Phytoprotection

Incidence of *Fusarium* species and levels of fumonisin B_1 in corn in the Samsun province of Turkey

Incidence d'espèces de *Fusarium* et des niveaux de fumosinine B_1 chez le maïs dans la province de Samsun en Turquie

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Article abstract

The contamination of corn with fumonisin produced by *Fusarium* species represents an important risk for humans and animals. The incidence of *Fusarium* spp. and contamination by fumonisin B_1 (FB_1) were studied in field samples from 70 fields of corn during the 2005 and 2006 preharvest seasons in the province of Samsun, Turkey. *Fusarium* was the predominant genus isolated from the field samples, with *F. verticillioides*, *F. proliferatum* and *F. subglutinans* being the most commonly isolated species. The occurrence of *Fusarium* spp. varied each year, from 97.14% to 78.57% in 2005 and 2006, respectively. The widespread occurrence of FB_1 was also observed across the Samsun province. All corn samples infected with *F. verticillioides*, *F. proliferatum* and *F. subglutinans* tested positive for FB_1, but none were infected with FB_2. Levels of FB_1 ranged from 0.28 to 8.48 mg kg^{-1} in 2005 and from 0.11 to 2.77 mg kg^{-1} in 2006. The concentration of FB_1 was lower than 2 mg kg^{-1} in 63.6% of the samples, 28.8% contained from 2 mg kg^{-1} to 5 mg kg^{-1}, while 7.6% contained more than 5 mg kg^{-1}. Our study shows that corn contamination with both *Fusarium* and FB_1 was present throughout the Samsun province, but it was strongly dependent on environmental and seasonal conditions. However, there was no *Fusarium* contamination in certain native white-type and popcorn-type cultivars in 2005 and 2006.

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The contamination of corn with fumonisin produced by *Fusarium* species represents an important risk for humans and animals. The incidence of *Fusarium* spp. and contamination by fumonisin B_1 (FB_1) were studied in field samples from 70 fields of corn during the 2005 and 2006 preharvest seasons in the province of Samsun, Turkey. *Fusarium* was the predominant genus isolated from the field samples, with *F. verticillioides*, *F. proliferatum* and *F. subglutinans* being the most commonly isolated species. The occurrence of *Fusarium* spp. varied each year, from 97.14% to 78.57% in 2005 and 2006, respectively. The widespread occurrence of FB_1 was also observed across the Samsun province. All corn samples infected with *F. verticillioides*, *F. proliferatum* and *F. subglutinans* tested positive for FB_1, but none were infected with FB_2. Levels of FB_1 ranged from 0.28 to 8.48 mg kg^{-1} in 2005 and from 0.11 to 2.77 mg kg^{-1} in 2006. The concentration of FB_1 was lower than 2 mg kg^{-1} in 63.6% of the samples, 28.8% contained from 2 mg kg^{-1} to 5 mg kg^{-1}, while 7.6% contained more than 5 mg kg^{-1}. Our study shows that corn contamination with both *Fusarium* and FB_1 was present throughout the Samsun province, but it was strongly dependent on environmental and seasonal conditions. However, there was no *Fusarium* contamination in certain native white-type and popcorn-type cultivars in 2005 and 2006.

Keywords: *Fusarium proliferatum*, *F. subglutinans*, *F. verticillioides*, HPLC, mycotoxins.

[Incidence d'espèces de *Fusarium* et des niveaux de fumosinine B_1 chez le maïs dans la province de Samsun en Turquie]

La contamination du maïs par la fumonisine produite par des espèces de *Fusarium* présente un risque important pour les humains et les animaux. L'incidence des espèces de *Fusarium* et la contamination par la fumonisine B_1 (FB_1) ont été étudiées durant la saison précédant la récolte en 2005 et 2006 dans des échantillons provenant de 70 champs de maïs de la province de Samsun, en Turquie. *Fusarium* était le genre prédominant dans les échantillons de champ, *F. verticillioides*, *F. proliferatum* et *F. subglutinans* étant les espèces les plus communément isolées. La présence d'espèces de *Fusarium* variait d'une année à l'autre, passant de 97,14 % à 78,57 % en 2005 et 2006, respectivement. La présence très répandue de FB_1 a également été observée dans la province de Samsun. Tous les échantillons de maïs infectés par *F. verticillioides*, *F. proliferatum* et *F. subglutinans* étaient contaminés par la FB_1, mais aucun n'était contaminé par la FB_2. Le niveau d'infection par la FB_1 variait entre 0,28 et 8,48 mg kg^{-1} en 2005 et entre 0,11 et 2,77 mg kg^{-1} en 2006. La concentration de FB_1 était inférieure à 2 mg kg^{-1} dans 63,6 % des échantillons, 28,8 % en contenait de 2 mg kg^{-1} à 5 mg kg^{-1}, alors que 7,6 % en contenait plus de 5 mg kg^{-1}. Notre étude montre que la contamination du maïs par le *Fusarium* et la FB_1 est répandue à travers la province de Samsun, mais qu'elle dépend fortement des conditions environnementales et saisonnières. Toutefois, certains cultivars indigènes de types blanc et popcorn n'étaient pas contaminés par le *Fusarium* en 2005 et 2006.

