culture was shaken every 3 days for 3 wk, by which time the medium was thoroughly colonized. It was then air-dried and crushed to pass through a 2-mm mesh sieve. This inoculum was used to infest soil. The culture of *F. moniliforme* was originally isolated from a sorghum plant affected by basal stalk rot and collected near Gunnedah, New South Wales. It was isolated on CLA and maintained on this medium by single-conidium transfers.

Soil characteristics, treatment, and moisture regimes. The sorghum was grown in a gray medium-heavy clay (pH 7.5) from Narrabri, New South Wales. The characteristics of this soil have been described (18). The moisture contents of this soil at −1.0 and −10.0 bars were 33 and 23%, respectively. In this paper, the moisture content of a soil sample is expressed as a percentge of the oven-dry weight of the sample. The soil was air-dried, crushed to pass through a 0.5-cm sieve and pasteurized with steam-air at 60 C for 30 min 10 days before planting. Infested soil was prepared by thoroughly mixing dry soil with crushed dry colonized CGM in the proportion 1:100, v/v (inoculum:soil). There were two soil-moisture regimes. In one regime (optimal) the soil was maintained at or near −1.0 bar by surface-watering from planting until the grain had matured. In the other regime (late stress), the soil was maintained at or near −1.0 bar from planting until flowering (two-thirds of head at anthesis). The soil was then allowed to dry to about −10 to −15 bars by withholding water so that plants were severely stressed by the middough stage, about 14 days after flowering. A fine spray of water was used to moisten the plants and rewet the soil to saturation 20 days after flowering. This final wetting of stressed plants was suggested by M. Boosalis (*personal communication*), who noted that severe stalk rot can be associated with late stress followed by wet conditions. Soil moisture was monitored by regular weighing of containers and was adjusted to −1.0 bar every 7 days until head emergence and then every 3 or 4 days until maturity.

Containers, planting, and experimental design. Forty galvanized steel containers (40 cm diam. × 50 cm high) that held 65 kg of soil at −1.0 bar were used. Twenty containers were filled with uninfested soil to a depth of 47 cm. The other 20 containers were filled with uninfested soil to a depth of 32 cm and a 15 cm layer of infested soil was added. Ten seeds treated with thiram of a hybrid (Yates 145) known to be susceptible to *F. moniliforme* basal stalk rot were planted 3 cm deep in a circle (15 cm diam.) in the dry soil in each container. A sample of this seed (200 grains) was plated on potato-dextrose agar (PDA) and found to be free of *F. moniliforme* and other fungal pathogens of sorghum. Procedures were adopted to minimize contamination of uninfested soil with infested soil. The soil in each container was then adjusted to −1.0 bar water potential by surface-watering and capilliary action through holes in the base of the container. The 20 containers with infested soil and the 20 with uninfested soil were divided equally between the two soil-moisture regimes, giving 10 replicate containers per treatment. The containers were arranged in a randomized block design in a greenhouse room maintained at 29 ± 3 C during the day and 20 ± 3 C at night. Soil temperatures were monitored by thermistors placed 3 and 15 cm deep in each of two containers, one in the optimal soil moisture and one in the late-stress treatment. Polystyrene sheets (15 mm thick) were cut and fitted between the containers to insulate the sides from solar radiation. This restricted the diurnal changes in soil temperatures to levels similar to those recorded in the field in summer. The experiment was undertaken during the summer months so that day length and light intensities would be similar to field conditions. Composite soil samples from 10 containers of infested and 10 of uninfested soil were collected before planting and at maturity. Each sample was mixed thoroughly and 5-g subsamples were dilution-plated on PPA to estimate the number of propagules of *F. moniliforme* per gram of soil (14).

Fertilizer. A liquid fertilizer was applied to the soil at 100 ml per container at 4 and 8 wk after emergence. This fertilizer contained 76 g NH_4NO_3, 38 g $Ca_3(PO_4)_2$, 10 g KCl and 8 g K_2SO_4/L water. A foliar spray, 1 g $ZnSO_4 \cdot 7H_2O$/L water, was also applied 4 and 8 wk after emergence.

