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Research Note

Effect of Sorting on Incidence and Occurrence of Fumonisins and *Fusarium verticillioides* on Maize from Nigeria†

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ABSTRACT

Fumonisin mycotoxins are commonly found on maize and pose a health risk to humans and domesticated animals. Visible sorting of grain has been suggested as a simple technique that can be used to reduce exposure to fumonisins. We collected maize samples in 2003 from different farms in the Kaduna state of Nigeria (Northern Guinea Savanna agroecological zone) that had been sorted by farmers as either good quality or poor quality. The amount of fumonisins and the presence of *Fusarium verticillioides* were determined for each sample. All 13 poor quality samples and the 5 good quality samples positive for fumonisins contained *F. verticillioides.* Twelve of 13 poor quality samples contained fumonisins (1.4 to 110 μg/g), as did the five good quality samples that were positive for *F. verticillioides* (0.2 to 3.7 μg of fumonisins per g). Thus, the visible sorting of grain as a technique to reduce the exposure of subsistence farmers to fumonisins could be successful if there were enough good quality grain available to permit the poor quality grain to be used for another purpose or discarded.

Fumonisins are well-known mycotoxins first described in 1988 by Gelderblom et al. *(12)* and now known to cause leukoencephalomalacia in horses *(15),* liver cancer in rats *(10, 11),* pulmonary edema in swine *(13),* and neurodegeneration in mice *(28).* In addition, fumonisins are known to be associated with esophageal cancer *(29)* and neural tube defects *(21)* in humans. Many countries recognize these risks by providing guidelines or regulations for fumonisin levels in both foods and feedstuffs *(9),* with levels of <2.0 μg/g usually recommended for human consumption. In countries of West Africa, such as Nigeria, where 65% of the population is rural and agrarian, government regulations regarding fumonisins are often unenforced and unenforceable in the local markets, where much of the population buys and sells food and food products. In such situations, relatively simple techniques that could be used to reduce fumonisin intake would be of great value.

Maize is the commodity most often contaminated with fumonisins in West Africa and worldwide. This crop is colonized endophytically by strains of *Fusarium verticillioides,* some of which can synthesize very high levels of fumonisins *(12). F. verticillioides* is commonly recovered in maize from West Africa, with reports of 90 to 95% of the *Fusarium* isolates recovered being *F. verticillioides (2, 19).* Given the general lack of drying and high quality storage facilities, the potential for high levels of fumonisin contamination is good. Among poorer families, maize may constitute a high proportion of the diet *(22),* which serves to further increase the total exposure to fumonisins.

Fumonisins can be recovered from both obviously diseased and apparently clean maize grains *(5),* although moldy grain usually is more heavily contaminated than are clean grain lots. Previous studies of fumonisin contamination from West Africa *(2, 3, 6, 17, 25, 26)* have analyzed composite samples rather than those that have been sorted on the basis of quality measures. Our objective in this study was to determine if Nigerian farmers could sort grain into good and poor quality categories that differed in the amount of fumonisins they contained. If so, then grain sorting could be used as a means to reduce the fumonisin exposure of these African subsistence farmers.

MATERIALS AND METHODS

Sample collection. Shelled maize kernels were collected in January 2003, 3 months after harvest, at Zaria, Samaru, Bomo, Sigau, and Galma in the Kaduna state of Nigeria (Northern Guinea Savanna agroecological zone). Although maize is grown throughout Nigeria, a disproportionately large amount of maize is grown in the Northern Guinea Savanna agroecological zone. This region has a single annual wet season (late May through September and October) with a mean annual rainfall of 1,100 mm, which coincides with the growing season, and a mean temperature range of 20 to 33°C. Farmers store their grain indoors either in bags or directly on the concrete or dirt floor of a hut. Twenty-seven grain samples (14 good quality and 13 poor quality) were collected from farmers' stores.

