Effects of conidial concentrations of *Fusarium moniliforme* on the growth of sorghum seedlings under greenhouse conditions

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Summary

Four sorghum cultivars were exposed to increasing concentrations of *Fusarium moniliforme* Sheldon. When grown in a greenhouse in pasteurized soil for eight weeks under optimum conditions, plant height and shoot and root growth of cv. DeKalb-59 sorghum seedlings were reduced by inoculum concentrations ≥ 1×10^3 conidia g^{-1} of soil mixture. Shoot dry weights of cvs. Asgrow 504, Contender and Seneca were reduced by inoculum concentrations as low as 10 conidia g^{-1} of soil mixture after nine weeks of growth. *F. moniliforme* infected sorghum at, or shortly after, emergence and caused seedling death or reduced seedling vigour. Effects incited by *F. moniliforme* during early stages of sorghum development were not compensated during later growth stages.

Introduction

Fusarium root and stalk rot is a serious disease of sorghum (Edmunds and Zummo 1975, Frederiksen 1986). The causal fungus, *Fusarium moniliforme* Sheldon, is distributed worldwide and is pathogenic to many crops, especially members of the Gramineae (Edmunds and Zummo 1975, Frederiksen 1986, Sumner 1968, Zummo 1984). *F. moniliforme* causes stalk rot, leaf blight, damping-off, seed rot and seedling blight of sorghum (Frederiksen 1986, Zummo 1984, Cuarezma-Teran 1984). The fungus also causes severe lesion development on the seminal root system and the mesocotyl (Burgess *et al.* 1981). Slight to extensive necrosis of the mesocotyl and red to purple discoloration of the coleoptile may develop on seedlings growing in infested soil (Trimboli and Burgess 1983).

Low soil temperatures, wet soil, high evapotranspiration, deep planting and poor seedbed tilth contribute to delayed germination and emergence and encourage seedling blight development (Burgess *et al.* 1981). These conditions enhance the survival of *F. moniliforme* (Nyvall and Kommedahl 1970). *F. moniliforme* can overwinter as conidia and mycelia in the soil and on crop debris (Manzo and Claflin 1984, Sumner 1968, Reed *et al.* 1983) which serve as primary sources of inoculum. Airborne conidia are also an important inoculum source in disease epidemiology. *F. moniliforme* is seedborne, but this inoculum source is not important for disease development (Manzo and Claflin 1984, Edmunds and Zummo 1975, Frederiksen 1986, Zummo 1984).

The pathogenic activity of this fungus, and the growth of saprophytic organisms which hasten the decay of crop residue, are enhanced by moderate temperatures in the spring and early summer (Manzo and Claflin 1984). Thus, environmental conditions which encourage plant growth also promote microbial activity and reduce the availability of substrate for long-term survival of microorganisms.

Fusarium moniliforme is the most frequently isolated *Fusarium* spp. from sorghum root and crown tissues in Mississippi (Cuarezma-Teran 1984, Bain 1973), Nebraska (Sumner 1968, Reed *et al.* 1983), Texas (Tullis 1951), Australia (Trimboli and Burgess 1983) and West Africa (Zummo 1984). The frequent occurrence of *F. moniliforme* in root and stalk tissues of sorghum is well documented. The mode of infection and colonization and inoculum densities which may affect sorghum growth require further investigation. Results of such investigations may be important in breeding programs for disease resistance or to establish inoculum potential in fields prior to sowing. This study was conducted to determine the response of seedling sorghum cultivars grown in a greenhouse to increasing inoculum concentrations of *F. moniliforme*.

Materials and methods

The isolate of *F. moniliforme* used in these studies was obtained from sorghum growing at the Plant Science Research Center at Mississippi State University, Starkville, MS, USA and was stored on potato dextrose agar (PDA) slants with an oil overlay. The culture was transferred to fresh PDA and grown at 20°C on a laboratory bench for three weeks. Conidia were obtained by adding 10 mL of sterile distilled water to a 9 cm diameter Petri plate culture and scraping the mycelial surface gently with a sterile dissecting needle. The resultant suspension was strained through four layers of cheesecloth into a 250 mL beaker. The conidial concentration was determined with a haemocytometer, and inoculum concentrations were prepared by serial dilution with sterile distilled water.

Samples of corncob grits (300 g) were autoclaved at 104 kPa for 20 minutes (Batson and Trevathan 1988). After cooling, each grit sample was infested by spraying 50 mL of inoculum evenly on the grit particles rotating in a seed-treater. Infested grits were spread onto sterile paper towels, placed under a laminar flow hood and allowed to air dry for 16 hours. Each sample of infested grit particles was thoroughly mixed with 1.7 kg of sterile soil mixture.

