Fumonisins in Argentinian Field-Trial Corn

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Seventeen corn samples, which formed part of a series of field trials conducted in Argentina, were analyzed for fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3). Combined fumonisin concentrations recorded in the samples ranged between 1585 and 9990 ng g^{-1}. The bulk of the samples (16/17) had combined fumonisin levels in excess of 2000 ng g^{-1}, with several having levels previously shown to be associated with outbreaks of equine leukoencephalomalacia. In addition, several isolates of *Fusarium moniliforme* and *Fusarium proliferatum* of Argentinian origin were analyzed to determine their fumonisin-producing ability. FB_1 was the major fumonisin analogue produced by the *F. moniliforme* isolates (50–8160 μg g^{-1}), but for each isolate, production levels of FB_3 exceeded the corresponding FB_2 levels. One of the three *F. proliferatum* isolates produced only FB_2. The data represent the first reports of the natural occurrence of the three major fumonisin analogues in Argentinian corn and the production of fumonisins by *Fusarium* isolates from Argentina.

INTRODUCTION

The fumonisins, a group of structurally related mycotoxins, were initially isolated from one of the most predominant fungal contaminants of corn, *Fusarium moniliforme* Sheldon (Gelderblom et al., 1988). To date, six fumonisins (two type A and four type B) have been characterized (Bezuidenhout et al., 1988; Gelderblom et al., 1988; Cawood et al., 1991; Plattner et al., 1992). Only three of these, fumonisin B_1 (FB_1; the major analogue), fumonisin B_2 (FB_2), and fumonisin B_3 (FB_3), appear to be produced in significant quantities under both cultural (Cawood et al., 1991) and natural (Sydenham et al., 1992b) conditions.

Fungal contamination of home-grown corn with *F. moniliforme* has been associated with the high incidence of human esophageal cancer in certain areas of the Transkei, southern Africa (Marasas et al., 1981, 1988a; Marasas, 1982). The neurotoxic syndrome equine leukoencephalomalacia (ELEM) has been associated with the consumption of *F. moniliforme*-contaminated feeds (Marasas et al., 1984). Culture material of *F. moniliforme* strain MRC 826 (from which the fumonisins were initially isolated) has been shown to be both hepatotoxic and hepatocarcinogenic to laboratory rats (Jaskiewicz et al., 1987) and to induce both ELEM and porcine pulmonary edema (PPE). ELEM, PPE, and the hepatotoxic and -carcinogenic effects in rats have been reproduced experimentally following the administration of pure FB_1 (Marasas et al., 1988b; Kellerman et al., 1990; Harrison et al., 1990; Gelderblom et al., 1991). In addition, FB_1 has been shown to induce diarrhea and reduce body weights in broiler chicks (Brown et al., 1992) and to inhibit sphingolipid biosynthesis in rat liver microsomes (Wang et al., 1991). Although no data yet exist on the extent to which the other major fumonisins may contribute to either ELEM, PPE, or other possible animal syndromes, recent studies have indicated that both FB_2 and FB_3 possess toxic and cancer-initiating potentials similar to those observed for FB_1 (Gelderblom et al., 1992).

In addition to *F. moniliforme*, five other *Fusarium* species, including *F. proliferatum* (Matsushima) Nirenberg, have been identified as fumonisin producers (Ross et al., 1990; Thiel et al., 1991a; Nelson et al., 1992). These studies have indicated that fumonisin production is restricted to *Fusarium* species either included in, or closely related to, the section Liseola (Thiel et al., 1991a; Nelson et al., 1992).

Analytical methods have been developed to monitor fumonisin contamination in various matrices (Gelderblom et al., 1988; Plattner et al., 1990; Shephard et al., 1990; Sydenham et al., 1990a, 1992b). Some of these methods have been used to determine fumonisin levels in a number of different products including animal feeds in Brazil (Sydenham et al., 1992a), South Africa (Shephard et al., 1990), and the United States (Harrison et al., 1990; Plattner et al., 1990; Wilson et al., 1990; Ross et al., 1991; Thiel et al., 1991a; Sydenham et al., 1992b), home-grown corn from esophageal cancer risk areas of the Transkei (Sydenham et al., 1990b; Rheeder et al., 1992), corn samples from the 1989 South African crop (Thiel et al., 1992), and commercially available human foodstuffs from Canada, Egypt, Peru, South Africa, and the United States (Sydenham et al., 1991). The data thus far indicate that the fumonisins occur in many countries in a wide variety of corn-based matrices at, on occasions, relatively high concentrations. Data on the levels at which the fumonisins naturally occur in food and feeds, coupled with the toxicological evidence, suggest that the fumonisins pose a potential threat to both animals and humans. The high-performance liquid chromatographic (HPLC) method of Shephard et al., (1990) was the subject of a recently completed collaborative study, in which the reproducibility characteristics of the method