Grain was sorted by farmers into good quality and poor qual-

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ity categories per their own quality considerations. According to farmers, good quality grain lots attract high prices in the market because they appear clean and are suitable for human consumption and planting. Poor quality grain fetches a lower price and shows visible signs of infection, moldiness, discoloration, insect damage, and bird damage in more than 10% of the grains. The exact proportion of good quality and poor quality grain in each farmer's stores was not determined, but the amount of poor quality grain generally exceeded that of the good quality grain. A 1.0- to 1.5-kg sample of grain of each quality type was returned to the laboratory and stored at 4°C in a cold room for 2 to 4 weeks before being analyzed. Diseased kernels were scored by counting the number of visibly moldy, rotted, or discolored kernels in a 200-kernel subsample.

Mycological analyses. Fifty kernels selected arbitrarily from each sample were surface disinfested by immersing them for 30 s in 1% NaOCl and then rinsing them twice in sterile distilled water; they were then transferred to peptone-pentachloronitrobenzene agar, a medium semiselective for *Fusarium (23).* The plates were incubated for 5 to 7 days under fluorescent lights on a 12-h day/night schedule at 22 to 24°C. Single spores from *Fusarium* colonies were transferred to carnation leaf agar *(8)* for identification on the basis of standard morphological criteria *(24).* At least 10 single-spore isolates of *Fusarium* species from each sample were identified.

Amplified fragment length polymorphisms. Identity of 48 arbitrarily selected strains as *F. verticillioides* was confirmed by comparison of amplified fragment length polymorphism fingerprint patterns with those of the standard mating type tester strains for this species (FGSC 7600 and FGSC 7603, Fungal Genetics Stock Center, School of Biological Sciences, University of Missouri–Kansas City). DNA was extracted as described by Kerényi et al. *(16).* An amplified fragment length polymorphism fingerprint *(31)* was generated by the *Eco*RI+TT and *Mse*I+AC primer pair for final amplification as previously described *(20).*

Fumonisin analysis. Total fumonisins were measured with a competitive direct enzyme-linked immunosorbent assay kit (CD-ELISA) in a microtiter plate format (Veratox for fumonisins, Neogen Corporation, Lansing, Mich.). Results obtained with this kit are comparable with those obtained with high-performance liquid chromatography *(1, 18).*

Approximately 100 g of grain from each sample was ground in a high-speed blender and divided arbitrarily into two subsamples. Each ground subsample was passed through a 20-mesh sieve, and 25 g of each subsample was extracted by blending at high speed with 125 ml of 70% methanol for 2 min. The extract was filtered through Whatman no. 1 filter paper (Whatman International Ltd., Maidstone, UK), and 15 ml of the filtrate was collected for further evaluation. The sample was diluted by adding 100 μl of extract to a sample dilution bottle containing 7.9 ml of 10:90 methanol-water, mixed by swirling the bottle, and processed according to the instructions for use of the CD-ELISA kit. Solutions containing known amounts of fumonisins served as the controls. Optical density at 650 nm was measured with a DYNATECH MR 250-microwell reader (Dynatech Laboratories Inc., Chantilly, Va.) and plotted against a standard curve generated with each microtiter plate from fumonisin solutions of known concentration to determine the amount of fumonisins present. Samples with more fumonisins than in the most concentrated standard were diluted appropriately with 70% methanol and reassayed. The minimum detection limit was 0.2 μg/g, while the recovery rate was 78 and 80% in two tests. The amount of variation between subsamples was <20%.

Statistical analysis was done with SAS software version 8.2 (SAS Institute, Cary, N.C.). A correlation coefficient analysis was made with the combined data from the 27 visibly good quality and visibly poor quality grain samples.

RESULTS

All the good quality samples contained <7% visibly diseased kernels, and 10 had <1% visibly diseased kernels. Among the visibly poor quality samples, only one had <10% diseased kernels, and eight had >30% visibly diseased kernels, with a maximum of 71% diseased kernels observed in a sample from Samaru. The good and poor quality samples also differed in the number of broken, off-color (yellow instead of the preferred white), small, and misshapen kernels, all of which were more frequent in the poor quality than in the good quality samples.

