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Mini-Review

Biology of the Fumonisins: Carcinogenic Metabolites of *Fusarium* Species

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The fungus *Fusarium verticillioides* (Sacc.) Nirenberg (Synonym: *F. moniliforme* Sheldon; teleomorph *Gibberella moniliformis* Wineland) is one of the most prevalent seed-borne fungi associated with corn (= maize, *Zea mays* L.) intended for human and animal consumption throughout the world (Marasas et al., 1984a). The fumonisins, a family of food-borne carcinogenic mycotoxins, were first isolated in 1988 from cultures of *F. verticillioides* strain MRC 826 at the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC) of the Medical Research Council (MRC) in Tygerberg, South Africa by Gelderblom et al. (1988a). During 1988, the structures of the fumonisins were elucidated in collaboration with the Council for Scientific and Industrial Research (CSIR) in Pretoria (Bezuidenhout et al., 1988) and fumonisin B_1 (FB_1) was shown to cause equine leukoencephalomalacia (ELEM) in collaboration with the Onderstepoort Veterinary Research Institute in South Africa (Marasas et al., 1988a). The isolation and chemical characterization of the fumonisins in South Africa in 1988 was the culmination of 18 years (1970-1988) of intensive dedicated research by a multidisciplinary team with members at each of the above three institutions. In this review, the events during this period that led to the isolation of the fumonisins in 1988 are described and the first 10 years (1988-1998) of fumonisin research are highlighted.

Events Leading to the Discovery of the Fumonisins in South Africa: 1970-1988

The predominant fungus isolated from moldy corn associated with a field outbreak in South Africa during 1970 of ELEM characterized by liquefactive necrotic lesions in the white matter of the cerebral hemispheres of horses (Marasas et al., 1984a), was *F. verticillioides* (Kellerman et al., 1972). The causative role of *F. verticillioides* in ELEM (Wilson, 1971) was subsequently confirmed with several South African isolates of the fungus and the pathognomonic pathological changes were described in detail (Kellerman et al., 1972; Marasas et al., 1976). The occurrence of bile duct proliferation, increased numbers of mitotic figures, multinucleated hepatocytes, and large, bizarre, hyperchromatic nuclei in the livers of these horses (Kellerman et al., 1972; Marasas et al., 1976) were the first indications that *F. verticillioides* may be a carcinogenic fungus.

Subsequently, we became involved in a study of the possible role of fungal toxins in the etiology of human esophageal cancer (EC) in the Transkei region of South Africa. The incidence rate of EC in males as well as females in the southern part of Transkei is among the highest in the world, whereas the rate in the northern part of Transkei is moderate to low (Jaskiewicz et al., 1987a; Makaula et al., 1996). The staple diet in both areas is home-grown corn and *F. verticillioides* was shown to be the most prevalent fungus in corn consumed by people in high EC incidence areas (Marasas et al., 1981).

In continuing investigations on the toxicology of *F. verticillioides* isolates from corn associated with field outbreaks of ELEM in horses, isolates from corn in high EC-risk areas in Transkei were included. One of these Transkeian isolates, designated *F. verticillioides* MRC 826, was soon found to cause ELEM experimentally in horses and porcine pulmonary edema (PPE) in pigs (Kriek et al., 1981a) and to be highly hepato- and cardiotoxic in rats (Kriek et al., 1981a, 1981b). In 1984, culture material of *F. verticillioides* MRC 826 was shown to be hepatocarcinogenic in rats and to cause primary hepatocellular carcinoma as well as cholangiocarcinoma (Marasas et al., 1984b).

Although chemical investigations on the mycotoxin(s) produced by *F. verticillioides* MRC826 were commenced in South Africa in July 1970, the chemical nature of the metabolite(s) responsible for ELEM still had not been elucidated when the fungus was shown to be carcinogenic in 1984. The isolation and chemical characterization of the mycotoxin(s) and carcinogen(s) produced by *F. verticillioides* then became a matter of paramount importance.

