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Mycotoxins produced by *Fusarium proliferatum* and *F. pseudonygamai* on maize, sorghum and pearl millet grains *in vitro*

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Highlights

- *Fusarium proliferatum* was isolated from maize and sorghum in Nigeria
- Irrespective of source, strains produce the same toxins on maize, sorghum or millet
- About half of the strains recovered from maize produce little or no fumonisins
- All of the strains recovered from sorghum produce fumonisins in culture
- **Some strains produced little or none of both toxins**

Abstract

Maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) are basic staple foods for many rural or poorer communities. These crops are susceptible to plant diseases caused by multiple species of *Fusarium*, some of which also produce mycotoxins, including fumonisins and moniliformin that are detrimental to both humans and domesticated animals. Eighteen potentially toxigenic *Fusarium* strains were isolated from maize (n = 10), sorghum (n = 7) and pearl millet (n = 1) growing in the same field in Nigeria. The 17 strains from maize and sorghum were all *F. proliferatum* and the one strain from pearl millet was *F. pseudonygamai*. Under conducive conditions, the 17 *F. proliferatum* strains produced fumonisins, 11 in relatively large quantities (700–17,000 mg total fumonisins, *i.e.*, $FB_1 + FB_2 + FB_3$/kg culture material), and six at < 45 mg/kg. Ten *F. proliferatum* strains produced > 100 mg of moniliformin per kg culture material with a maximum of 8,900 mg/kg culture material. All strains could use all grains for growth and toxin production, regardless of the host from which they were isolated. Isolates varied in the amount of toxin produced on each substrate, with toxin production a property of the strain and not the host from which the strain was recovered. However, the extent to which a

toxin-producing phenotype could be altered by the grain on which the fungus was grown is consistent with subtle genetic × environment interactions that require a larger data set than the one presented here to rigorously identify. In conclusion, there is significant variation in the ability of strains of *F. proliferatum* to produce fumonisins and moniliformin on maize, sorghum and millet. If the amount of toxin produced on the various grains in this study reflects real-world settings, *e.g.*, poor storage, then the consumers of these contaminated grains could be exposed to mycotoxin levels that greatly exceed the tolerable daily intakes.

Keywords: fumonisin, moniliformin, *Fusarium*, subsistence farming

Abbreviations:

FB: Fumonisin B mycotoxins

FB_1: Fumonisin B_1

FB_2: Fumonisin B_2

FB_3: Fumonisin B_3

HPLC: High-performance liquid chromatography

MON: Moniliformin

PMTDI: Provisional maximum tolerable daily intake

1. Introduction

The occurrence of *Fusarium* species on staple cereal crops and their ability to produce mycotoxins that have detrimental health effects for both humans and animals make it important to evaluate their potential toxin production on diverse crops intended for human and animal consumption (Cendoya et al., 2017; Marasas et al., 2012; Vismer et al., 2004). Maize (*Zea mays*), sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) are staple cereal crops grown as human foods and animal feeds worldwide, with one of these grains often serving as the primary calorie source for subsistence farmers in Africa, South Asia or Central and South America.

These crops are subject to fungal diseases such as stalk rots, ear rots and grain mold, which may cause great economic losses (Frederiksen and Odvody, 2000; Jidda, 2017; Leslie, 2003; Munkvold and White, 2016). Multiple species in the genus *Fusarium* can cause **grain mold** and ear rots of maize, sorghum and millet. The most important species associated with maize are *Fusarium verticillioides* and *F. proliferatum*, whereas with sorghum and millet diseases the most important species are *F. thapsinum*, *F. proliferatum*, *F. andiyazi* and *F. pseudonygamai*, and, to a lesser extent, *F. verticillioides*, *F. nygamai* and *F. napiforme* (Leslie et al., 2005; Leslie and Summerell, 2006). *Fusarium* species produce numerous mycotoxins, including fumonisins and moniliformin that have negative health effects on both humans and domesticated animals that consume the agricultural crops infected with these fungi (Marasas et al. 2008; Nagaraj et al., 1996; Peltonen et al., 2010).