Nine of the 14 visibly good quality grain samples contained no detectable fumonisins (Table 1). The remaining five good quality grain samples contained fumonisins at levels of 0.2 to 3.7 μg/g, with only one sample containing greater than the 2.0-μg/g level commonly recommended for grain meant for human consumption *(9).* The 13 poor quality grain samples (Table 1) contained fumonisin levels of 1.4 to 110 μg/g, with only one sample containing fumonisins at a level of <2.0 μg/g. The highest level of fumonisins detected was 110 μg/g in a sample from Bomo. The mean fumonisin level of the poor quality grain samples was 31 μg/g. Fumonisin levels were significantly correlated ($P < 0.0001$; $r = 0.697$) with the percentage of visibly diseased kernels.

F. verticillioides was recovered from every sample that was positive for fumonisins (Table 1). Amplified fragment length polymorphism fingerprints from representative strains were used to confirm the morphological identifications. In banding patterns, strains from the Nigerian maize samples were, on average, 90% similar to those of the standard mating type tester strains for this species and always at least 75% similar. These similarity values are expected if both the strains from the Nigerian maize samples and the tester strains belong to the same species *(20).*

DISCUSSION

Our results are consistent with previous reports *(4, 7, 14),* which state that fumonisin toxins are not uniformly distributed throughout a grain lot and that sorting can be an effective way to reduce fumonisins in food. Farmers were not given specific directions for sorting, but the independent criteria they used resulted in similar results in terms of qualitatively high and low levels of fumonisin contamination. Nor were they asked to sort a common grain lot. Rather, they sorted their own grain, which could vary considerably in quality from farmer to farmer. When the farmers sorted their grain into those of good and poor quality, then the poor quality grain was more heavily contaminated with fumonisins in 11 of 13 cases. The two exceptions (from Samaru) (Table 1) were samples in which the incidence of diseased kernels and fumonisin contamination

TABLE 1. *Occurrence of fumonisins and percent visibly diseased kernels in maize samples from Kaduna, Nigeria*

	Good quality			Poor quality		
Location	Disease (%)[a]	Fumonisin (μg/g)[b]	*F. verticillioides*[c]	Disease (%)	Fumonisin (μg/g)	*F. verticillioides*
Bomo	0.9	<0.2[d]	ND[e]	37	36	+
	0	<0.2	ND	38	110	+
Galma	0.3	0.2	+	15	17	+
Samaru	0.6	3.7	+	4.5	1.4	+
	1.1	0.2	+	10	<0.2	+
	0.3	<0.2	ND	47	9.6	+
	1.7	<0.2	ND	71	80	+
Sigau	6.7	<0.2	ND	44	22	+
	0	1.2	+	21	21	+
	0.3	<0.2	ND	43	38	+
	0.7	<0.2	ND	50	5.9	+
	0.4	<0.2	ND	35	18	+
	0	0.4	+	30	49	+
	6.4	<0.2	ND	ND	ND	ND
Mean	1.4	0.4		34	31	
SE[f]	0.6	0.3		5.1	9.0	

[a] Percentage of visibly diseased kernels.
[b] Mean of two subsamples analyzed independently.
[c] Presence (+) or absence (−) of *F. verticillioides.*
[d] Detection limit for CD-ELISA test was 0.2 μg/g.
[e] ND, no data. This sample was all considered good quality by the farmer.
[f] Standard error of the mean.

was relatively low overall. The good quality grain usually was free of detectable fumonisins. The one case of good quality grain with a significant level of fumonisin contamination is consistent with previous findings, i.e., that even good quality grain can be contaminated with fumonisins *(5).*

The poor quality samples were much more heavily contaminated with fumonisins than were the good quality samples, with only 2 of 13 samples containing fumonisin levels of <2.0 μg/g and with 2 of 13 samples containing fumonisin levels of >50 μg/g. If the good quality and poor quality grain were consumed equally, then those persons consuming the poor quality maize would be exposed, on average, to ~100-fold more fumonisins than those who consumed the good quality maize. In some cases, the exposure could be as much as ~300-fold higher than for those who consume an equivalent amount of the average good quality maize.