Mots clés : CLHP, *Fusarium proliferatum*, *F. subglutinans*, *F. verticillioides*, mycotoxines.

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INTRODUCTION

Corn (*Zea mays* L.) is an important crop in the Black Sea region of Turkey (Anonymous 2007). It was introduced into Turkey from the Americas in the 1600s and was included in traditional farming systems. In Turkey, about 590,000 ha of corn are planted every year, which yield an estimated 300,000 t of grain annually (Anonymous 2008). Ear rots caused by a number of fungi not only decrease yields but they also have the potential to contaminate grain with mycotoxins that can adversely affect human and animal health (Kedera *et al.* 1999; Macdonald and Chapman 1997; Njuguna *et al.* 1990; Ross *et al.* 1990). The prevalence and density of *Fusarium verticillioides* (Sacc.) Nirenberg in field samples was 49.25% for the Bolu province and 62.67% for the Zonguldak province of the West Black Sea region of Turkey in 1992 (Aktas *et al.* 1994). Since the International Agency for Research on Cancer (IARC) declared fumonisins as a 2B carcinogen, legal limits in corn have recently been defined by the European Union as 2.0 mg kg^{-1} in grain, 1.0 mg kg^{-1} in corn meal and flour, 0.4 mg kg^{-1} in corn-based products for human consumption, and 0.2 mg kg^{-1} in baby food.

The production of mycotoxins in corn is often influenced by moisture, temperature and nutrient factors unfavourable to growing corn (Miller *et al.* 1993). Fumonisins are common contaminants of corn-based food and feed in the United States, China, Europe, South America and Africa (Fandohan *et al.* 2003; Shephard *et al.* 1996; Sydenham *et al.* 1994; Visconti and Doko 1994). Fumonisins inhibit the biosynthesis of sphingolipids and can cause a variety of diseases in animals that eat contaminated feed (Desjardins *et al.* 1998). Consumption of corn contaminated with high levels of fumonisins causes esophageal cancer in humans in parts of the world where corn is a staple food (Munkvold and Desjardins 1997). Fifteen *Fusarium* species have been reported to produce fumonisins (Marasas 2001). Eight of these species are in the section Liseola of *Fusarium*, including *F. verticillioides* (Syn = *F. moniliforme*) mating population A, MP-A; *F. sacchari* (E.J. Butler & Hafiz Khan) W. Gams, MP-B; *F. fujikuroi* Nirenberg, MP-C; *F. proliferatum* (T. Matsush.) Nirenberg ex Gerlach 7 Nirenberg, MP-D; *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Tousson & Marasas, MP-E; and *F. subglutinans sensu lato* isolated from teosinte seed. These are all part of the *Gibberella fujikuroi* (Sawada) Wollenw. (teleomorphs) species complex. *Fusarium verticillioides* is a common pathogen on corn causing root, stalk and ear rots worldwide (Munkvold and Desjardins 1997). *Fusarium proliferatum* is similar to *F. verticillioides* in many respects, but the chains of microconidia are usually shorter than those of *F. verticillioides* and are often formed in pairs from polyphialides, resulting in a characteristic "V" shape (Burgess *et al.* 1994). *Fusarium subglutinans* is common in the cooler areas of subtropical regions of eastern Australia and in temperate areas. It