Sorghum cv. DeKalb-59 (DK-59) was exposed to inoculum concentrations of 0, 1×10^3, 1×10^4 and 1×10^5 conidia g^{-1} of soil mixture in the first experiment. Asgrow cvs. A504, Contender and Seneca, were used for all tests in the second experiment and were exposed to inoculum concentrations of 0, 10, 1×10^2 and 1×10^3 conidia g^{-1} of soil mixture. Ten seeds treated with thiram fungicide were planted into 15 cm diameter clay pots containing a pasteurized mixture of soil and sand (1:1 v/v). Osmocote, 14-14-14 NPK, fertilizer (Sierra Chemical Company, 1001 Yosemite Drive, Milpitas, CA 95035, USA) was applied at the rate of 2 mg g^{-1} of soil mixture. Approximately 300 g of the infested soil-grit mixture was added to the top 2 cm of each pot to cover the seeds. To minimize pot-to-pot contamination, an additional 1 cm layer of sterile soil mixture was added to cover the infested soil in each pot. All experiments were conducted during the spring in a greenhouse. Plants were thinned to two per pot 1 week after emergence. Plant height from the soil-line to the tip of the uppermost blade was measured weekly for eight weeks after planting in the first experiment and at 2, 3, 6 and 9 weeks after planting in the second experiment. Seedling emergence data were recorded seven days after planting in the second experiment. Plants were uprooted, shaken free of soil, and shoot and root fresh weights were recorded at the end of each experiment. Shoots and roots were placed in paper bags and dried in a hot air drier at 40°C for 96 hours, after which dry weights were recorded.

Incidence of infection and extent of colonization by *F. moniliforme* were determined at termination of the second experiment by plating root and crown tissue segments on carnation leaf agar (CLA) medium. Four root sections, 5 mm long, were excised from the interface of apparently healthy and symptomatic tissues. A 5 mm stem section was taken above the point of attachment of the first leaf. Prior to

Table 1. Plant height, shoot and root fresh and dry weights of sorghum cv. DeKalb-59 exposed to increasing inoculum concentrations of *Fusarium moniliforme* for eight weeks in a greenhouse.

Inoculum concentration[A]	Height[B] (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
0	63 a[C]	6.5 a	1.44 a	3.4 a	2.98 a
1×10^3	60 b	5.7 b	1.25 bc	2.3 b	1.12 b
1×10^4	59 b	5.2 b	1.12 c	2.1 b	0.86 b
1×10^5	60 b	5.7 b	1.28 b	2.2 b	0.96 b
FPLSD	2	0.6	0.15	0.3	0.57

[A] Conidia per gram of soil mixture.
[B] Measured from the base of the plant to the tip of the uppermost leaf.
[C] Means represent results of two experiments. Means in columns followed by the same letter are not significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$).

Table 2. Comparison of parameter estimates for the rate of increase in height and of mean height of sorghum cv. DeKalb-59 at different inoculum concentrations of *Fusarium moniliforme* over eight weeks.

Inoculum concentration[A]	Mean height (cm)	Slope[B] (b_1)
0	44 a[C]	8.21
1×10^3	41 b	8.12
1×10^4	40 b	7.86
1×10^5	41 b	7.95

[A] Conidia per gram of soil mixture.
[B] Slopes (b_1) are different from 0; H_0: slope = 0 at $P \leq 0.05$.
[C] Means not followed by a common letter are significantly different according to the t-test as calculated by the LSMeans option of SAS® Release 6.03.

Table 3. Comparison of parameter estimates for the rate of increase in height and of mean height of three sorghum cultivars exposed to different inoculum concentrations of *Fusarium moniliforme* over nine weeks.

	Cultivars					
	Asgrow 504		Contender		Seneca	
Inoculum concentration[A]	Mean height (cm)	b_1	Mean height (cm)	b_1	Mean height (cm)	b_1
0	71.04	11.11[B]	71.70 a[C]	12.16	67.62	11.33
10	69.02	10.80	66.54 b	12.60	65.53	12.09
1×10^2	70.42	12.80	60.89 c	11.51	65.26	11.18
1×10^3	68.40	10.99	66.16 b	11.22	65.32	11.42

[A] Conidia per gram of soil mixture.
[B] Slopes (b_1) are different from 0; H_0: slope = 0 at $P \leq 0.05$.
[C] Means within a column not followed by a common letter are significantly different according to the t-test as calculated by the LSMeans option of SAS® Release 6.03.