The percentage of diseased kernels did not increase as dramatically as did the fumonisin levels, although the two values were significantly correlated. This is contrary to previous reports of poor correlation between visibly diseased kernels and fumonisin production in South Africa where multiple *Fusarium* spp., both fumonisin producers and nonproducers, infect maize *(29, 30).* The significant correlation in our study implies that fumonisin-producing *F. verticillioides* was the predominant colonizer of grains in Nigeria, which is similar to the situation in neighboring Ghana, where 95% of the *Fusarium* isolates from maize were *F. verticillioides (19).* Among the 14 good quality samples, the highest proportion of diseased kernels was 6.7%, with only 4 of 14 samples having >1% diseased kernels. Of these four samples, only one had a detectable amount of fumonisins. The two poor quality samples that had low levels of fumonisins also had ≤10% diseased kernels. The remaining 11 poor quality samples all had ≥15% diseased kernels, but the relationship between percent diseased kernels and amount of fumonisin contamination is not a linear one, since samples with 30, 35, 37, and 38% diseased kernels were contaminated with fumonisins at levels of 49, 18, 36, and 110 μg/g, respectively.

All of the samples that contained fumonisins also were positive for *F. verticillioides,* which means that fungal inocula would be present and could colonize any stored grain, should environmental conditions permit it. Some of the samples we examined were stored in bags of woven polypropylene. These bags, unlike those of woven jute, do not support fungal growth in the material that composes the bag, which ensures that the fungi recovered in the grain were in or on the grains before they were placed in the bag *(27).* Thus, the amounts of fumonisins that we measured should be viewed as minimums, and it is significantly possible that toxin levels can increase further during storage between harvest and consumption.

Previous studies of fumonisins in West Africa *(2, 6, 17, 25, 26)* have found fumonisin contamination levels of 0.07 to 26 μg/g, although moldy grain samples have been reported with levels of 52 μg/g *(17).* Our samples were all sorted before analysis, and the exact proportions of good and poor quality grain within each farmer's stores are not known. From casual observation, we estimate that the poor quality samples from each location would constitute 50%

or more of the total grain available to the farmer. If we assume a 50:50 composite of good and poor quality grain, then the grain we sampled would contain fumonisin levels of <0.2 to 55 μg/g, and only 3 of 14 would contain fumonisin levels of <2 μg/g. If a farmer relies on the sale of grain in the local market or to traders to earn cash, then the good quality grain is much more likely to be sold than the poor quality grain. At least some of the good quality grain would be used to plant the next year's crop, so most farmers would have <50% of their total harvest available for consumption if they ate only the good quality grain. Some of the poor quality grain could be used to feed domesticated animals (chickens, pigs, goats, and sheep), but the fumonisin levels in some of the sorted grain were high enough that the growth and health of the animals consuming it certainly would suffer. It also is likely that at least some of the poor quality grain would be used for human consumption; however, this would probably occur after it had been milled to flour to reduce the appearance of imperfections. Additionally, it is likely that the diet of those who consumed such flour would contain a significantly higher amount of fumonisins than is considered safe. Thus, sorting as a technique to reduce exposure to fumonisins will be successful only if there is enough good quality grain available to permit the poor quality grain to be used for another purpose or discarded.

In conclusion, sorting grain is an appropriate technique for use by subsistence farmers of West Africa to reduce their exposure to fumonisin mycotoxins, but it will be effective in reducing overall fumonisin exposure only if the good quality grain alone is consumed. The diversion of some grain to other uses, e.g., animal feed, and the use of relatively simple decontamination procedures, e.g., nixtamalization *(4, 14, 32),* could enable more of the crop to be used safely than at present. Making this information available to rural African consumers, whose local markets are not subject to effective government regulations, will be necessary to improve the long-term safety of the food that they are consuming.

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