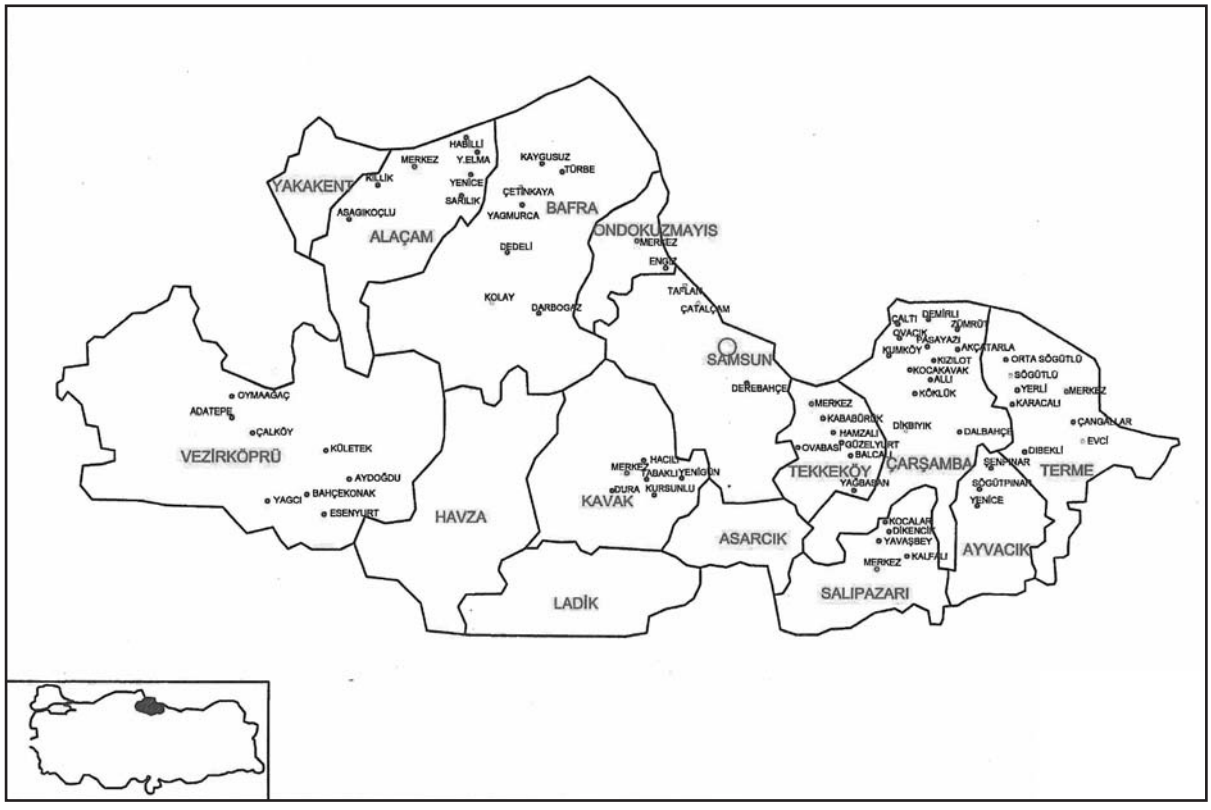

Figure 1. Map of the Samsun province of Turkey showing the sampling sites in 2005 and 2006.

is also associated with stalk rot and cob rot of corn and can be seed-borne (Burgess *et al.* 1994; Francis and Burgess 1975).

The most important producers of fumonisins are *F. verticillioides* and *F. proliferatum* because of their overall high levels of production, wide geographical distribution, frequent occurrence on corn, and association with known animal mycotoxicoses (Ross *et al.* 1992). The highest yield of FB_1 to be reported for a *Fusarium* species was obtained from a Spanish corn isolate of *F. proliferatum* cultured on whole corn. This isolate also produced the highest published yield of FB_2. Some isolates of *F. subglutinans*, *F. thapsinum* Klittich, J.F. Leslie, P.E. Nelson & Marasas, *F. anthophilum* (A. Braun) Wollenw., *F. globosum* Rheeder, Marasas & P.E. Nelson, *F. dlamini* Marasas, *F. napiforme* Marasas, P.E. Nelson & Rabie, *F. oxysporum* var. *redolens* (Wollenw.) W.L. Gordon, and *F. polyphialidicum* Marasas, P.E. Nelson, Toussoun & P.S. van Wyk were also found to produce FB_1 in the same study (Rheeder *et al.* 2002).

Oruc *et al.* (2006) analyzed 26 corn samples by competitive ELISA and found that fumonisin levels ranged from 0.80 to 356.8 mg kg^{-1} in corn from Turkey and from 4 to 263 mg kg^{-1} in imported corn. In Turkey, very little research has been done on the occurrence of fumonisin in maize. In a study conducted by Omurtag (2001), detected levels of FB_1 in the country were between 0.25 and 2.66 mg kg^{-1} in 25.6% of the 82 samples analyzed, but FB_2 was detected only in a single corn meal sample at 0.55 ppm. Arici *et al.* (2004) reported that fumonisin contamination ranged from 0.8 to 273 mg kg^{-1} in low-processed products and from 0.3 to 76.8 mg kg^{-1} in processed products out of a total of 92 corn-based food products in Turkey. There is a great need for additional investigation in Turkey, at least in the Black Sea region where corn production and consumption are predominant. The objectives of this project were: (i) to determine the infection levels and distribution of *Fusarium* spp. in field samples of corn in the Samsun province; and (ii) to investigate natural levels and incidence of FB attributable to various isolates of *F. verticillioides*, *F. proliferatum* and *F. subglutinans* in grain from farmers' fields in the Samsun province.

MATERIALS AND METHODS

Surveys and sample collections

Surveys were conducted in 11 districts of the Samsun province during the week before harvest in August 2005 and September 2006 (Fig. 1). A total of 140 corn samples were collected from these locations (Table 1). At least 10 corn cobs (10 cobs from < 1 ha fields, 20 cobs from 2 to 5 ha fields, 30 cobs from > 5 ha fields) were collected at each sampling location. Corn cobs were hand-shelled and the kernels from each cob were air-dried for 2 wk on the laboratory bench. Samples were stored in a cold room at 4°C until analysis.