Disease assessment. Three plants were removed from each container at the three- and five-leaf stages and two at the eight-leaf stage. Some of these plants were selected at random at each growth stage for plating. The two remaining plants were removed after the grain had matured. Incidence of infection and extent of colonization of stalks by *F. moniliforme* was assessed by plating segments of tissue on CLA after plants were washed in water for 30 min and surface-sterilized in 0.3% sodium hypochlorite in 10% EtOH (roots, 4 min; stalks, 10 min). At the three-leaf stage, one segment of tissue from the mesocotyl, coleoptile, and crown of one seedling from each container was plated. At the five-leaf stage, one segment from the mesocotyl and crown of each of 20 plants, one per alternate container, were plated; the coleoptile had disintegrated at this stage. Nodal roots were not well established until after the five-leaf stage. At the eight-leaf stage, one segment from a nodal root, the crown, and the leaf-sheath of the lowest secondary leaf from each of 20 plants, one per alternate container, was plated. At maturity, one segment from each of six locations within the stalk, 2, 5, 10, 20, 30, and 60 cm above the crown, of all plants was plated. In addition, one segment from each of four lesions on the nodal roots of each mature plant was plated.

The severity of stalk rot was assessed at maturity using a 0–10 scale where 0 = normal firm stalk and 10 = stalk in which the pith had disintegrated completely. Severity of root rot was also assessed at maturity using a 0–10 scale where 0 = healthy root system and 10 = completely necrotic root system. The weight of air-dry grain from each plant was determined.

RESULTS

Infection and colonization. At the three-leaf stage, *F. moniliforme* was isolated from 95% of mesocotyl, 90% of

coleoptile, and none of the crown segments from seedlings grown in infested soil. At the five-leaf stage, the pathogen was isolated from 85% of mesocotyl and 5% of crown segments from plants in infested soil. The fungus was not isolated from plants in uninfested soil at the three- and five-leaf stages. At the eight-leaf stage, *F. moniliforme* was isolated from 70% of nodal root, 15% of crown, and 85% of leaf-sheath segments from plants in infested soil. The pathogen was also isolated from 5% of nodal root, none of the crown, and 20% of leaf-sheath segments from plants in uninfested soil. Thus, some contamination of the uninfested soil by *F. moniliforme* occurred before the eight-leaf stage. At maturity, *F. moniliforme* was isolated from 50 and 60% of segments from nodal-root lesions from plants in infested and uninfested soil, respectively, and grown under optimal soil moisture throughout. In contrast, *F. moniliforme* was isolated from 96 and 80% of such lesions from plants in infested and uninfested soil, respectively, grown under optimal soil moisture until flowering, then subjected to moisture stress followed by rewetting.

Although *F. moniliforme* was established earlier in plants grown in infested soil, there was no difference in its frequency of isolation from the stalks of mature plants grown in infested versus uninfested soil within each moisture regime (Table 1). Isolation data indicated, however, that the plants subjected to late stress were more extensively colonized by the fungus than plants grown under optimal soil moisture throughout the season (Table 1). This data also indicated that the fungus colonized further than 60 cm from the crown in a small proportion of stalks in all treatments except that involving late stress and infested soil. We failed to isolate *F. moniliforme* from some of the stalks that were rotted severely, presumably because they readily absorbed the surface-sterilant that was used. Microscopic examination of the interior of such stalks revealed microconidia similar to those of *F. moniliforme* on the loose vascular strands. The isolation data and microscopic observations indicated that the fungus was present in all stalks subjected to late stress. We believe the isolation data for stalks of plants grown under optimal soil moisture throughout reflect the true extent of colonization of these stalks.

