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Mycotoxigenic Potential of Ten *Fusarium* species Grown on Sorghum and *in vitro*

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Abstract: The objective of this study was to determine the mycotoxigenic potential of 12 *Fusarium* isolates (10 species), including six isolates (4 species) from sorghum. The species were: *F. thapsinum*, *F. semitectum*, *F. proliferatum* and *F. chlamydosporum* isolated from molded sorghum seed; *F. poae*, *F. graminearum* and *F. sporotrichioides* from barley seed with *Fusarium* head blight; *F. acuminatum* from wheat seed; *F. verticillioides* from infected corn seed; and *F. nygamai* isolated from soil. Fumonisin and zearalenone concentrations were measured following incubation on autoclaved sorghum seed for 21 days at 25°C, while fusaric acid was measured in mycelia harvested from Czapek Dox broth cultures. *F. thapsinum* (SC8 and CS121) and *F. semitectum* (SC7) produced fusaric acid only (4.59-64.13 mg g^{-1}). *F. graminearum* (KB172) and *F. semitectum* (CS152) produced zearalenone only (73.4 and 799.3 μg g^{-1}, respectively). *F. proliferatum* (CS183), *F. verticillioides* (TX02) and *F. nygamai* produced both fumonisin (1.92-6.05 μg g^{-1}) and fusaric acid (39.4-234.17 mg g^{-1}). *F. poae* (KB652), *F. acuminatum* (Ark), *F. chlamydosporum* (CS102) and *F. sporotrichioides* (KB662) did not produce any of these three mycotoxins. Five of the six *Fusarium* isolates (three species) isolated from sorghum had mycotoxigenic potential. *Fusarium* spp. naturally occurring on sorghum in the field have the potential to contribute to mycotoxin contamination, either singly or in combination.

Key words: *Fusarium verticillioides*, *Sorghum bicolor*, mycotoxin, fumonisin, zearalenone, fusaric acid

INTRODUCTION

The mycotoxins, fumonisin, zearalenone and fusaric acid, are secondary metabolites produced by a number of *Fusarium* species and are frequent contaminants of grains of several plants (Abbas *et al*., 1999; Benneth and Klich, 2003; Desjardins, 2006; Miller, 1995; Sweeney and Dobson, 1998). Fumonisin contamination of sorghum has been identified in many locations, including Brazil (da Silva *et al*., 2000), India (Bhat *et al*., 1997; Shetty and Bhat, 1997), Ethiopia (Ayalew *et al*., 2006) and Botswana (Siame *et al*., 1998). Zearalenone-contaminated sorghum poisoned pigs in Australia (Blaney *et al*., 1984). Zearalenone contamination of sorghum has been documented in Colombia (Diaz and Céspedes, 1997), as well as in the United States (Bowman and Hagler, 1991; Hagler *et al.,* 1987; McMillian *et al*., 1983; Schroeder and Hein, 1975; Shotwell *et al*.,1980).

Fusaric acid, produced by several *Fusarium* species, was first noticed for its phytotoxicity to rice (Desjardins, 2006). This toxin is a pathogenicity factor, causing wilting in tomato plants infected with *F. oxysporum* f. sp. *lycopersici* (Fakhouri *et al*., 2003). To date, no outbreaks of human or animal disease have been linked to consumption of foods or feeds contaminated with fusaric acid (Desjardins, 2006). However, a synergistic toxic interaction of fusaric acid and fumonisin on developing chicks has been reported (Bacon *et al*. (1995). There is a lack of information of the ability of *Fusarium* species isolated from sorghum to produce fusaric acid, in comparison with fumonisin and zearalenone. Thus, this study was initiated to assess the ability of four *Fusarium* species isolated from sorghum to produce these mycotoxins, in comparison with six other species from other sources.

Single-spore cultures of *F. thapsinum*, *F. semitectum*, *F. proliferatum* and *F. chlamydosporum* isolated from sorghum seed with grain mold, *F. poae*, *F. graminearum* and *F. sporotrichioides* from barley kernels with symptoms of *Fusarium* head blight,

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F. acuminatum from wheat, *F. verticillioides* from corn kernels and *F. nygamai* isolated from soil and maintained on dried, colonized Whatman No. 2 filter papers stored in a freezer at -7°C were used in this study. Identities of isolates from sorghum were confirmed by Jane Juba at the *Fusarium* Research Center, The Pennsylvania State University.

Fumonisin and zearalenone production by isolates was evaluated by growing them on sorghum seed. Three hundred milliliters of water was added to 100 g of sorghum seed in 500 mL Erlenmeyer flasks and placed on laboratory benches for 36-48 h. The flasks were then drained and autoclaved at 121°C for 30 min and then autoclaved again on the following day. Flasks were inoculated with plugs from agar cultures and incubated for 21 days at 25°C. Every 3 days, the flasks were shaken thoroughly to facilitate complete colonization by the isolate. Three flasks were used per isolate. Non-inoculated, autoclaved sorghum was included as a control. The experiment was repeated once. Fumonisin and zearalenone were quantified using a competitive direct enzyme linked immunosorbent assay in a microwell format, following recommended protocol (Neogen Corp., Lansing MI).

To measure fusaric acid production by isolates, conidia (2×10^5) were added to 250 mL flasks containing 50 mL Czapek Dox liquid medium and incubated at ambient room temperature with shaking (250 rpm). Three flasks were used per isolate. Fusaric acid was quantified using a modification of the method of Notz *et al*. (2002). The concentration of fusaric acid in each flask was expressed in mg g^{-1} dry weight of mycelia after 15 days of incubation.