Fungal isolation

Between 35 and 40 kernels from each sample were surface-disinfected by immersing the kernels in 10% commercial sodium hypochlorite solution for 3 min, and rinsing in sterile distilled water for 20 s. They were then transferred onto three plastic Petri plates containing two layers of blotter paper. The samples were first incubated at 21°C for 24 h and then frozen at -20°C for another 24 h to inhibit seed germination. The Petri plates were then placed in the incubator at 23°C for 7-10 d and exposed to a 15:9 h light:dark

Table 1. Prevalence of *Fusarium* species contamination in 4200 corn seeds from 140 fields in 2005 and 2006 surveys conducted in the Samsun province of Turkey

Fusarium species	2005[1]				2006[1]				Total contaminated seed ratio	Total contaminated fields
	Contaminated seeds		Contaminated fields		Contaminated seeds		Contaminated fields			
	(nb)	(%)	(nb)	(%)	(nb)	(%)	(nb)	(%)	(%)	(%)
F. verticillioides	180	9.04	30	42.85	43	2.04	12	17.14	5.54 c[2]	30.00 c[2]
F. proliferatum	91	4.33	22	31.42	30	1.42	7	10.00	2.88 c	20.71 c
F. subglutinans	37	1.76	9	12.85	35	1.66	8	11.42	1.71 bc	12.14 abc
F. solani	13	0.61	5	7.14	47	2.23	17	24.28	1.42 abc	15.71 bc
F. poae	1	0.04	1	1.42	1	0.04	1	1.42	0.04 a	1.42 ab
F. acuminatum	1	0.04	1	1.42	0	0	0	0	0.02 a	0.71 a
F. oxysporum	0	0	0	0	9	0.42	5	7.14	0.21 ab	3.57 ab
F. sporotrichioides	0	0	0	0	1	0.04	1	1.42	0.02 a	0.71 a
F. equiseti	0	0	0	0	2	0.09	1	1.42	0.04 a	0.71 a
F. semitectum	0	0	0	0	2	0.09	1	1.42	0.04 a	0.71 a
F. graminearum	0	0	0	0	11	0.52	1	1.42	0.26 ab	0.71 a
F. culmorum	0	0	0	0	3	0.14	1	1.42	0.07 a	0.71 a
Total	**333**	**15.85**	**68**	**97.14**	**184**	**8.76**	**55**	**78.57**	**12.30**	**87.85**

[1] Means are not significantly different between 2005 and 2006 at $P < 0.01$ and $P < 0.05$ (*t*-test).
[2] Means are significantly different at $P < 0.01$ (Duncan test).

cycle (Aktaş and Tunali 1990). Fungal colonies with mycelium resembling *Fusarium* spp. were transferred onto Synthetic Nutrient Agar (SNA) (Gerlach and Nirenberg 1982).

Fungi were examined with stereo binocular (Euromex Model KTD, 45147 W 10X) and compound microscopes (Leica DMLS). The identification of *Fusarium* spp. was confirmed using keys by Booth (1977), Burgess *et al.* (1994), Gerlach and Nirenberg (1982), and Leslie and Summerall (2006). Fungus species other than *Fusarium* that developed on kernels after incubation were transferred onto potato dextrose agar (PDA) medium for identification and their identity was determined according to keys found in Barnett and Hunter (1998) and Ellis (1976). Stock cultures of *Fusarium* species were single-spored according to the method described by Burgess *et al.* (1994). Cultures were maintained on silica gel (Windels *et al.* 1988) and stored at -20°C in 2 mL vials.

Mycotoxin analyses

Mycotoxin analyses were performed with high performance liquid chromatography (HPLC) using fluorescence and diode array detectors. Fumonisins were analysed by HPLC using the method described by Shephard *et al.* (1990) with some modifications. Ground corn samples (50 g) were weighed, and 1 g was placed into a 15 mL centrifuge tube for fumonisin extraction. Each sample was extracted with water:acetonitrile (1:1 vol:vol 5mL g^{-1} corn) by shaking for 1 h on an end-over-end shaker. The extract was centrifuged at 2000 rpm for 5 min, and 1 mL was transferred into a glass tube. Sample clean-up prior to analysis was done using Bond-Elute SAX cartridge (100 cc 200 mg^{-1}) and eluted with 0.5% acetic acid in methanol. The solution was evaporated and the residue was derivatized with ortho-phthalaldehyde (OPA). The OPA (Sigma, St. Louis, MO, USA) reagent was prepared by dissolving OPA (20 mg) in methanol (500 µL) and adding 2.5 mL of 0.1 M sodium tetraborate and 25 µL 2-mercaptoethanol. A 50 µL aliquot of the sample was placed in the HPLC system. The HPLC system consisted of a model HP1100 and autoinjector, a Lichcorb 5 µm C8 reversed-phase 12.5 x 4 mm column, and a fluorescence detector. Samples from each isolate were analyzed for FB_1 and FB_2 using this HPLC method. Results were expressed in terms of levels of FB_1 and FB_2 produced by each isolate. The detection limit was 0.10 µg g^{-1} for FB_1 and FB_2. A deoxynivalenol (DON) analysis was also performed for samples that had shown the presence of *F. graminearum* Schwabe and *F. culmorum* (Wm.G. Sm.) Sacc. using HPLC with diode array detection with a modification of the procedure described by Chang (1984) for deoxynivalenol analysis. A 1 g sample was taken from well-mixed, finely ground material. To prepare extracts, 5 mL of methanol/water was added to the sample in glass tubes, which were then subjected to end-over-end mixing for 1 h and centrifuged for 5 min at 2000 rpm. The supernatant solution was passed through a filter and the filtrate used for clean-up procedures. A 2 mL extract was washed with ethyl acetate. The solution was evaporated to dryness in a vacuum and the residues were treated with dichloromethane. A prepared Pasteur pipette was pre-washed with toluene/acetone and then washed with dichloromethane. The sample solution was added to a test tube with a toluene/acetone mixture. This solution was discharged, after which dichloromethane/methanol was treated and collected into a test tube and mixed. This solution was evaporated to dryness and the residue dissolved in a 0.5 mL methanol/water solution. It was then transferred into vials for analysis by HPLC. The detection limit was 0.10 µg g^{-1} for DON.