Fumonisins have been widely studied since their discovery in 1988 (Gelderblom et al., 1988). In humans, fumonisins are considered possibly carcinogenic to humans (a Group 2B carcinogen) by IARC (Ostry et al., 2017), and are associated with neural tube defects in newborns (Gelineau-van Waes et al., 2009). Toxin production is subject to both genetic (Proctor et al.,

2004) and environmental (Marín et al., 2004) controls, with most of these studies conducted with *F. verticillioides*. Strains of *F. proliferatum* that produce high and low amounts of fumonisins are well-known from field settings (Leslie et al., 1992; Nelson et al., 1992; Thiel et al., 1991). Numerous environmental conditions can alter fumonisin production by *F. proliferatum* including substrate (Cendoya et al., 2017; Lazzaro et al., 2013; Li et al., 2017a), light (Fanelli et al., 2011; Matic et al., 2013), pH (Keller et al., 1997; Li et al., 2017b), water potential (Marín et al., 1995; Samapundo et al., 2005), oxygenation (Keller et al., 1997) temperature (Ryu et al., 1999; Samapundo et al., 2005), and exposure to preservatives (Marín et al., 1999; Etcheverry et al., 2002) or plant extracts (Stepien et al., 2015), amongst others.

Moniliformin was first described in 1973 (Cole et al., 1973) but has been studied much less extensively than have the fumonisins. Moniliformin inhibits thiamine pyrophosphatase dependent enzymes in the tricarboxylic acid cycle (Pirrung et al., 1996). The highest reported production of moniliformin is by isolates of *F. nygamai* from millet in Africa (4,300-18,200 mg/kg)(Marasas et al., 1991). In general, poultry are the most sensitive animals to moniliformin and problems resulting from exposure to this toxin often are those heart associated (Gruber-Dorninger et al., 2017). Moniliformin is not currently viewed as a major health threat in Europe given levels in normal diets there (Peltonen et al., 2010), but human exposure to the toxin in subsistence diets could be much higher. **For this reason, investigation into its production by fungal strains isolated from African crops is important.**

The equivalence of strains recovered from different hosts in terms of toxin production is usually assumed. This assumption is difficult to test because there are other variables that can confound the comparisons such as geographic and climate differences. The group of strains in this study were isolated from three different hosts growing in adjacent rows on the same farm in

Nigeria (Vismer et al., 2015). Thus, neither geographic nor climate variables are present for the origins of the strains used in our comparisons. Similarly, there are known differences in the amount of toxin that can be recovered from individual contaminated grains. There are two possible hypotheses regarding the differences. One is that differences result from differences between the strains that colonize the different host plants. Alternatively, nutrient composition and accessibility within the grain may differentially enhance or inhibit toxin production.

The objective of this study was to determine whether isolates of *Fusarium* from maize, sorghum and pearl millet could produce fumonisins and/or moniliformin on these grains *in vitro*, and whether the amounts of toxin produced varied by strain, substrate or both. Our working hypothesis was that all of the strains would produce one or both toxins on all three grains and that there would be a hierarchy in the capacity of strains to produce toxins that would not be affected by the substrate upon which the strain was grown. This study advances the field by identifying potential genotype × environment interactions for toxin production by strains of *F. proliferatum*, and confirming the threat posed by contaminated grains to consumers of these grains.

2. Materials and methods

2.1 Source of Fusarium *strains*

Eighteen fungal strains were evaluated. These strains were from cereals grown in Nigeria (Vismer et al., 2015) – ten of *F. proliferatum* from maize, seven of *F. proliferatum* from sorghum, and a *F. pseudonygamai* isolate from pearl millet (Table 1).