Data for the levels of fumonisin, zearalenone and fusaric acid were analyzed using the command PROC ANOVA (SAS version 9.1, SAS Institute, Cary, NC) to determine the differences in mycotoxin production by the *Fusarium* species. Mean comparisons were based on Tukey's Studentized Range test at the 5% probability level.

Fumonisin was produced in sorghum seed cultures by *F. nygamai*, *F. proliferatum* and *F. verticillioides*, but not by the other *Fusarium* species (Table 1). *Fusarium nygamai* produced a significantly higher level of fumonisin than *F. verticillioides*. Zearalenone was produced in sorghum seed cultures only by *F. semitectum* (CS152) and *F. graminearum* (Table 1). Six of the 12 *Fusarium* species in this study produced fusaric acid in Czapek Dox broth (Table 1). *Fusarium proliferatum* produced the highest level of fusaric acid, 234.17 mg g^{-1} dry weight of mycelia; whereas *F. thapsinum* (CS121) produced the lowest level, 4.59 mg g^{-1} dry weight of mycelia. The other species producing fusaric acid included *F. nygamai*, *F. semitectum* (SC7), *F. verticillioides* and *F. thapsinum* (SC8).

This study was initiated to assess the mycotoxin-producing potential of ten *Fusarium* species when cultured on sorghum. Four species were obtained from sorghum kernels with grain mold. Six species were capable of producing at least one type of mycotoxin. Our findings confirm the mycotoxin-producing capability of *Fusarium* spp. reported by other workers (Abbas *et al*., 1999; Bennett and Klich, 2003; Desjardins, 2006; Sweeney and Dobson, 1998). Additionally, we report for the first time the production of fusaric acid by an isolate of *F. semitectum* (SC7).

We found quantitative differences in toxin-producing potential among isolates of *F. thapsinum*. This confirms earlier work with other species. All 8 isolates of *Fusarium* (Gibbosum and Semitectum) isolated from the sorghum seed produced varying amounts of zearalenone on autoclaved sorghum, with one isolate producing 3030 $\mu g\ g^{-1}$ (McMillan *et al*., 1983). Three of five isolates of *F. equiseti* produced 0.363-0.667 $\mu g\ g^{-1}$ zearalenone in sterile corn culture (Shotwell *et al*., 1980). Fumonisin concentrations on sorghum seed inoculated with *F. moniliforme* ranged from 8.25-125.31 $\mu g\ g^{-1}$ (Bhat *et al*., 2000), while 47% of *F. moniliforme* isolates from Burundi sorghum samples produced 3-374 $\mu g\ g^{-1}$

Table 1: Production of mycotoxins by *Fusarium* species

Fusarium sp.	Source	Code	Fumonisin ($\mu g\ g^{-1}$)	Zearalenone ($\mu g\ g^{-1}$)	Fusaric acid (mg g^{-1})
F. acuminatum	Wheat	Ark	ND[1]	ND	ND
F. chlamydosporum	Sorghum	CS102	ND	ND	ND
F. graminearum	Barley	KB172	ND	73.4b[2]	ND
F. nygamai	Soil	6.05a[2]		ND	109.98b
F. poae	Barley	KB652	ND	ND	ND
F. proliferatum	Sorghum	CS183	4.85ab	ND	234.17a
F. semitectum	Sorghum	CS152	ND	799.3a	ND
F. semitectum	Sorghum	SC7	ND	ND	64.13bc
F. sporotrichioides	Barley	KB662	ND	ND	ND
F. thapsinum	Sorghum	SC8	ND	ND	9.98bc
F. thapsinum	Sorghum	CS121	ND	ND	4.59bc
F. verticillioides	Corn		1.92b	ND	39.40bc

[1]ND = non-detectable, [2]Means within a column followed by the same letter (s) are not significantly different based on Tukey's Studentized Range test at the 5% probability level

fumonisins in rice culture (Munimbazi and Bullerman, 1996). Leslie *et al.* (2005) evaluated five *Fusarium* species from sorghum for toxigenicity. These species, until recently, were grouped as *F. moniliforme*. They found that *F. nygamai* and *F. verticillioides* produced fumonisin, while *F. andiyazi*, *F. pseudonygami* and *F. thapsinum* did not. *F. graminearum* isolated from sorghum in Australia produced zearalenone in culture on either maize or sorghum (Blaney and Dodman, 2002). The isolate of *F. graminearum* used in this study originated from barley.

Factors affecting *Fusarium* mycotoxin production in sorghum in the field are not well known. Hagler *et al.* (1987) found zearalenone contamination associated with wet weather during flowering and grain fill, but there was no head blight and grain mold was not severe. Grain mold was significantly correlated in two combined years with zearalenone contamination in several locations in North Carolina, with levels of contamination up to 3099 ng g^{-1}, but contamination also occurred in the absence of grain mold (Bowman and Hagler, 1991).

Mixtures of mycotoxins in grains can occur and there is the possibility of interactions in some cases (Miller, 1995). In addition to contamination in the field, there is also the possibility of post-harvest accumulation of mycotoxins. Areas with poor food handling procedures, poor grain storage, the presence of toxigenic fungi and rain at the time of harvest are factors contributing to mycotoxin contamination (Abbas *et al.*, 1999; Benneth and Klich, 2003; Bhat *et al.*, 1997).

This study evaluated the mycotoxigenic potential of ten *Fusarium* species in pure culture. Toxigenicity under field conditions could be different and could be affected by factors such as weather, timing of infection and interaction with other microorganisms.

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