RESULTS

Results of the surveys conducted in 2005 and 2006 in a total of 140 fields from 11 districts of the Samsun province are presented in Table 1. Microscopic analysis showed that *Fusarium* was the predominant fungal genus in corn ears during the 2005 and 2006 pre-harvest seasons in the Samsun province.

The occurrence of *Fusarium* species differed significantly between the years 2005 and 2006 ($P < 0.05$). *Fusarium verticillioides* was the most commonly isolated fungus in 2005, while *F. solani* (Mart.) Sacc. was most commonly isolated during the 2006 pre-harvest season (Table 1). *Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans* were found during both years, but were more frequent in 2005. Occurrence of those three species in 2005 was 42.9, 31.4 and 12.9%, respectively, and 17.1, 10.0 and 11.4% in 2006. The number of kernels contaminated with *Fusarium* species was 333 in 2005 and 184 in 2006 (Table 1). *Fusarium oxysporum*, *F. equiseti* (Corda) Sacc., *F. sporotrichioides* Sherb. and *F. semitectum* Berk. & Ravenal were detected only in 2006, while *F. acuminatum* Ellis & Everh. was found only in 2005 in one location. A total of 517 *Fusarium* isolates were obtained from the 2005 and 2006 samples. The number of isolates for each species was 233 *F. verticillioides*, 121 *F. proliferatum*, 72 *F. subglutinans*, 60 *F. solani*, 11 *F. graminearum*, 9 *F. oxysporum*, 3 *F. culmorum*, 2 *F. poae* (Peck), Wollenweb. in Lewis, 2 *F. equiseti*, 1 *F. sporotrichioides*, and 1 *F. acuminatum* for both years. Incidence of *Fusarium* spp. in field samples was 97.14% in 2005 and 78.57% in 2006 (Table 1). The 2-yr mean value was 87.85%.

Other non-*Fusarium* species were identified in 16.73% of the field samples in 2005 and 2006, including *Penicillium* spp. (5.76%), *Mucor* spp. (2.76%), *Alternaria* spp. (1.95%), *Acremonium strictum* W. Gams (1.45%), *Alternaria alternata* (Fr.:Fr.) Keissl. (1.38%), *Rhizophus* sp. (1.16%), *Aspergillus niger* Tiegh. (0.92%), *Trichoderma harzianum* Rifai (0.50%), *Verticillium* sp. (0.21%), *Aspergillus flavus* Link:Fr. (0.19%), *Chaetomium globosum* Kunze (0.19%), *Epicoccum purpurascens* Ehrenb. (0.14%), *Cladosporium* sp. (0.04%), and *Nigrospora oryzae* (Berk. & Broome) Petch (0.02 %).

Fusarium graminearum and *F. culmorum* were not found in 2005. They were found only in different locations of a single field in 2006. The concentration of deoxynivalenol in grain was non-detectable in samples infected by these two *Fusarium* species.

