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Fumonisins and Fungal Species in Corn from Sonora, Mexico

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Fumonisins are mycotoxins produced primarily by strains of *Fusarium moniliforme*, *F. proliferatum*, and other *Fusarium* species commonly associated with corn and other cereal grains. Surveys in different parts of the world indicate the common occurrence of fumonisins. Of the fifteen fumonisin analogues currently described (Bezuidenhouth et al. 1988, Gelderblom et al. 1988, Cawood et al. 1991, Plattner 1995, Abbas and Shier, 1997, Musser and Plattner, 1997) only fumonisin B_1 (FB_1) and, to a lesser extent, FB_2, which may comprise 15-35% of FB_1 have been reported to occur naturally in significant levels in corn and corn-based products (Sydenham et al. 1991, Thiel et al. 1992, Stack 1998, Visconti and Doko, 1994). FB_1 is the most abundant and usually accounts for about 70% of the total fumonisins found in cultures and in naturally contaminated corn samples (Ross et al. 1992). Both fumonisins are considered to have cancer promoting activity (Bacon and Nelson 1994, Gelderblom et al. 1992).

Fumonisins have been epidemiologically and experimentally associated with equine leucoencephalomalacia (ELEM) and porcine pulmonary edema syndrome (Bezuidenhout et al. 1988; Norred and Voss 1994). Fumonisin levels in corn-based products have been associated with an increased risk of human esophageal cancer in the Transkei region in the Republic of South Africa, where FB_1 concentration reached up to 11.1 mg/Kg (Sydenham et al. 1990). The Food and Drug Administration currently is reviewing a draft of maximum allowable levels of fumonisins in corn products destined for animal or human consumption (U.S. FDA/CFSAN 2000). In Mexico, Rosiles et al. (1998) reported an outbreak of ELEM in donkeys, and fumonisins were responsible. The state of Sonora, located in northwest Mexico, is one of the main agricultural areas of this country; consequently, a large number of storage facilities are distributed throughout its territory. From the grains produced in Sonora, maize is one of the most commonly stored at farms for human consumption and as livestock feed. At the beginning of this work, there were no previous reports of the presence of fumonisins in Sonora.

MATERIALS AND METHODS

Forty-eight and 142 corn samples of 4-5 kg each, from various locations in Sonora were obtained for the 1999 and 2000 harvest, respectively. These corn samples

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were a random selection taken directly from the plant at commercial fields or from trucks at the grain elevators.

Upon receipt of samples, moisture content was determined using a Steinlite moisture tester. Samples with high moisture content were oven-dried at 40°C to ≤13.0% (wet basis). Samples were sieved in a 12/64" round aluminum sieve to remove dust, weed seeds, and other impurities. Also, samples were hand-cleaned of non-corn materials, then homogenized in the Boerner divider and 250 g were taken for fungi determination, and the rest were stored at 5°C in plastic bags.

One-hundred of visually intact corn kernels from each sample were surface disinfected by washing/shaking for 1 minute in 2% sodium hypochlorite (NaOCl), then rinsed with sterile distilled water (Sauer and Burroughs 1982). The kernels were aseptically plated germ-side-up in Malt Salt Agar (MS6T) (DIFCO Laboratories, Detroit) containing 6% NaCl and 200 ppm of Tergitol NPX, and incubated for 7 days at room temperature (20-24°C). The number of fungal colonies present on each grain in the plates were identified and counted. Fungal identification was done according to their physical characteristics using the key of Toussoun and Nelson (1976), and Nelson et al. (1990). Each sample was duplicated and results are mean values.

To determine *Fusarium* species, agar sections with the *Fusarium* sp. colonies were transferred to Potato Dextrose Agar (PDA, DIFCO Lab., Detroit) slant tubes prepared based on the procedure by Nelson (1992). After 18 days of incubation, the conidia and conidiophores were compared for identification and confirmation according to Nelson et al. (1983).

A 1.5 kg subsample of the corn samples, where *Fusarium moniliforme* was detected, was ground finely using a Wiley mill with a 2 mm mesh. To avoid contamination between samples, 250 g of the next sample to be ground was used to clean the mill, and this fraction was discarded; then, the sample for fumonisin analysis was collected. Ground samples were placed in plastic bags and stored at 5°C until analyzed. Extraction procedures and FB_1 analysis were based on the Fumonitest® Immunoaffinity Column method from VICAM (Fumonitest Manual).

Fifty g of ground corn sample was mixed with 5 g of NaCl and 100 ml of extraction solvent (80% methanol/water) and blended at high speed for 1 min. The blended material was filtered (No. 4 Whatman paper), and 25 ml of the extract was collected. Five milliliters of the extract was pipeted into a cup and mixed with 20 ml of a wash buffer (25 g NaCl + 5 g bicarbonate + 0.1 ml of Tween dissolved in 1 L of water) and passed through a microfiber filter and a clear filtrate was collected in a clean cup.

A Fumonitest® affinity chromatography column was attached to the outlet of 10 ml reservoir on a pump stand. Then 10 ml of the extract was pipeted into the reservoir and passed through the column at a slow flow rate of about 1 drop per second. The column was washed twice with 10 ml of the wash buffer, and after

that with 10 ml of water. Washes were discarded. The fumonisin was eluted from the column using 1.0 ml of HPLC grade methanol and collected into a cuvette to which 1.0 ml of Fumonitest developer A and B mixture was also added. The cuvette was vortexed and placed into a calibrated Torbex FX-100 series 3 fluorometer to read the fumonisin concentration.