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Table I. Fumonisin Levels in Corn Samples Harvested from Field Trials Conducted in Two Localities of the Province of Buenos Aires, Argentina, in 1991

sample no.	*F. moniliforme*, propagules g^{-1} ($\times 10^3$)	fumonisin concn, ng g^{-1}				% fumonisin		
		FB_1	FB_2	FB_3	total[a]	FB_1	FB_2	FB_3
9 de Julio district								
1[b]	11	1745	775	205	2725	64.1	28.4	7.5
2	300	1900	775	230	2905	65.4	26.7	7.9
3	60	2735	935	410	4080	67.0	22.9	10.1
4	120	2320	795	290	3405	68.2	23.3	8.5
5	10	1820	725	275	2820	64.5	25.7	9.8
6	60	2210	1910	440	4560	48.5	41.9	9.6
7	100	2880	925	145	3950	72.9	23.4	3.7
8[b]	10	1655	545	110	2310	71.6	23.6	4.8
9	140	4235	1750	540	6525	64.9	26.8	8.3
10[b]	700	3960	1545	640	6145	64.5	25.1	10.4
11	20	1740	600	130	2470	70.4	24.3	5.3
12	40	2385	845	330	3560	67.0	23.7	9.3
Pergamino district								
1	50	1110	325	150	1585	70.0	20.5	9.5
2	50	2530	735	315	3580	70.7	20.5	8.8
3	400	5450	2680	800	8930	61.0	30.0	9.0
4	80	6695	2440	855	9990	67.0	24.4	8.6
5	100	3535	1030	450	5015	70.5	20.5	9.0

[a] Total, combined FB_1 + FB_2 + FB_3 levels determined for each sample. [b] Minor contamination of the sample with *F. proliferatum*.

were assessed (Thiel et al., 1993). This culminated in a review and subsequent further optimization of the method to include the codetermination of the third most abundant fumonisin mycotoxin, FB_3 (Sydenham et al., 1992b).

To further assess the worldwide occurrence of the fumonisins, this optimized method was applied to a number of corn samples which formed part of a series of field trials undertaken in Argentina during 1991. An assessment was also made of the fumonisin-producing ability of several *F. moniliforme* and *F. proliferatum* isolates of Argentinian origin. This paper reports the results of these analyses.

MATERIALS AND METHODS

Corn Samples. During February 1991, field trials were conducted in the "9 de Julio" and "Pergamino" districts of the Province of Buenos Aires, Argentina, using commercial and experimental corn hybrids. At harvest, randomized corn samples were withdrawn from the trials in accordance with the procedure published by Apro et al. (1987). Subsamples of about 1 kg each were prepared for analyses by grinding in a laboratory mill. Approximately 100 g each of 17 of these samples was supplied to the laboratories of the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), Medical Research Council, Tygerberg, South Africa, and maintained at 5 °C prior to analysis.

Mycology. Subsamples (1 g) of each ground corn sample were mixed with 9 mL of sterile distilled water. Dilutions with additional sterile distilled water were prepared and aliquots (1 mL) added to Petri dishes containing malt extract agar (15 mL). The dishes were incubated at 25 °C for 5 days, and any observed *Fusarium* colonies were counted. Representative colonies of *F. moniliforme* and *F. proliferatum* were transferred to carnation leaf water agar and their identities verified according to the classification system of Nelson et al. (1983).

Fungal Cultures. Twelve strains of *F. moniliforme* and three of *F. proliferatum*, isolated from corn from the 1990 Argentinian crop and grown on carnation leaf water agar, were stored on sterilized soil and subsequently submitted to the laboratories of PROMEC. Each strain was single-spored, lyophilized, and used to inoculate yellow corn kernels as previously described (Thiel et al., 1991a). The identify of each strain was verified according to the classification system of Nelson et al. (1983).

Mycotoxin Analyses. Subsamples of the 17 corn samples were subsequently analyzed for the presence of FB_1, FB_2, and FB_3, according to the method of Sydenham et al. (1992b). Briefly, aqueous methanol extracts were prepared for each of the samples, aliquots of which were applied to conditioned strong anion exchange (SAX) solid-phase extraction cartridges. The cartridges were washed to remove corn-intrinsic compounds, and the fumonisins selectively eluted with an acetic acid/methanol solution, the eluates being collected and evaporated to dryness. *o*-Phthaldialdehyde (OPA) derivatives of each purified sample extract were prepared and analyzed by reversed-phase HPLC utilizing fluorescence detection. Identification and quantification of the fumonisins were by comparison of the retention times and peak areas observed in the samples with those observed for similarly prepared authentic fumonisin standards—isolated and purified from culture material of *F. moniliforme* strain MRC 826 as previously described (Gelderblom et al., 1988; Cawood et al., 1991). Subsamples of each of the corn cultures of 15 *Fusarium* strains were similarly analyzed with minor alterations made to the method in accordance with those prescribed by Shephard et al. (1990).