Table 4. Effect of *Fusarium moniliforme* on shoot dry weight of sorghum nine weeks after planting.

Inoculum concentration[A]	Shoot dry weight (g)
0	48.7 a[B]
10	39.7 b
1×10^2	37.7 b
1×10^3	40.4 b
FPLSD	7.4

[A] Conidia per gram of soil mixture.
[B] Means not followed by a common letter are significantly different according to Fisher's protected least significant difference test ($P \leq 0.05$).

plating, samples were washed in water, placed in 100% ethyl alcohol (EtOH) for three seconds, surface disinfested in 0.5% sodium hypochlorite for 90 seconds, rinsed in sterile distilled water and blotted dry on sterile filter paper. Stem sections were sliced into four cross sections, 2 mm thick. Plated tissue samples were incubated on a laboratory bench for three weeks. A scale of 0–4 was used to rank colonization by *F. moniliforme*, where 0 = no colonization, and 1, 2, 3 or 4 colonies, respectively, on each culture plate.

Each experiment was repeated and data were combined for analysis. Plant height, fresh and dry weights of shoots and roots, and per cent shoot and root colonization were subjected to analysis of variance. Means were separated by Fisher's protected least significant difference (FPLSD) test (Steele and Torrie 1980, Gilligan 1986). Repeated plant height measurements were analysed by regression analysis. Per cent seedling emergence data were transformed to arcsin then subjected to analysis of variance and means were separated by FPLSD test.

Results

The effect of treatment with *F. moniliforme* was significant for all growth parameters measured on cv. DK-59. Results were similar for repetitions of this experiment and data were combined for presentation in Table 1. When cv. DK-59 was exposed to *F. moniliforme* at inoculum concentrations $\geq 1 \times 10^3$ conidia g^{-1} of soil mixture under optimum growing conditions in a greenhouse, plant height and shoot and root growth were significantly reduced after eight weeks. During destructive sampling at termination of the experiment, affected plants were chlorotic and necrosis was observed on the root systems.

Slopes for the rate of increase in height of cv. DK-59 exposed to different inoculum concentrations were all significantly different from zero (Table 2). Comparison between slopes showed no significant differences between the growth rate of untreated control plants and treated plants. Mean height over eight weeks was significantly greater for control plants than for plants grown in soil mixture containing *F. moniliforme*.

Plant heights after nine weeks ranged from 107 to 119 cm for cvs. A504, Contender and Seneca. Slope parameters of plant growth over the nine weeks were not affected by treatments. Mean height of all three cultivars was reduced; height of cv. Contender was lowered significantly by treatment with *F. moniliforme* (Table 3).

Shoot fresh weights did not differ significantly after nine weeks among inoculum concentrations, but the mean for cv. Contender was significantly lower than that of cv. Seneca (123, 121 and 108 g for Seneca, A504 and Contender,

respectively). Shoot dry weights were affected by treatment with *F. moniliforme* and were significantly reduced by all inoculum concentrations (Table 4).

Cultivar and inoculum concentration significantly affected root fresh weight at concentrations of 10 conidia g^{-1} of soil mixture or greater (Table 5). The reductions were statistically significant for cv. A504. Root fresh weight of cv. Contender was lower than for cvs. A504 or Seneca. There were no significant main effects for any variables tested for root dry weights. Root dry weights were reduced by all *F. moniliforme* inoculum concentrations, but differences were not significant.

Cultivar and inoculum concentration significantly affected the emergence of seedlings seven days after planting. Emergence of cvs. A504 and Seneca was greater than emergence of cv. Contender (Table 6). Inoculum concentrations of 10 and 1×10^2 conidia g^{-1} of soil mixture significantly reduced the emergence of cvs. Seneca and Contender, respectively, seven days after planting.

There was no significant effect of cultivar on shoot or root colonization by *F. moniliforme*. All conidial concentrations resulted in significantly increased levels of shoot and root colonization (Table 7). In the absence of soil inoculation, colonization of shoots was twice as high as root colonization. Colonization of shoots and roots was similar when *F. moniliforme* inoculum was added to the soil mixture.

Table 5. Root fresh weight of sorghum cultivars exposed to increasing inoculum concentrations of *Fusarium moniliforme* nine weeks after planting in a greenhouse.

Inoculum concentration[A]	Root fresh weight (g)		
	Asgrow 504	Contender	Seneca
0	39.4 a[B]	31.4	35.7
10	31.3 b	27.8	33.4
1×10^2	28.9 b	27.1	30.8
1×10^3	32.4 b	29.6	31.1
FPLSD	6.8	n.s.	n.s.