Symptom development. At the three- and five-leaf stages, plants growing in infested soil showed slight to extensive necrosis of the mesocotyl and obviously red to purple discoloration of the coleoptile, whereas plants in uninfested soil were symptomless. At the eight-leaf stage, slight root necrosis and obvious reddening of the blades and sheaths of senescent lower leaves were observed in plants growing in infested soil. Plants growing in uninfested soil at this stage were symptomless except for a slight reddening of the sheaths of lower leaves. At maturity, severe root rot was recorded in all plants in the late-stress treatment in infested and uninfested soil (Table 2). Root rot was present but was not severe in plants grown in infested and uninfested soil under optimal soil moisture throughout.

Typical basal stalk rot developed in plants subjected to late stress followed by rewetting in both infested and uninfested soil (Table 2). The symptoms were identical to those reported in the field in Texas (17) and in Australia (15). Field symptoms are described in the introduction to this article. Stalk rot did not develop in plants grown under optimal soil moisture from planting until maturity. There was only one stalk per plant in all treatments.

The first signs of moisture stress in plants in the late-stress treatment were recorded 8–10 days after the final application of water and about 7 days after anthesis was complete. With the onset of stress, leaves faded from bright green to dull gray and the margins rolled inward. The distal 10–15 cm of the leaf then collapsed and shriveled into a grey-brown spike. A few days later, these plants senesced completely. The lower leaves were the first to show signs of stress and senesce. One or two days after the flag leaf senesced, the lower stalk and the nodal roots had become spongy and the stalk and leaves developed a distinctive bleached (straw-colored) appearance. Bleaching of the entire plant is a typical field symptom. Grain development had ceased by this stage. The stressed plants were then moistened for 24 hr with a fine spray that also wet the soil to saturation. Subsequently, there was a further

Table 1. Frequency of isolation of *Fusarium moniliforme* from stalks of mature sorghum plants grown under two soil moisture regimes in *F. moniliforme*-infested and uninfested soil in the greenhouse

	Soil-moisture regime			
	Optimal (about −1 bar) throughout growing season		Optimal (about −1 bar) until flowering, then allowed to dry (about −10 to −15 bars) to subject plants to stress followed by rewetting	
Height above crown (cm)	Infested soil	Uninfested soil[x]	Infested soil	Uninfested soil
2	9[y]	6	15[z]	13[z]
5	6	3	12[z]	15[z]
10	6	1	12[z]	11[z]
20	1	1	6	8
30	3	1	5	6
60	4	4	0	6

[x] Uninfested pasteurized soil was contaminated by *F. moniliforme* during the growing season, presumably by airborne inoculum.
[y] Number of stalks *F. moniliforme* positive of 20 stalks assayed on carnation-leaf agar.
[z] Isolation of pathogen presumed to be adversely affected by absorption of surface-sterilant by rotted stalks. Microscopic observations indicated all stalks colonized by *F. moniliforme* at these locations.

Table 2. Basal stalk and root rot severity and grain yield of stalks of mature sorghum plants grown under two soil moisture regimes in *Fusarium moniliforme*-infested and uninfested soil in the greenhouse

	Soil-moisture regime			
	Optimal (about −1 bar) throughout growing season		Optimal (about −1 bar) until flowering, then allowed to dry (about −10 to −15 bars) to subject plants to stress followed by rewetting	
	Infested soil	Uninfested soil[w]	Infested soil	Uninfested soil
Mean stalk rot severity[x]	0	0	7.0	5.5
Mean root rot severity[y]	3.7	3.6	8.4	8.0
Mean air-dry grain weight based on 20 plants (g)	34.0 a[z]	29.3 a	15.6 b	13.9 b

[w] Uninfested pasteurized soil was contaminated by *F. moniliforme* during the growing season, presumably by airborne inoculum.
[x] Based on 20 stalks and a 0–10 scale: 0 = normal firm stalk, 10 = rotted stalk in which pith has completely disintegrated, leaving loose vascular strands inside a hollow stalk.
[y] Based on 20 plants and a 0–10 scale: 0 = healthy root system, 10 = completely necrotic root system.
[z] Means followed by a common letter are not significantly different at $P = 0.01$. Data were analyzed as a randomized complete block design with subsamples.

deterioration in the integrity of the lower stalk and nodal roots. The latter developed a deep brown discoloration. Sporodochia of *F. moniliforme* formed on the base of the stalk and exposed areas of the nodal roots of a few plants. Some of the rotted stalks lodged before the final sampling and the stressed plants senesced 2–3 wk before the plants grown under optimal conditions reached maturity.