Statistical Analyses. Analyses of covariance were performed on log transformed data with the occurrence of FB_1, FB_2, FB_3, and total fumonisins in the corn samples and the concomitant degree of *F. moniliforme* contamination, as covariates. The probability levels reported correspond to the most conservative (i.e., least significant) obtained.

RESULTS AND DISCUSSION

During 1990, field trials were conducted in two districts of the Buenos Aires Province, with the purpose of determining correlations between corn-plant morphology and aflatoxin contamination for each particular genotype. Mycological evaluations of Argentinian corn have previously indicated *F. proliferatum* to be the major *Fusarium* fungal contaminant, with *F. moniliforme* contamination being relatively minor (H. H. L. González, unpublished data). However, examination of the 17 submitted field-trial samples identified *F. moniliforme* as the predominant fungal contaminant, with only minor cocontamination with *F. proliferatum* being recorded in three of the samples (Table I). Both *F. proliferatum* and *F. moniliforme* belong in the section Liseola and are differentiated mainly on the basis of the presence or absence of polyphialides (Nelson et al., 1983). It has been suggested that the relatively minor morphological differences between these two species have resulted in their mutual misidentification (Marasas et al., 1984, 1988c).

The degree of *F. moniliforme* contamination of the 17 corn samples (10 000–700 000 propagules g^{-1}; Table I) was significantly correlated with the levels of FB_1 ($r = 0.596$; $p < 0.05$), FB_2 ($r = 0.550$; $p < 0.05$), FB_3 ($r = 0.597$; $p < 0.05$), and total fumonisins ($r = 0.612$; $p < 0.01$). These

Table II. Fumonisin Concentrations in Corn Cultures of *F. moniliforme* and *F. proliferatum* Isolated from Argentinian Corn

MRC no.[a] and *Fusarium* spp.	fumonisin concn, μg g^{-1}				% fumonisin		
	FB_1	FB_2	FB_3	total[b]	FB_1	FB_2	FB_3
control[c]	0.4	0.1	nd[d]				
F. moniliforme							
6307	90	10	25	125	72.0	8.0	20.0
6308	50	5	15	70	71.4	7.2	21.4
6309	1500	205	400	2105	71.3	9.7	19.0
6310	595	110	305	1010	58.9	10.9	30.2
6311	75	10	35	120	62.5	8.3	29.2
6412	130	5	20	155	83.9	3.2	12.9
6413	1860	240	650	2750	67.6	8.7	23.7
6414	280	20	100	400	70.0	5.0	25.0
6415	85	10	30	125	68.0	8.0	24.0
6416	8160	1380	1430	10970	74.4	12.6	13.0
6419	2530	385	680	3595	70.4	10.7	18.9
6420	295	10	30	335	88.0	3.0	9.0
F. proliferatum							
6417	160	35	5	200	80.0	17.5	2.5
6418	315	30	100	445	70.8	6.7	22.5
6421	nd[e]	30	nd[e]	30	0.0	100.0	0.0

[a] MRC no. accession number of lyophilized cultures in the collection of the Medical Research Council, Tygerberg, South Africa. [b] Total, $FB_1 + FB_2 + FB_3$ concentrations. [c] Control, corn used for the preparation of the cultures. [d] nd, not detected in control corn (<50 ng g^{-1}). [e] nd, not detected in culture material (<1 μg g^{-1}).

data imply that fumonisin contamination of the Argentinian samples was due mainly to contamination with *F. moniliforme*, and the results are similar to those previously reported for Transkeian home-grown corn and commercial corn samples from various countries (Sydenham et al., 1990b, 1991). The levels of FB_1 in the samples were highly significantly correlated with the concurrent levels of both FB_2 ($r = 0.887$; $p < 0.001$) and FB_3 ($r = 0.846$; $p < 0.001$), while FB_2 levels were similarly correlated with those for FB_3 ($r = 0.864$; $p < 0.001$).

Table I lists the results obtained for the fumonisin analyses of the 17 corn samples. The levels of fumonisin contamination ranged between 1110 and 6695 ng g^{-1} for FB_1, between 325 and 2680 ng g^{-1} for FB_2, and between 130 and 855 ng g^{-1} for FB_3, with total fumonisin concentrations (i.e., $FB_1 + FB_2 + FB_3$) ranging between 1585 and 9990 ng g^{-1} (Table I). No statistically significant differences for either the individual or combined fumonisin concentrations were obtained between the two field-trial regions.