The urgency of the matter was accentuated even further when researchers in USA reported that corn implicated in field outbreaks of ELEM and naturally infected by *F. verticillioides*, also was hepatocarcinogenic in rats (Wilson et al., 1985). The pathological changes in these rats were iden-

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tical to those that had been described previously in rats fed culture material of *F. verticillioides* MRC 826 (Marasas et al., 1984b). This provided evidence that the unidentified carcinogen(s) produced by *F. verticillioides* was present not only in culture material of *F. verticillioides* MRC 826, but also occurred naturally in corn in USA.

In 1984, the following myotoxins were known to be produced by "*F. moniliforme*" according to the literature: Deoxynivalenol, diacetoxyscirpenol, fusaric acid, fusarins A, B, C and D, fusariocins, gibberellins, moniliformin, T-2 Toxin and zearalenone (Marasas et al., 1984a). We knew, however, that *F. verticillioides* MRC 826 does not produce moniliformin, trichothecenes or zearalenone (Kriek et al., 1981b; Marasas, 1986; Marasas et al., 1984a). We also knew that a chloroform/isopropanol extract of culture material of *F. verticillioides* MRC 826 was highly mutagenic to *Salmonella typhimurium* in the Ames test (Gelderblomet et al., 1983, 1984a). Consequently intensive efforts were made to isolate and characterize the mutagen(s) because most mutagens are also carcinogens. A group of structurally related compounds, the fusarins, were isolated and chemically characterized. One of these, fusarin C, was very promising because it was highly mutagenic in the Ames test and occurred naturally in Transkeian corn (Gelderblom et al., 1984a, 1984b) as well as in corn from USA that had been shown to be hepatocarcinogenic in rats (Thiel et al., 1986). Consequently short-term carcinogenicity assays with fusarin C as well as long-term trials in rats with culture material of *F. verticillioides* MRC 1069 that contained high levels of fusarin C were performed (Gelderblom et al., 1986; Jaskiewicz et al., 1987b). However, no evidence of the carcinogenicity of fusarin C could be found. It was concluded that fusarin C was not the carcinogenic metabolite present in culture material of *F. verticillioides* MRC 826. In view of the findings that fusarin C was not carcinogenic and the fact that it is heat and light sensitive, it was concluded that fusarin C does not pose a threat to human health.

Thus the search for the elusive *F. verticillioides* carcinogen continued. During our investigations on the carcinogenicity of fusarin C, a short-term initiation/promotion assay in rat liver was developed. This involved partial hepatectomy of rats followed by administration of an initiator such as diethylnitrosamine (DEN) followed by administration of a promotor such as phenobarbital. After 14 weeks, the gamma glutamyltranspeptidase (GGT) activity was determined histochemically in the liver (Gelderblom et al., 1986, 1988a, 1988b). During the application of this short-term carcinogenicity assay, it was found that culture material of *F. verticillioides* MRC 826 initiated the formation of early lesions in the liver and exhibited cancer promoting activity. The short-term cancer initiation/promoting assay, using DEN as initiator and the ability of fractions of extracts of the culture material to selectively stimulate the development of GGT altered foci in rat liver, was used to purify the active principle(s) (Gelderblom et al., 1988a).

All these efforts finally met with success in 1988 when the chemical nature of the carcinogen was unravelled. Fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2), novel mycotoxins with cancer-promoting activity in rat liver, were isolated from cultures of *F. verticillioides* MRC 826 at PROMEC, MRC, Tygerberg, South Africa (Gelderblom et al., 1988a). The structures of FB_1 and FB_2 were elucidated in collaboration with the CSIR, Pretoria, South Africa (Bezuidenhout et al., 1988).

The elucidation of the chemical structure of the fumonisins together with the demonstration of the biological activity of FB_1 in 1988, was the end of an era and the beginning of a new one. During the period 1970-1988 the toxicity of culture material of *F. verticillioides* MRC 826 to horses and pigs and the carcinogenicity to rats were established. During the period 1988-1991 the stage was set for a new era of research on the biological activity of chemically characterized fumonisins rather than crude fungal cultures and/or extracts.