The FB_1 levels were analyzed using the General Linear Models (GLM) procedure for unbalanced data (Milliken and Johnson 1992). Comparisons were made to determine whether FB_1 levels were affected significantly by year of harvest and location.

RESULTS AND DISCUSSION

The percentage of kernels surface disinfected invaded by molds were significantly different ($P \leq 0.05$) among locations (Table 1).

Table 1. Number of samples collected and percentage of mold invasion.

	Number of samples		% Mold invasion	
Location	1998/1999	1999/2000	1998/1999	1999/2000
Rio Sonora	7	No samples	27[a]	No samples
Moctezuma	1	5	91[c]	67[c]
Hermosillo	3	2	42[b]	32[a]
Valle del Yaqui	33	132	47[b]	39[a]
Valle del Mayo	5	10	41[b]	49[b]

Values followed by the same letter in a column are not significantly different at 5% level.

The field fungi data showed that certain fungi genera and species were much more prevalent and widespread than others such as *Fusarium* sp., *Penicillium* sp., and *Alternaria* sp. Other fungi species found in low amount were *Rhyzopus, Mucor, Cladosporium, Helmintosporium* and *Nigrospora.*

Among the *Fusarium* species, *F. moniliforme* was the most abundant and frequently isolated from the corn samples. Samples from Moctezuma collected in 1998/1999 crop had the highest levels of *Fusarium* (67%), *Aspergillus* and *Penicillium*, but in samples from the 1999/2000 crop, *Penicillium* was not detected. In the 1998/1999 crop samples from Hermosillo, *Fusarium, Aspergillus*, and *Penicillium* were also detected. *Aspergillus* was the most prevalent genus (74%). However, for the 1999/2000 crop, the genus *Fusarium* was the main genus invading the samples (70%).

Aspergillus sp. was the most common storage fungi. The *Aspergillus* group species observed were *A. flavus*, *A. niger*, *A. glaucus*, and *A. ochraceus*. *Penicillium* sp. also was observed in many samples. The degree of fungi invasion varied among locations and year. In the 1998/1999 samples from Valle del Yaqui *Aspergillus, Fusarium,* and *Alternaria* were among the most common genus

detected. However, for the 1999/2000 crop samples, *Fusarium* presence was higher than *Aspergillus, Penicillium*, and *Alternaria*. Similar results were obtained in samples from Valle del Mayo.

Samples analyzed for fumonisins were selected on the presence of *F. moniliforme*. Results indicated that there were difference between crop years (1998/1999 and 1999/2000) and among locations (Table 2). The means of fumonisin B_1 levels for 1999/2000 corn was significantly ($P \leq 0.05$) lower than the mean of 1998/1999 corn samples (1.5 and 2.6 ppm, respectively). There was no correlation between fumonisin content and the number of *F. moniliforme* colonies in each sample. This indicated that the number of molds present in the kernels is not directly related with the amount of mycotoxin that can be detected in the sample. Samples collected in 1998/1999 crop at Moctezuma had the highest total fumonisin (6.8 ppm) and those from Valle del Mayo, the lowest (1.5 ppm). However, for 1999/2000 crop samples, there were differences in fumonisin levels. The highest value detected (8.8 ppm) was in a sample collected in the 1998/1999 crop from the Rio Sonora. In Mexico, fumonisins were first reported only at Oaxaca by Rosiles et al. (1998) in ranges from 0.67 to 13.3 ppm, which were higher than those detected in our study.

Table 2. Total fumonisins and FB1 levels (ppm) in corn samples from Sonora, Mexico.

Locality	1998/1999		1999/2000	
	Total Fumonisins (ppm)	FB1 (ppm)	Total Fumonisins (ppm)	FB1 (ppm)
Río Sonora	4.7^b	3.5^b	No samples	
Moctezuma	6.8^c	4.8^c	2.0^b	1.4^b
Hermosillo	2.2^a	1.5^a	2.0^b	1.4^b
Valle del Yaqui	3.3^a	2.3^a	0.5^a	0.4^a
Valle del Mayo	1.5^a	1.1^a	4.8^c	4.4^c

Values followed by the same letter in a column are not significantly different at 5% level.

The fungi genera detected invading corn samples in high proportions were *Fusarium, Aspergillus,* and *Alternaria. Alternaria* spp. was found in all locations and crops and was present in high numbers. *Aspergillus flavus*, *A. niger*, *A. glaucus*, and *A. ochraceus* were commonly found in most of the samples, but the level of invasion in terms of number of colonies per sample was low. The genera *Cladosporium, Rhyzopus, Mucor, Helminsthosporium, Cephalosporium*, and *Nigrospora* were less frequently isolated. The species present on the grains varied among locations and the year crops.

Samples collected at Moctezuma location had the highest fungi invasion in both years. *F. moniliforme* was detected in all locations and nearly in all the samples analyzed in both years. Fumonisin B_1 was detected in all the samples analyzed regardless the year crop and locality. The range of fumonisins in the samples were

from less than 1 to 8.8 ppm. This indicates that fumonisins could be frequently present on corn in Sonora. The 1998/1999 corn samples had the lowest FB_1 level, but in 1999/2000 fungal levels rose significantly.

This is the first report of fumonisins in corn from Sonora, Mexico, and the FB_1 levels detected in some samples were higher than the levels considered safe for horses.

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