The occurrence of *F. verticillioides* and *F. proliferatum* decreased significantly from 2005 to 2006. All 66

Table 2. The presence of fumonisin-producing *Fusarium* species, infection ratio and fumonisin B_1 level from field samples of corn collected in 2005 and 2006 in the Samsun province of Turkey

Year	Site number	Site name	*Fusarium* spp. and infection ratio (%)			Fumonisin B_1 level in each sample (mg kg^{-1})
			F. ver.[1]	*F. prol.*[1]	*F. subg.*[1]	
2005	1	Merkez- Derebehçe-1	3.33	3.33	0	0.85
	2	Merkez- Taflan-2	0	0	40.00	1.93
	3	Merkez-Taflan-3	6.66	0	0	0.99
	4	Merkez-Taflan-4	13.33	6.66	0	1.38
	5	Alaçam-Killik-3	3.33	0	3.33	**2.79**[2]
	6	Alaçam-Merkez-5	0	13.33	0	1.38
	7	Ayvacık-Söğütpınar-2	0	6.66	0	1.00
	8	Ayvacık-Söğütpınar-3	0	0	3.33	1.28
	9	Bafra-Çetinkaya-1	16.66	0	0	**2.57**[2]
	10	Bafra-Çetinkaya-2	3.33	0	0	**2.93**[2]
	11	Bafra-Dedeli-5	46.66	20.00	0	**3.76**[2]
	12	Bafra-Yağmurca-6	0	6.66	0	1.57
	13	Bafra-Yağmurca-7	20.00	0	0	**3.42**[2]
	14	Bafra-Yağmurca-8	0	16.66	0	0.57
	15	Bafra-Yağmurca-9	0	3.33	0	**3.87**[2]
	16	Çarşamba-Allı-1	36.66	0	0	0.53
	17	Çarşamba-Çaltı-2	0	6.66	0	1.57
	18	Çarşamba-Dalbahçe-3	20.00	0	0	0.49
	19	Çarşamba-Kızılot-4	0	0	16.66	**4.15**[2]
	20	Çarşamba-Ovacık-5	66.66	0	0	1.39
	21	Çarşamba-Ovacık-6	30.00	0	0	0.63
	22	Çarşamba- Ovacık -7	0	3.33	0	1.06
	23	Çarşamba-Zümrüt-8	26.66	0	0	**2.19**[2]
	24	Kavak-Tabaklı-3	6.66	0	0	**2.79**[2]
	25	Kavak-Yenigün-4	6.66	0	0	**2.78**[2]
	26	Ondokuzmayıs-Merkez-1	0	0	3.33	1.53
	27	Ondokuzmayıs-Merkez-2	0	0	3.33	1.76
	28	Salıpazarı-Kalfalı-1	23.33	0	0	**6.67**[2]
	29	Salıpazarı-Merkez-2	36.66	16.66	0	**3.74**[2]
	30	Salıpazarı-Yavaşbey-3	50.00	33.33	0	**2.30**[2]
	31	Tekkeköy-Hamzalı-1	0	10.00	13.33	**3.31**[2]
	32	Tekkeköy-Kababürük-2	3.33	13.33	0	**4.02**[2]
	33	Tekkeköy-Kababürük-3	30.00	53.33	0	**5.04**[2]
	34	Tekkeköy-Merkez-5	23.33	20.00	0	**3.80**[2]
	35	Tekkeköy-Ovabaşı-6	26.66	36.66	0	**5.83**[2]
	36	Tekkeköy-Ovabaşı-7	0	0	36.66	**2.02**[2]
	37	Terme-Çangallar-2	10.00	0	0	1.61
	38	Terme-Çangallar-3	3.33	0	0	0.37
	39	Terme-Merkez-9	33.33	0	0	0.28
	40	Terme-Ortasöğütlü-10	13.33	6.66	0	0.92
	41	Vezirköprü-Aydoğdu-1	3.33	6.66	3.33	**8.48**[2]
	42	Vezirköprü-Aydoğdu-2	0	6.66	0	**7.20**[2]
	43	Vezirköprü-Esenyurt-5	20.00	10.00	0	**2.22**[2]
	44	Vezirköprü-Kületek-6	6.66	3.33	0	1.01
	45	Vezirköprü-Kületek-7	43.33	0	0	1.08

Table 2. Continued

Year	Site number	Site name	*Fusarium* spp. and infection ratio (%)			Fumonisin B_1 level in each sample (mg kg^{-1})
			F. ver.[1]	*F. prol.*[1]	*F. subg.*[1]	
2006	46	Merkez-Çatalçam-1	3.33	10.00	0	0.11
	47	Merkez-Çatalçam-2	13.33	0	0	1.03
	48	Merkez-Derebahçe-3	6.66	20.00	0	0.16
	49	Merkez-Taflan-5	3.33	13.33	0	0.59
	50	Merkez-Taflan-6	0	0	3.33	0.87
	51	Merkez-Taflan-7	10.00	0	0	0.95
	52	Bafra-Çetinkaya-3	0	0	6.66	0.35
	53	Bafra-Kaygusuz-5	0	0	26.66	0.49
	54	Bafra-Yağmurca-9(2)	3.33	0	0	0.73
	55	Çarşamba-Kızılot-6	6.66	0	0	1.25
	56	Çarşamba-Kızılot-8	0	0	6.66	**2.77**[2]
	57	Çarşamba-Köklük-11	0	0	3.33	1.07
	58	Çarşamba-Paşayazı-13	3.33	0	0	1.77
	59	Terme-Evci-2	6.66	0	6.66	0.54
	60	Terme-Evci-3	3.33	0	0	1.23
	61	Terme-Evci-4	0	0	53.33	1.03
	62	Terme-Merkez-5	0	6.66	10.00	1.98
	63	Vezirköprü-Adatepe-1	33.33	30.00	0	0.64
	64	Vezirköprü-Adatepe-2	46.66	0	0	**2.01**[2]
	65	Vezirköprü-Bahçekonak-4	0	10.00	0	0.89
	66	Vezirköprü-Yagcı-6	0	10.00	0	1.99