The First Ten Years of Research: 1988-1998

Biological activity and natural occurrence: 1988-1991. Pure FB_1 was first shown to cause ELEM in horses by intravenous injection in 1988 (Marasas et al., 1988a) and by oral dosing in 1990 (Kellerman et al., 1990). Pure FB_1 was shown to cause PPE in pigs by intravenous injection in 1990 (Harrison et al., 1990) and to cause liver cancer in male BD IX rats at a dietary level of 50 μg/g (Gelderblom et al., 1991).

The natural occurrence of FB1 was first reported in home-grown corn from Transkei (Sydenham et al., 1990a). The first quantitative and sensitive HPLC method for the simultaneous determination of FB_1 and FB_2 in naturally contaminated corn and mixed feed was developed (Shephard et al., 1990) By using this analytical method, it was shown that home-grown corn from high incidence areas of EC in Transkei contained significantly higher levels of both FB_1 and FB_2 than corresponding samples from low incidence areas (Sydenham et al., 1990b). In 1981, a correlation was shown between the incidence of the fungus *F. verticillioides* in home-grown corn and EC rate in the Transkei (Marasas et al., 1981). In 1990, a correlation was shown between the levels of FB_1 and FB_2 in home-grown corn and EC rate in the Transkei (Sydenham et al., 1990b).

It is remarkable that the fumonisins were launched into international importance shortly after their discovery in South Africa in 1988 by events that occurred in the USA during 1989 and 1990. These events occurred shortly after

the publication of the structure of the fumonisins in 1988 but just before the publication of a sensitive chemical analytical method for FB_1 and FB_2 in June 1990 (Bezuidenhout et al., 1988; Shephard et al., 1990).

The great American outbreaks: 1989-1990. During the fall of 1989 and winter of 1990 widespread and large-scale outbreaks of ELEM and PPE occurred in the USA and large numbers of horses and pigs died due to the consumption of commercial mixed feeds containing fumonisin-contaminated corn screenings of the 1989 US corn crop (Harrison et al., 1990; Ross et al., 1990, 1991a, 1991b; Wilson et al., 1990a, 1990b).

Great activity and IARC evaluation: 1990-1993. The disastrous consequences of the contamination of the 1989 US corn crop with high levels of fumonisins triggered a great deal of interest in and research on the fumonisins in USA and elsewhere (Jackson & Bennett, 1990; Norred et al., 1990; Plattner et al., 1990; Vesonder et al., 1990; Voss et al., 1990a, 1990b; Wang et al., 1991). This resulted in a sharp increase in the number of publications dealing with the fumonisins (Marasas, 1996) and several comprehensive reviews (Gelderblom et al., 1992, 1996; Marasas 1995, 1996; Merrill et al., 1993; Nelson et al., 1993; Norred, 1993; Riley et al., 1993). The First Conference on Fumonisins was held in Ames, Iowa, USA from September 6-7, 1990. This was followed by several other international conferences on fumonisins (Riley & Richard, 1992; Jackson et al., 1996).

The International Agency for Research on Cancer (IARC) in Lyon, France evaluated the "Toxins produced by *Fusarium moniliforme*" as "Group 2B carcinogens", i.e. probably carcinogenic to humans in 1993 (IARC, 1993).

Esophageal cancer in Transkei revisited: 1981-1998. At this juncture, I should like to return to the Transkei in South Africa where we first demonstrated a link between *F. verticillioides* and EC in 1981 (Marasas et al., 1981) and between fumonisins and EC in 1990 (Sydenham et al., 1990b). These correlations between the incidence of *F. verticillioides* and fumonisin levels in home-grown corn and EC rate were confirmed in 1988 (Marasas et al., 1988b) and 1992 (Rheeder et al., 1992). The data obtained in Transkei over 6 seasons between 1976-1989 are summarized in Tables 1 and 2.