[A] Conidia per gram of soil mixture.
[B] Means in columns not followed by a common letter are significantly different according to Fisher's protected least significant difference test (P≤0.05).
n.s. = not significant.

Table 6. Effect of inoculum concentration of *Fusarium moniliforme* on percent seedling emergence of three sorghum cultivars seven days after planting.

Inoculum concentration[A]	Seedling emergence (%)		
	Asgrow 504	Contender	Seneca
0	92	67 a[B]	92 ab
10	55	33 ab	57 c
1×10^2	72	27 b	65 bc
1×10^3	67	68 a	100 a
FPLSD	n.s.	26	27

[A] Conidia per gram of soil mixture.
[B] Means in columns not followed by a common letter are significantly different according to Fisher's protected least significant difference test (P≤0.05).
n.s. = not significant.

Table 7. Colonization of shoots of sorghum plants following exposure to increasing inoculum concentrations of *Fusarium moniliforme* for nine weeks.

Inoculum concentration[A]	Shoot colonization	Root colonization
0	63.2 b[B]	33.3 b
10	82.6 a	88.2 a
1×10^2	86.1 a	86.8 a
1×10^3	86.8 a	91.0 a
FPLSD	10.7	10.0

[A] Conidia per gram of soil mixture.
[B] Per cent colonization from twelve plants of three cultivars in two repetitions. Means in columns not followed by a common letter are significantly different according to Fisher's protected least significant difference test (P≤0.05)

Discussion

Trimboli and Burgess (1983) previously reported that symptoms of basal stalk rot of field-grown sorghum occurred between planting and flowering when crops developed under optimal or near-optimal conditions and were subsequently subjected to moisture stress. Root rot occurred on sorghum plants grown in soil infested with *F. moniliforme* under optimal soil moisture conditions, but disease expression was not severe. Field observations indicated root rot was not significant prior to anthesis. Superficial lesions, which gave the appearance of severe root rot, affected only the outer cortex, and the stele was not damaged (Trimboli and Burgess 1985).

In addition to symptom development on roots, a response to *F. moniliforme* was recorded on cv. DK-59 seedlings to 1×10^3 conidia g^{-1} of soil mixture for all growth parameters measured. We postulated that the soil inoculum density threshold for effects of *F. moniliforme* on seedling sorghum could be below this concentration under conditions described for this experiment. Results of the second experiment with cvs. A504, Contender and Seneca confirmed the soil inoculum density threshold to be below 1×10^3 conidia g^{-1} of soil mixture, and responses to concentrations as low as 10 conidia were possible depending on the growth parameter measured.

Based on results of our studies, *F. moniliforme* infects sorghum at or shortly after emergence causing seedling death or reducing seedling vigour under optimal or near-optimal growing conditions. This conclusion is based in part on emergence data recorded seven days after planting for cultivars grown for nine weeks. Although slopes of the growth rate of cv. DK-59 over eight weeks were not significantly different, they were not identical. Mean height of plants exposed to the various treatments was significantly different from untreated controls, suggesting that effects incited early during sorghum development were not compensated for during later growth stages. The response of plants grown for nine weeks supports the findings of those grown for eight weeks. The fact that mean height responses were only significant for cv. Contender and not for cvs. A504 or Seneca, may indicate a genetic influence.

Fusarium moniliforme has been isolated from symptomless sorghum plants (Trimboli 1981). Trimboli and Burgess (1983) attributed the infection of plants grown in noninfected soil to contamination by airborne inoculum. This is a likely source of infection of plants grown in noninfested soil mixture in our study since such sources could not be excluded in the greenhouse. Additionally, the higher colonization frequency of shoots

compared to roots supports the likelihood of an airborne, exogeneous inoculum source.

Trimboli and Burgess (1983) reported that the response of sorghum to *F. moniliforme* was dependent upon the parameter measured. They found no statistically significant difference between the mean grain weight of plants grown in infested or noninfested soil exposed to several soil-moisture regimes. Although not grown to maturity, the plants in our study had significantly different growth patterns in early developmental stages. This may be particularly important to producers who grow sorghum principally as forage. Whether these plants subsequently express differences in grain yield is undetermined at this time. Our results confirm the necessity to measure several growth parameters to account for natural variability and accurately assess the effects of organisms such as *F. moniliforme* on crop growth. It is generally accepted that *F. moniliforme* infects sorghum in early developmental stages and subsequently takes advantage of preferential growth conditions to incite disease. Our results support early, measurable effects on sorghum seedlings which occur even under optimum growing conditions.

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