[1] *F. ver.*: *Fusarium verticillioides*; *F. prol.*: *Fusarium proliferatum*; *F. subg.*: *Fusarium subglutinans*.
[2] Levels of FB_1 higher than the European Union's limit.

samples naturally contaminated with *F. verticillioides*, *F. proliferatum* and *F. subglutinans* were found to be FB_1 positive, with levels ranging from 0.28 to 8.48 mg kg^{-1} in 2005 and from 0.11 to 2.77 mg kg^{-1} in 2006. None of them contained FB_2. The concentration of FB_1 in 63.6% of the samples was below 2 mg kg^{-1}, 28.8% of the samples contained from 2 to 5 mg kg^{-1}, while 7.6% of the samples contained more than 5 mg kg^{-1} (Table 2). A positive and significant correlation was found between the FB_1 level in corn and the occurrence of *F. verticillioides*, *F. proliferatum* and *F. subglutinans*. However, there was no correlation between the level of infection with those three fungi and the concentration of FB_1 detected in the same sample (Table 2). Thirty isolates of *F. verticillioides*, 22 isolates of *F. proliferatum* and 9 isolates of *F. subglutinans* were obtained in 2005, while 12 isolates of *F. verticillioides*, 7 isolates of *F. proliferatum* and 8 isolates of *F. subglutinans* were obtained in 2006 in the Samsun province (Table 2).

A *t*-test on mean transformed (square root (toxin level + 1)) toxin levels showed a significant difference between 2005 and 2006 (Table 3). The amount of toxin produced by the three *Fusarium* spp. was significantly higher in 2005 compared with 2006. No differences between locations were registered among FB_1 levels.

Weather data

Mean monthly temperatures from July to October were generally similar for both years (Fig. 2). Monthly rainfall amounts during August and October were consistently lower in 2006. No rain was recorded in August 2006 while 114.2 mm of rain had been recorded in August 2005. Average rainfall for the last 30 yr (1978-2008) was 37.5 mm for August in the Samsun province. During the months of July and September, total precipitation was slightly lower in 2005 than in 2006.

FB_1 was found in all samples from the Çarşamba, Salıpazarı and Merkez districts, in six out of seven samples from Tekkeköy, seven out of nine samples from Bafra, and two out of four samples from the Kavak and Ondokuz Mayıs districts. In the Alaçam, Ayvacık and Terme districts, FB_1 was found in less than 50% of the samples in 2005 (Table 2). In 2006, FB_1 was found in six out of seven samples in the Merkez district, four out of six samples in Vezirköprü, and four out of seven samples in Terme. In contrast, FB_1 was not found in Alaçam, Kavak, Ondokuzmayıs, Salıpazarı and Tekkeköy in 2006 (Table 3).