It is clear from Table 1 that *F. verticillioides* is significantly more prevalent in good as well as in moldy corn from high than from low EC incidence areas in Transkei. Similarly, fumonisin levels in good as well as moldy corn from high EC incidence areas are significantly higher than in low incidence areas (Table 2). The data clearly identify the high EC incidence area, comprising Kentani and Butterworth districts in the southern Transkei, as an ecological zone which favours the infection of corn ears by *F. verticillioides* and the concomitant production of fumonisins in the infected kernels. Conversely, the low incidence area, comprising Bizana and Lusikisiki districts in the northern Transkei, is not favourable for the development of *F. verticillioides* ear rot and fumonisin production in corn. In fact, the low EC area was found to be much more conducive to another *Fusarium* sp. that causes ear rot of corn, ie *F. graminearum* Schwabe, and the production of three mycotoxins by this fungus, ie deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) in corn than the high EC area (Table 3). The two areas are compared with respect to some climatic, geographic, geological and soil fertility factors that may be, or may not be, important in determining the mycotoxicological differences between the areas in Table 4.

Incidence rates of EC in high (Kentani district) and low (Bizana district) incidence areas in Transkei during the period 1955-1990 are compared in Table 5. It is clear that in 1955-1959 the two areas were distinctly different with respect to EC incidence rates in both males and females, i.e.

Table 1. Incidence of *Fusarium verticillioides* in home-grown corn from Transkei[a]

Season	Mean % kernals infected		P <
	Low EC area	High EC area	
	Good corn		
1976	5.0	41.5	0.0001
1979	5.0	23.1	0.01
1985	8.3	42.0	0.001
1986	9.0	43.0	0.01
1989	8.9	41.2	0.01
	Moldy corn		
1977	17.0	25.7	0.005
1979	9.8	33.4	NS
1985	34.5	67.7	0.01
1989	21.4	61.7	0.01

Abbreviation: EC = Esophageal Cancer
[a]Data from Rheeder et al. (1992)

Table 2. Levels of fumonisins in home-grown corn from Transkei[a]

Season	Mean fumonisin level (μg/g)[b]		P <
	Low EC area	High EC area	
	Good corn		
1985	0.3	2.1	0.001
1989	0.6	2.0	NS
	Moldy corn		
1985	9.0	31.5	0.01
1989	5.1	67.4	0.005

Abbreviation. EC = Esophageal cancer
[a]Data from Rheeder et al. (1992)
[b]Total fumonisins in positive samples

Table 3. Incidence of *Fusarium graminearum* and levels of DON, NIV and ZEA in moldy corn from Transkei in 1985[a]

	Low EC area	High EC area	P <
F. graminearum (%)	34.9	8.0	0.01
DON (μg/g)	2.9	0.3	NS
NIV (μg/g)	4.6	1.8	0.05
ZEA (μg/g)	1.2	0.4	0.01

Abbreviations: DON = Deoxynivalenol; NIV = Nivalenol; ZEA = Zearalenone; EC = Esophageal cancer
[a]Data from Sydenham et al. (1990b) and Rheeder et al. (1992)

very low (2.6 and 1.8) in Bizana and very high (54.2 and 30.3) in Kentani. The rates in Kentani have consistently stayed very high and in 1985-1990 were very similar (55.6 and 22.1) to those recorded in 1955-1959. In Bizana, however, the incidence rates in both males and females increased markedly and in 1985-1990 were not much different (37.0 and 11.7) from those in Kentani. Because of the numerous problems and pitfalls associated with cancer registry in remote rural areas of Africa, it is not clear whether the increased EC rates in Bizana are real or reflections of changes in cancer registry patterns due to demographic, socio-economic, political and/or other factors.