The stalks were cut longitudinally after surface-sterilization and before plating. The pith tissue of the lower 15–20 cm of rotted stalks had disintegrated completely, leaving the vascular strands loose in a hollow cylinder. In most instances, the pith tissue in the lower internodes initially developed a mauve to grey discoloration that faded to a pink-buff color as the pith disintegrated.

Grain yield. There was no statistically significant ($P = 0.01$) difference between the mean grain weight per head of plants in infested versus uninfested soil within each soil-moisture regime (Table 2). The mean grain weight per head of plants grown under optimal soil moisture throughout, however, was about twice that of plants grown under optimal soil moisture and then subjected to stress after flowering (Table 2). There was no significant ($P = 0.01$) correlation between grain yield per stalk and severity of stalk rot.

Soil temperatures. The soil temperatures recorded at 3 and 15 cm were within the range 29 ± 3 C during the day and 20 ± 3 C at night. The records indicate that temperatures at 3 cm were slightly higher than those at 15 cm during the day. The day temperatures in dry soil in the late-stress treatment were higher than temperatures in soil at −1 bar; however, there were not sufficient thermistors for accurate estimation of these differences.

Levels of *F. moniliforme* in soil. About 5×10^5 propagules of *F. moniliforme* per gram of soil were detected in the infested soil at planting using a dilution-plate technique, whereas the fungus was not detected in the uninfested soil. At maturity, about 10^4 and 10^3 propagules per gram of *F. moniliforme* were detected in the infested and uninfested soils, respectively.

DISCUSSION

Typical symptoms of basal stalk rot and root rot of grain sorghum were reproduced in plants grown under optimal soil moisture until flowering, then subjected to severe moisture stress followed by rewetting. Stalk rot did not develop in plants grown under optimal conditions from planting until maturity, although many of these plants were infected and partly colonized by *F. moniliforme*. Furthermore, the isolation data indicate that early infection and establishment of the fungus in the plant may not be essential for stalk rot development. These findings are in agreement with field observations and isolation data (15).

Infection of plants grown in uninfested soil by *F. moniliforme* can probably be attributed to the contamination of this soil and/or the plants by airborne inoculum. Such inoculum could have brought about infection directly after deposition on the stalks and exposed areas of the nodal roots or indirectly after colonization of the uninfested soil. Microconidia, which formed on necrotic-coleoptile remains and on the original inoculum exposed on the soil surface, were presumably the main source of airborne inoculum. The greenhouse air conditioning provided adequate air movement for dispersal.

The isolation data indicate that the fungus extensively colonized the stalks of plants subjected to late stress followed by rewetting and some of the stalks grown under optimal conditions. Trimboli (15) also found that rotted stalks are extensively colonized under field conditions, thus providing abundant inoculum for overseasoning. Because the fungus was able to colonize some of the symptomless plants grown under optimal soil moisture, a finding that is also in agreement with field isolation data (15), we can assume that inoculum will be available for overseasoning from crops unaffected by stalk rot.

Recovery of *F. moniliforme* from rotted stalks was less than expected on the basis of symptoms and we believe this was a result of the absorption of the surface-sterilant by the stalks. To avoid this problem, we suggest that stalks be surface-sterilized not by submerging in 0.3% sodium hypochlorite in 10% EtOH but by swabbing the stalk lightly with 95% EtOH before plating. This suggestion is being evaluated.

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