Table 1. Fumonisin production (mg/kg) by *Fusarium proliferatum* and *F. pseudonygamai* strains isolated from Nigerian grain and grown on maize, white sorghum, red sorghum and pearl millet patties. Results given as fumonisin B_1, total fumonisins, and the relative amounts of fumonisins B_1, B_2 and B_3.

MRC No.[1]	Maize			White Sorghum			Red Sorghum			Pearl Millet		
	FB_1	**Total**	**FB_1:FB_2:FB_3**	**FB_1**	**Total**	**FB_1:FB_2:FB_3**	**FB_1**	**Total**	**FB_1:FB_2:FB_3**	**FB_1**	**Total**	**FB_1:FB_2:FB_3**
***F. proliferatum* isolated from maize (*n* = 10)**												
8737	16	**26**	100:44:19	37	**46**	100:14:11	7	**10**	100:29:14	4	**5**	100:25:0
8738	3	**3**	100:0:0	4	**4**	100:0:0	1	**1**	100:0:0	2	**2**	100:0:0
8739	2	**2**	100:0:0	2	**2**	100:0:0	3	**3**	100:0:0	nd	nd	0:0:0
8740	1	**1**	100:0:0	nd[2]	nd	0:0:0	1	**1**	100:0:0	nd	nd	0:0:0
8741	1	**1**	100:0:0	1	**1**	100:0:0	1	**1**	100:0:0	1	**1**	100:0:0
8742	13,600	**17,400**	100:25:3	4,630	**6,110**	100:27:5	2,210	**2,860**	100:23:6	2,900	**3,800**	100:27:3
8743	1,910	**2,580**	100:28:8	1,480	**2,170**	100:32:14	2,040	**2,880**	100:32:9	1,630	**2,200**	100:27:10
8744	1,310	**1,690**	100:12:17	2,440	**3,160**	100:11:18	1,690	**2,130**	100:14:12	1,800	**2,420**	100:13:21
8745	508	**694**	100:31:5	1,410	**1,760**	100:20:5	1,060	**1,350**	100:22:6	1,810	**2,300**	100:23:4
8746	3,060	**3,600**	100:14:5	2,510	**2,970**	100:13:5	2,470	**2,910**	100:12:6	2,750	**3,250**	100:14:4
***F. proliferatum* isolated from sorghum (*n* = 7)**												
8726	4,040	**6,480**	100:56:4	3,450	**5,480**	100:55:4	2,850	**4,370**	100:49:5	4,000	**6,470**	100:58:4
8727	1,540	**2,100**	100:30:6	1,790	**2,260**	100:16:10	2,280	**3,010**	100:23:8	2,410	**3,140**	100:24:7
8728	2,080	**3,370**	100:59:3	1,830	**2,910**	100:53:6	4,290	**6,480**	100:46:5	1,660	**2,600**	100:53:4
8729	2,780	**4,020**	100:42:3	3,210	**5,070**	100:55:3	3,460	**4,840**	100:36:3	4,040	**6,100**	100:48:3

MRC No.[1]	Maize			White Sorghum			Red Sorghum			Pearl Millet		
	FB_1	**Total**	**FB_1:FB_2:FB_3**	**FB_1**	**Total**	**FB_1:FB_2:FB_3**	**FB_1**	**Total**	**FB_1:FB_2:FB_3**	**FB_1**	**Total**	**FB_1:FB_2:FB_3**
8730	1,530	**2,140**	100:36:4	1,880	**2,760**	100:43:4	2,560	**3,520**	100:34:4	1,890	**2,600**	100:33:5
8731	6,110	**8,390**	100:21:16	5,680	**7,890**	100:21:18	3,990	**5,740**	100:21:23	3,120	**4,390**	100:22:19
8732	24	**41**	100:67:4	22	**33**	100:41:9	24	**34**	100:33:8	21	**31**	100:38:10
***F. pseudonygamai* isolated from pearl millet (*n* = 1)**												
8723	2	**3**	100:50:0	19	**27**	100:32:5	29	**43**	100:41:7	3	**5**	100:33:33
***F. verticillioides* positive control (*n* = 1)**												
826	10,300	**13,600**	100:25:7	5,600	**8,100**	100:32:12	5,220	**7,550**	100:31:13	6,600	**9,090**	100:29:9

[1]Medical Research Council (MRC) culture collection number; [2] nd = not detected (detection limit < 1 mg/kg).