In a comparative study of the incidence of esophageal cytological abnormalities determined by means of brush biopsies in residents of the two areas in 1985, it was found that mild and advanced cytological changes occurred much more frequently in the high EC area (Kentani, 50.0 and 36.7%, respectively) than in the low EC area (Bizana, 12.5 and 4.2%, respectively) (Marasas et al., 1988b). It can be assumed that residents of both areas in Transkei have nutritional deficiencies for several mineral elements and vitamins as indicated by the blood biochemical parameters in Table 6. The esophageal cytological changes were determined in the occupants of 12 households in each area, and at the same time samples of good and moldy corn were collected from each household and analysed for *F. verticillioides* and fumonisins (Table 7). The incidence of advanced

Table 5. Esophageal cancer incidence rates in Transkei[a]

Period	ASIR			
	Low EC area Bizana district		High EC area Kenitani district	
	Males	Females	Males	Females
1955-1959	2.6	1.8	54.2	30.3
1965-1969	10.5	4.4	39.7	16.1
1981-1984	19.5	15.0	45.0	23.3
1985-1990	37.0	11.7	55.6	22.1

Abbreviations: ASIR = Age standardised incidence rate/100,000/annum; EC = Esophageal cancer
[a]Data from Makaula et al. (1996)

esophageal cytological changes were higher in the occupants of the 12 households in the high than in the low EC area and so were the levels of *F. verticillioides* and of fumonisins in the corn from the households in the high EC area (Table 7). Although the occupants of the households in both areas probably suffered from underlying nutritional deficiencies (Table 6), the residents of Kentani district in the high EC area had higher levels of fumonisins in the home-grown corn stored in their houses, and also had higher incidences of esophageal cytological abnormalities than residents of Bizana district in the low EC area (Table 7).

Although FB_1 and FB_2 were first reported to occur natu-

Table 6. Some blood biochemical parameters of populations at risk for EC in Transkei[a]

Blood biochmical parameters	Normal values	Transkei
Selenium (ng/ml)	112-210	58-69
Vitamin A (μg/dl)	20-70	25-40
Vitamin B_{12} (μg/dl)	220-750	227-366
Vitamin E (μg/dl)	> 6.0	3.7-5.3
Rolic acid (ng/ml)	210-980	242-307

Abbreviation: EC = Esophageal cancer
[a]Data from Jaskiewicz et al. (1988a, b)

Table 4. Ecological characteristics of low and high EC incidence areas in Transkei[a]

Characteristic	Low EC area Bizana district	High EC area Kenitani district
Altitude (m)	600-900	200-400
Averagerainfall (m)	800-950	900-1,000
Geology	Ecca, Dwyka	Beaufort
General soil features	Red, yellow/brown apedal with orthic epipedon (Hutton, Clovelly, Griffin)	Weakly developed soils on rock (Glenrosa, Mispah, Swartland)
Vegetation	Coastal Forest and Thornveld	Thornveld - Bushveld
Soil fertility factors	Soil organic matter and level of Al and Fe significantly higher	Soil pH and levels of Mn, Ni, Mg, Ca and K significantly higher

Abbreviation: EC = Esophageal cancer
[a]Data from Rheeder et al. (1994)

Table 7. Incidence of esophageal cytological abnormalities, *Fusarium verticillioides* and fumonisins in home-grown corn in low and high EC areas in Transkei[a]

	Low EC area		High EC area	
	Bizana district		Kenitani district	
Advanced cytological change (%)[b]	4.2		36.5	
	Good corn	Mold corn	Good corn	Moldy corn
F. verticillioides (%)[c]	8.3	34.5	42.0	67.7
Fumonisins (μg/g)[c]	0.3	9.0	2.1	31.5

Abbreviation: EC = Esophageal cancer
[a] Data from Marasas et al. (1988b) and Rheeder et al. (1992)
[b] In occupants of 12 households in each area in 1985
[c] In samples of good and moldy corn from each household

rally in corn from Transkei (Sydenham et al., 1990a), fumonisins have subsequently also been reported to occur in home-grown and/or commercial corn in several other high EC incidence areas in the world (Table 8). It is clear that fumonisins are known to occur in corn consumed by humans at risk for EC in some areas in Africa, Asia, Europe and the USA. The question remains whether some individuals who take in higher levels of fumonisins in corn consumed as the staple diet than others, are at higher risk to develop cytological abnormalities in the esophagus that may terminate in EC. The first step required to answer this question is the assessment of human fumonisin intake. Two approaches are being taken to do this, i.e. calculation of the probable daily intake (PDI) and the measurement of biomarkers for fumonisin exposure in humans (Marasas, 1997).