With the exception of one sample (no. 6, Table I), in which the FB_1 and FB_2 levels were similar, the proportion of FB_1 and FB_2 in the samples (expressed as percentages of the total fumonisin concentrations) fell within relatively narrow ranges of 61.0–72.9% and 20.5–30.0%, respectively (Table I). The FB_3 concentrations in the samples accounted for between 3.7 and 10.4% of the total fumonisin levels in the samples (Table I). These data confirmed again that FB_1 is the major fumonisin analogue produced under natural conditions (Thiel et al., 1992a). Limited data yet exist on the natural occurrence of FB_3 in corn, although a recent report has shown FB_3 levels of between 50 and 2650 ng g^{-1} (corresponding to 3.5–18.0% of the combined FB_1, FB_2, and FB_3 levels), in 12 of 13 horse-feed samples associated with field outbreaks of ELEM in the United States (Sydenham et al., 1992b).

Due to the matrix differences, the results presented in Table I should not be compared with those obtained from either animal feeds or commercial foodstuffs. They may, however, be compared with the results obtained from other field samples, such as those of the 128 samples representative of the 1989 South African corn crop (Thiel et al., 1992). Although the combined fumonisin concentrations in the South African samples ranged between 0 and 7020 ng g^{-1}, over 80% had combined FB_1 and FB_2 levels lower than 500 ng g^{-1}, with almost 95% having combined concentrations below 2000 ng g^{-1}. Although FB_3 levels were not reported for the South African samples, it is unlikely that their inclusion would have drastically altered the general trend, with respect to the total fumonisin contamination of the samples. These figures for the South African samples contrast sharply with those listed in Table I, where only 1 of 17 samples had combined fumonisin levels below 2000 ng g^{-1}. The 17 corn samples had fumonisin levels similar to those previously reported for home-grown corn (fractionated for human consumption), collected from high esophageal cancer risk areas of the Transkei, southern Africa (Sydenham et al., 1990b; Rheeder et al., 1992). In addition, several samples (no. 9 and 10 from the 9 de Julio district and no. 3 and 4 from the Pergamino district; Table I) had individual and combined fumonisin levels previously shown to be associated with outbreaks of ELEM (Thiel et al., 1991b; Sydenham et al., 1992b), a disease that has occurred in some South American countries (Marasas et al., 1984; Monina et al., 1981; Rodríguez 1945; Sydenham et al., 1992a).

Fumonisin levels produced in corn cultures by 12 *F. moniliforme* and 3 *F. proliferatum* isolates originating from Argentinian corn are listed in Table II. The *F. moniliforme* cultures produced 50–8160 μg g^{-1} FB_1, 5–1380 μg g^{-1} FB_2, and 15–1430 μg g^{-1} FB_3. With the exception of isolate MRC 6416, which produced one of the highest yet recorded levels of FB_1, the levels of FB_1 and FB_2 in the *F. moniliforme* cultures were similar to those previously reported (Shephard et al., 1990; Thiel et al., 1991a,b; Nelson et al., 1991, 1992; Ross et al., 1992; Sydenham et al., 1992a). Fumonisin B_3 levels exceeded the corresponding FB_2 levels in each of the 12 *F. moniliforme* cultures, corresponding to 9–30.2% of the combined fumonisin B_1, B_2, and B_3 levels. Similar observations have previously been reported for a series of Brazilian cultures where "in 16 of the 26 cultures the FB_3 levels exceeded their FB_2 concentrations" (Sydenham et al., 1992a). This trend with respect to the ratios of FB_2/FB_3 was not, however, similarly reflected in the naturally fumonisin-contaminated samples from either Argentina (Table I) or Brazil (Sydenham et al., 1992a).

Each of the three *F. proliferatum* isolates produced different ratios of the fumonisin toxins. For isolates MRC 6417 and MRC 6418 (Table II), FB_1 concentrations were higher than the corresponding FB_2 levels. In MRC 6418, the FB_3 concentration was greater than the corresponding

FB_2 concentration. Similar observations have been reported for other *F. proliferatum* isolates (Ross et al., 1992). Isolate MRC 6421 differed from the other two, in that it produced only FB_2 (Table II). Previous papers have illustrated FB_3 to be the major and, in the case of two isolates, the only fumonisin produced by isolates of *F. proliferatum* (Ross et al., 1992). This is the first report of the identification of a FB_2-only producing *F. proliferatum* isolate, although Ross et al. (1992) have previously reported that a single *F. proliferatum* isolate produced predominantly FB_2.

Even though corn is one of the major crops grown in Argentina, human dietary consumption within that country is relatively low when compared to wheat-based products (Gallo et al., 1993). The present data suggest that *F. moniliforme* is the major fungal contaminant of Argentinian corn. Though based on relatively few field-trial samples, obtained from two locations, the data clearly indicate for the first time that the fumonisin mycotoxins occur in corn grown and harvested in Argentina and suggest that additional data concerning fumonisin contamination of Argentinian corn are required. Studies might include the evaluation of seasonal or geographical variations or the determination of the effect that use of different hybrid seed might have on fumonisin levels.

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