2.2 Preparation of grain patty cultures

Grade one cereals used for preparation of patties were purchased on the open market without regard to cultivar and were analyzed for fumonisins and moniliformin. Only grain with no detectable mycotoxins was used to prepare patties. Maize patties were prepared as previously described (Alberts et al., 1993, 1994) with 30 g ground yellow maize that had been autoclaved in 30 ml distilled water for 1 hr at 121°C on two consecutive days.

Patties composed of white sorghum, red sorghum, or pearl millet were developed and optimised by following the same principles as for the maize patties. Pearl millet patties were prepared in the same manner as maize patties. For sorghum patties, 30 g of ground meal in 35 ml water was placed in an 18-cm diameter Pyrex® Petri dish and autoclaved for 1 hour at 121°C on two consecutive days.

The water activity (a_w) for the patties was between 0.98 and 0.99, which is well above known minimums for fumonisin production (Marín et al., 2004). These levels also enabled toxin production at high levels by control strains MRC 826 and MRC 8279 (Tables 1 and 2).

Thirty Petri-dish cultures per strain, for each of the four grains (120 Petri-dish cultures per strain), were used to minimize variation in toxin production. Each patty was inoculated with a standardized spore suspension of lyophilized conidia from a freeze-dried culture. The contents of a storage vial containing freeze-dried spores were resuspended in 2 ml of sterile distilled water. The spore suspension was diluted 1:100 with distilled water. One ml of the diluted spore suspension was used to inoculate each grain patty by **spreading** the diluted spore suspension evenly across the surface of the patty. Thirty patties inoculated with *F. verticillioides* MRC 826, a high fumonisin producer (Alberts et al., 1990), and another thirty patties inoculated with *F. napiforme* MRC 8279, a high moniliformin producer (Leslie et al., 2002), were included as

positive controls. Five non-inoculated patties of each grain type (20 patties total) were included as negative controls.

Patty cultures and controls were incubated stationary on a laboratory bench at 25°C for 21 days in darkness and then oven dried overnight at 60°C. After drying, the 30 patty cultures for each strain/substrate combination were pooled, mixed well, and ground in a small electric laboratory mill (Falling AB S12611, Stockholm, Sweden) to a powder fine enough to pass through a 20 mesh screen. Ground powder was stored at 4°C until analysed. The mill was thoroughly cleaned following grinding of every different strain/substrate combination sample. Immediately prior to chemical analysis of any ground material, the entire strain/substrate sample was mixed thoroughly and a 1.5 g subsample taken for further evaluation. Fumonisin production typically varies ± 10% in solid substrate cultures such as the ones used in this study (Vismer et al., 2004).

2.3 Chemical analyses

High-performance liquid chromatography (HPLC) was used to analyse the harvested patty meal for the production of fumonisins and moniliformin. The method for fumonisin B_1, fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3), was as described in Vismer et al. (2015) and based on the methods of Shephard et al. (1990) and Sydenham et al. (1996) modified for ***in vitro*** culture material (ten-fold less sample taken for analysis).