Table 8. Fumonisin levels reported in corn from different high risk areas for EC

Area	Maximum total fumonisins (μg/g)	Reference
China, Linxian country	155	Chu & Li (1994)
Transkei, Kentani district	117	Rheeder et al. (1992)
Zimbabwe	4	Sydenham et al. (1993)
Italy, Pordenone province	4	Pascale et al. (1995)
USA, Charlestone, SC	2	Sydenham et al. (1991)

Abbreviation: EC = Esophageal cancer

Human fumonisin intake can be calculated from analyses of naturally occurring levels of fumonisins in corn and data on corn intake and expressed as the PDI (Table 9). Both the level of fumonisins in the corn and the amount of corn consumed influence the PDI, i.e. the higher the level and the larger the amount consumed, the higher the PDI. Large variations are apparent in the PDI, ranging from 1.2 μg/kg bw/d in urban South Africans consuming commercial corn, to 354.9 μg/kg bw/d in rural South Africans consuming moldy home-grown corn. The fact of the matter is that the rural population in Transkei who are at the highest risk for EC in South Africa, have the highest daily corn intakes, and also consume home-grown corn containing the highest levels of fumonisins. Thus the PDI can be a useful estimate of the fumonisin intake of population groups, such as the rural population in Transkei, and although it can be applied to individuals by means of food from-the-plate analyses, this is not easy to do.

Biomarkers are more accurate as measures of individual intakes, but biomarkers for human fumonisin intakes are unfortunately not yet available. However, fumonisins disrupt sphingolipid biosynthesis (Wang et al., 1991), and the resulting elevation in the sphinganine/sphingosine (Sa/So) ratio in serum, plasma or urine has been used as a biomarker in animals, including non-human primates (Shephard et al., 1996, 1998). Moreover, analytical techniques have been developed to determine Sa/So levels in humans, and ratios of 0.09-0.78 have been reported in serum (Castegnaro et al., 1998) and 0.04-0.60 in urine (Castegnaro et al., 1996; Solfrizzo et al., 1997).

In a recent paper by Van der Westhuizen et al. (1999), Sa/So ratios were reported in the plasma and urine of residents of Kentani district in the high EC incidence area in Transkei (Table 10). Although the mean values were similar to those reported in human serum and urine above, the upper ranges of the ratios in both plasma (2.97) and urine (5.75) were much higher than those previously reported. It remains to be determined whether the

- Sa/So ratio is sensitive enough as a biomarker of human intake of fumonisins at the levels of contamination examined to date (Table 10).
- Variation in the ratio between individuals and within an individual over time can be accommodated.

Table 9. Probable daily intake (PDI) of fumonisins in home-grown and commercial corn by rural and urban South Africans[a]

	Fumonisin levels in Corns (μg/g)	Corn intake (g/70 kg bw/d)	PDI (g/kg bw/d)
South Africa - rural (moldy corn)	54.0	460	354.9
South Africa - rural (good corn)	7.1	460	46.6
South Africa - urban	0.3	276	1.2

Abbreviation: PDI = Probable daily intake.
[a] Data from Marasas (1997)

Table 10. Sphinganine/sphingosine ratios in humans at risk for EC in Transkei[a]

Plasma[b]	Urine[b]
0.01-2.97	0.01-5.75
0.34 ± 0.36	0.41 ± 0.72
Total fumonisins in corn samples (μg/g)[c]	
Good corn: 0-7.2	? = 0.6
Moldy Corn: 0.03-37.88	? = 4.8

Abbreviation: EC = Esophageal cancer
[a] Data from Van der Westhuizen et al (1999)
[b] Determined in 150 residents of Kentani district, Transkei in 1997
[c] Determined in samples of good and moldy home-grown corn collected in Kentani district, Transkei in 1997

• Individuals with high Sa/So ratios in high incidence areas of EC in Transkei have high intakes of fumonisins and are at high risk for EC.

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