Determination of moniliformin was based on the method of Parich et al. (2003). Briefly, 1.5 g of ground culture sample was extracted by homogenization with 100 ml acetonitrile:water (84:16). The extract was concentrated to dryness on a rotary evaporator at 50°C, the residue dissolved in methanol and cleaned-up on a preconditioned strong anion exchange (SAX) solid phase extraction

cartridge (ISOLUTE SAX 7 500 mg, Biotage, Uppsala, Sweden). After the extract had passed through the column it was washed (2 ml 0.1 M phosphoric acid and 2 ml water) and dried for 15 min by pulling air through the column. Finally, the toxin was eluted an aqueous solution of 366 mM **potassium dihydrogen phosphate (KH_2PO_4)** and 3.3% (w/w) tetrabutylammonium hydroxide at pH 7.0. The moniliformin was separated by reversed-phase ion-pair HPLC with UV detection at 229 nm and a mobile phase of 1:1 acetonitrile: 732 mM KH_2PO_4 aqueous 6.6% (w/w) tetrabutylammonium hydroxide. Recoveries at 600 mg/kg ranged from 85 to 96% with repeatability of 4.4%.

2.4 Molecular analyses

The identity of the eighteen *Fusarium* strains, originally isolated from maize, sorghum and pearl millet from Nigeria, were determined morphologically and confirmed with AFLP patterns, and partial DNA sequences of the Translocation Elongation Factor (*TEF*)-1α and β-tubulin genes (Vismer et al., 2015).

3. Results

3.1 Fumonisins

The known high-producing strain (MRC 826) of *F. verticillioides* produced the most fumonisins on maize, followed by pearl millet, white sorghum and red sorghum, with the amounts produced on red and on white sorghum generally similar. The strain of *F. pseudonygamai* from pearl millet produced low levels of fumonisins on all four substrates. No fumonisins were detected in the non-inoculated patties indicating that the controls worked as anticipated.

Five of the 10 strains recovered from maize produced large amounts of total fumonisins (> 600

mg/kg) on all of the substrates and the other five produced < 50 mg/kg on any of the substrates (Table 1). Strains in the low-producing group usually produced only fumonisin B_1.

When the amounts of fumonisins produced by the high-producing strains from maize on different substrates were compared, there were few general patterns. MRC 8742 produced the most fumonisins on all of the substrates except for red sorghum. MRC 8746 was usually the second-highest producer, but with numbers that could be less than 25% of the amount of fumonisins produced by MRC 8742. MRC 8745 was consistently the lowest fumonisin producer of any of the strains in this group. The range in the amount of toxin produced also varied by substrate, with the range from high to low greatest on maize, ~16,700 mg/kg, and least, ~1,500 mg/kg, on red sorghum. Four of five strains produced more fumonisins on white sorghum than they did on red sorghum.

Of the seven strains from sorghum, one was in the low category and the other six were in the high category. The gap between the high and low producing classes amongst the sorghum strains was larger than for the maize strains, with the lowest producer in the high group producing at least 2,000 mg/kg of fumonisins more than the sole member of the low group. As with the high producing maize strains, there were no general patterns associated with strain or substrate. No strain produced the most toxin on more than two substrates, while MRC 8727 was the lowest producer on all of the substrates except pearl millet.

The ratio of fumonisin isomers (Table 1) was relatively constant across substrate but varied whether the strains were initially recovered from maize or sorghum. For the maize strains, the average ratio across all of the substrates was 100:22:9 (FB_1:FB_2:FB_3). The most disparate ratio was 100:14:4 for MRC 8746 on pearl millet, and the least disparate ratio was 100:32:14 for MRC 8743 on white sorghum. For the sorghum strains the average ratio was 100:40:7 across all substrates.

The most disparate ratio was 100:16:10 for MRC 8727 on white sorghum and the least disparate ratio was 100:58:4 for MRC 8726 on pearl millet and 100:59:3 for MRC 8728 on maize.

3.2 Moniliformin

The known high-producing strain of *F. napiforme* (MRC 8279) produced the most moniliformin on pearl millet, followed by white sorghum, red sorghum and maize, with the amounts produced on red and on white sorghum similar (Table 2). No moniliformin was detected in the non-inoculated patties indicating that the controls worked as anticipated. Of all of the newly reported strains, the strain of *F. pseudonygamai* from pearl millet produced the highest levels of moniliformin on all four substrates although these levels were much lower than those produced by the *F. napiforme* control.

The *F. proliferatum* strains from maize produced either no moniliformin (6/10 strains) or levels of moniliformin that were < 10% of that produced by the *F. napiforme* positive control (Table 2). Toxin production by the four producing strains ranged from 6 to 1,960 mg/kg, depending on the substrate. There was a hierarchy among the strains with MRC 8744 producing the most moniliformin, followed by MRC 8746, MRC 8745 and MRC 8743. There was no clear hierarchy for the substrates in terms of one always resulting in more (or less) toxin than was produced on the other three substrates.

The *F. proliferatum* strains from sorghum all produced at least some moniliformin although maximum levels usually were no more than 25-33% of the amount produced by the *F. napiforme* control. Three of the strains recovered from sorghum produced the same or lesser amounts of moniliformin than did the strains recovered from maize. For the remaining four strains there was no clear high or low producing strain, and no substrate was clearly preferable over another in terms

of toxin production.

Table 2. Moniliformin production by *Fusarium proliferatum* and *F. pseudonygamai* strains isolated from Nigerian grain, grown on maize, white sorghum, red sorghum, and pearl millet patties. *F. napiforme* (MRC 8279), a known high moniliformin producer, was used as a positive control.

	Moniliformin Levels in Grain Patties (mg/kg)			
MRC number[1]	**Maize**	**White Sorghum**	**Red Sorghum**	**Pearl Millet**
***F. proliferatum* isolated from maize (*n* = 10)**				
MRC 8737	nd[2]	nd	nd	nd
MRC 8738	nd	nd	nd	nd
MRC 8739	nd	nd	nd	nd
MRC 8740	nd	nd	nd	nd
MRC 8741	nd	nd	nd	nd
MRC 8742	nd	nd	nd	nd
MRC 8743	21	27	61	6
MRC 8744	1,420	1,960	1,030	1,620
MRC 8745	115	265	97	107
MRC 8746	924	1,430	546	1,440
***F. proliferatum* isolated from sorghum (*n* = 7)**				
MRC 8726	3,560	5,430	6,110	1,300
MRC 8727	86	648	557	852
MRC 8728	1,200	5,000	2,840	4,000
MRC 8729	4,960	3,030	4,640	4,090
MRC 8730	6,102	4,250	4,010	8,890
MRC 8731	1,240	1,750	1,400	2,270
MRC 8732	761	1,170	1,420	1,100
***F. pseudonygamai* isolated from pearl millet (*n* = 1)**				
MRC 8723	19,200	22,600	13,800	21, 400
***F. napiforme* positive control (*n* = 1)**				
MRC 8279	23,000	27,700	27,300	33,400

[1] Medical Research Council (MRC) culture collection number; [2] nd = not detected (detection limit < 1 mg/kg)

4. Discussion

Fungal metabolism is a complicated process with multiple levels of controls that enable strains to respond individually to variations in environmental factors such as substrate, pH, oxygen availability, water potential, temperature and light. Mycotoxin production by *Fusarium* spp. is commonly tested on a synthetic medium, e.g. Vismer et al., (2004). Other natural substrates tested include cracked maize kernels (Desjardins et al., 2007), maize grits (Fotso et al., 2002), parboiled rice (Abbas et al., 1989), and wheat kernels (Cendoya et al., 2017). In the present study we focused on mycotoxin production on several naturally occurring substrates not previously evaluated – pearl millet, red sorghum and white sorghum. These grains are important food sources in semi-arid regions of Africa and South Asia, but risks of mycotoxin contamination in these grains often are inferred from results obtained with maize. The fungi colonizing these grains frequently differ, with differences often attributed to differences in geographic locations from which samples were taken.

In this study, the strains used were all collected from the same farmer's field, where maize, sorghum and pearl millet were grown side by side as part of an earlier assessment of toxin contamination of different crops under field conditions (Bandyopadhyay et al., 2007; Vismer et al., 2015). Thus, the strains colonizing each crop were selected from the same field soil populations, although different strains could have been brought in with the different seeds. With the correct combination of substrate and strain, production of fumonisins could be as high as (mg/kg) 17,400 on maize, 7,890 on white sorghum, 6,480 on red sorghum and 6,470 on pearl millet (Table 1). Thus, all four grains are potentially susceptible to high levels of fumonisin contamination if the correct strain of *Fusarium* is present under conducive, *e.g.*, poor storage, environmental conditions.

Fusarium pseudonygamai is commonly recovered from pearl millet in Africa (Leslie and Summerell, 2006), and also has been reported from pearl millet grown in the United States (Jurjevic et al., 2005). In general, pearl millet is not highly contaminated with fumonisins (Jurjevic et al., 2005; Vismer et al., 2015; Wilson et al., 2006), although there are some reports of exceptionally high levels (Chilaka et al., 2016). However, *F. pseudonygamai* is associated with high levels of moniliformin under field and laboratory conditions (Fotso et al., 2002; Leslie et al., 2005), and moniliformin also occurs in millet under field conditions (Wilson et al., 2006; Warth et al., 2012). *F. pseudonygamai* and *F. proliferatum* were both recovered from pearl millet grown in Georgia (USA). The plant samples from which the fungi were recovered contained no fumonisins (Jurjevic et al., 2005); moniliformin was not assayed. Moniliformin has also been reported in millet grown in trials in the USA (Wilson et al., 2006).

The seventeen *F. proliferatum* strains analysed fell into two large classes (Tables 1 and 2). With respect to fumonisin production, there were eleven high producers and six low to negligible producers, whereas for moniliformin, there were 11 producers and 6 non-producers. Of the six non-producers of moniliformin, four were also low to negligible fumonisin producers. Otherwise, there were no clear patterns regarding either strain or substrate in terms of toxin production. Means and medians for producing strains usually were similar for producing strains on a common substrate, with exceptions usually due to the relatively small number of observations in a class or to exceptionally high production by a strain on a particular substrate, *e.g.*, MRC 8742 on maize. Simple rank statistics and rank correlation analyses do not yield any statistically significant results. These patterns are consistent with both the strain (genotype) and the environment (substrate) playing a major role in determining the amount of toxin produced. By far the highest amount of fumonisin was produced by MRC 8742 on maize,

but this strain ranked second on white sorghum, fourth on pearl millet and ninth on red sorghum. The highest ranked strain on white sorghum, MRC 8731, ranked second on both maize and red sorghum, and third on pearl millet. The top ranked strain on red sorghum, MRC 8728, ranked sixth on maize and white sorghum, and seventh on red sorghum. The highest ranked fumonisin producer on pearl millet, MRC 8726, ranked third on maize and fourth on each of red and white sorghum. A number of components could play a role in these differences including ability to utilize available carbon and nitrogen sources, ability to utilize/break down polyphenols, and ability to break down or avoid other plant defence compounds.

Patterns for moniliformin production are similarly variable with MRC 8730 the highest ranked on maize and pearl millet and ranked third on both white and red sorghum. The top-ranked strain on both white and red sorghum, MRC 8726, ranked third on maize and seventh on pearl millet.

A **genetic x environment (GxE)** interaction for mycotoxin production that depends on host line and fungal strain can change the way that populations would behave and make it more difficult to predict potential levels of contamination. Analysis of fungal populations on multiple host lines could help in locations where defined hybrids and cultivars are used, but such analyses would be difficult, if not impossible, in less-developed countries where farmers plant traditional mixtures of multiple genetic lines that are carried over locally from year-to-year. This study provides a foreshadowing of these questions since the grain used to make the patties was purchased in the open market in South Africa without regard to host cultivar or variety and the variation we observed is due to just the fungal side of the host-plant interaction.

A second significant observation is that those strains of *F. proliferatum*, which may synthesize very little or none of both fumonisins and moniliformin, occur naturally. Such strains might have a potential utility in a biological control strategy such as the

AflaGuard/AflaSafe technologies (Abbas et al., 2017; Ojiambo et al., 2018) now being used to reduce aflatoxin contamination. The utility of such strains would be increased if there was a common mechanism limiting toxin production, rather than independent mutations in the structural portions of each biosynthetic pathway. Genetic studies with these non-producing strains might also be useful in identifying mutations that have a phenotype beyond rendering non-functional a critical enzyme in a toxin biosynthesis pathway.

Our findings also have implications for human consumption of contaminated grain. Even though our environment was "ideal" for fungal growth and toxin production, such settings also may occur if grain is stored under poor conditions and/or for an extended period of time. In humans, the Joint FAO/WHO Expert Committee on Food Additives has established a provisional maximum tolerable daily intake (PMTDI) for fumonisins at 2 µg/kg body weight/day (JECFA, 2011), or 120 µg/person/day given an average weight of 60 kg/individual. In Africa, overall daily maize consumption has been given as 106 g (WHO, 2003) or, in subsistence areas, a mean daily consumption of the order 450 g is typical (Shephard et al., 2019). Consumption at these levels would require maize below 1.13 mg/kg and 0.27 mg/kg, respectively, to meet the PMTDI. Grain contaminated at the higher levels seen in this study would cause the PMTDI to be exceeded by orders of magnitude and render the maize unfit for human consumption. On average, lesser amounts of sorghum (27 g/day) and millet (52 g/day) are consumed in Africa. Nevertheless, consumption of these grains at the higher fumonisin contamination levels achieved here, would similarly cause the PMTDI to be exceeded by a large margin**. Whether such highly moldly grain would be consumed by subsistence farmers would depend on whether clean grain supplies were available. Even if they were available, molded grains are frequently added to beer production, resulting in the consumption of the water soluble fumonisins (and**

moniliformin).

For moniliformin, there is much less certainty surrounding safe levels of exposure due to a relative paucity of data, although at present the toxin is not one of major concern (EFSA CONTAM, 2017). These conclusions, however, are based on a European diet which would include less than 10% of the maize consumed per day by an African and no sorghum or millet. A published estimate for a provisional tolerable daily intake (TDI) of 0.1 mg/kg body weight for moniliformin was based on a single rodent study (Peltonen et al., 2010). This is equivalent to 6 mg/kg body weight for an average adult. Based on average African maize, sorghum and millet consumption in the above paragraph, these cereals should not be contaminated at levels above 56.6 mg/kg, 222 mg/kg or 115 mg/kg, respectively, in order for consumers' exposure not to exceed the TDI. Of the 18 *Fusarium* isolates, 11 produced moniliformin levels above 56.6 mg/kg on maize, with a maximum of 19200 mg/kg by MRC 8723. The corresponding numbers of isolates producing unacceptably high moniliformin levels in white sorghum, red sorghum and pearl millet were 11 (maximum 22600 mg/kg), 10 (maximum 13800 mg/kg) and 10 (maximum 21400 mg/kg) of the 18 isolates, respectively. Given the much higher cereal consumption of subsistence farmers, moniliformin exposure in these communities has the potential to be well above the proposed TDI. Thus both fumonisin and moniliformin contamination could pose health threats to poor and rural populations in Africa.

In conclusion, we found that isolates of *Fusarium* recovered from maize, sorghum and pearl millet all could produce fumonisins and/or moniliformin on these grains *in vitro* under conditions that mimic those found when these grains are stored poorly. The amounts of toxin produced varied by both strain and substrate in a manner that suggested a potential G×E interaction. The toxin levels produced were high enough for those consuming contaminated grain

to potentially be exposed to mycotoxin levels that exceed the TDI, especially for the fumonisins.

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Declaration of interest

The authors declare that there is no actual or potential conflict of interest.

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