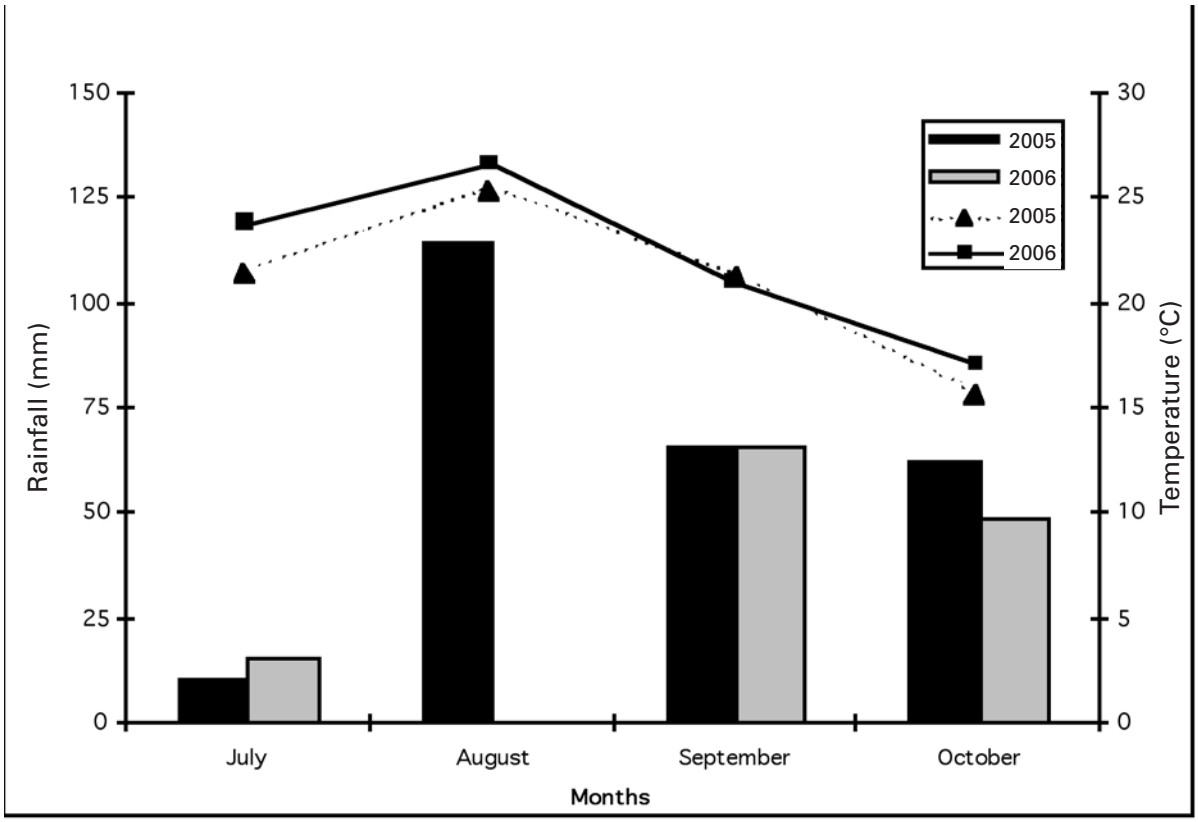

Figure 2. Monthly rainfall and temperature during the 2005-2006 experiment period in the Samsun province (data obtained from a governmental meteorology station).

Table 3. Number of corn samples collected in 2005 and 2006 and Fumonisin B_1 contamination at collection sites in the Samsun province of Turkey

	2005[1]			2006[1]		
Location	Samples collected (nb)	Samples contaminated with FB_1 (nb)	Mean FB_1 levels (mg kg^{-1})[2]	Samples collected (nb)	Samples contaminated with FB_1 (nb)	Mean FB_1 levels (mg kg^{-1})[2]
Merkez	4	4	1.28	7	6	0.61
Alaçam	5	2	2.08	4	0	0
Ayvacık	7	2	1.14	ns[3]	ns	ns
Bafra	9	7	2.67	12	3	0.52
Çarşamba	8	8	1.50	16	4	1.71
Kavak	4	2	2.78	4	0	0
Ondokuzmayıs	4	2	1.64	4	0	0
Salıpazarı	3	3	4.23	3	0	0
Tekkeköy	7	6	4.00	7	0	0
Terme	12	4	0.79	7	4	1.19
Vezirköprü	7	5	3.99	6	4	1.38
Total	**70**	**45**		**70**	**21**	

[1] Means are significantly different between 2005 and 2006 at $P < 0.01$ (*t*-test).
[2] Means are not significant at $P < 0.01$ and $P < 0.05$ (*F*-test).
[3] ns = no collection of samples.

DISCUSSION

Fusarium verticillioides, *F. proliferatum* and *F. subglutinans* were responsible for the formation of fumonisin at the time of harvest in corn fields of the Samsun province. All tested strains of those three fungi produced FB_1. The highest level of FB_1 was found in sample 41 (Vezirköprü-Aydoğdu 1). This sample contained all three species. The mycotoxin committee of the American Association of Veterinary Diagnosticians recommends that concentrations greater than 4 mg kg^{-1} should not be fed to horses and pigs (Riley *et al.* 1993). According to this advisory level, at least seven samples of corn from this study should be considered hazardous to feed horses and pigs.

Other surveys carried out in many parts of the world have revealed that these are the fumonisin-producing *Fusarium* species most frequently isolated from corn in tropical and subtropical zones (Shephard *et al.* 1996). *Fusarium verticillioides* and *F. proliferatum* co-occur worldwide in corn (Leslie *et al.* 1990), probably because they have similar optimum growth conditions. In our study, both fungi were found together in 17 samples. However, it is also common to find one without the other, such as was the case in our study. Some publications indicate that *F. subglutinans* does not produce fumonisin, but it produces other important mycotoxins (Marasas *et al.* 1984). However, Rheeder *et al.* (2002) reported that *F. subglutinans* can produce FB_1 in corn kernels. In our study, *F. subglutinans* was found alone in 12 samples that contained FB_1 (0.87 to 4.15 mg kg^{-1}) (Table 2).

The role of humidity for fumonisin production in corn is clearly important. Variation in *Fusarium* spp. presence and fumonisin contamination from one season to another was observed in 2005 and 2006. *Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans* incidence was higher in 2005 than in 2006. Fumonisin B_1 contamination was also higher in 2005 than in 2006. In our study, average rainfall during the survey period was higher in August 2005 (114.2 mm) than in August 2006 (0.0 mm). In a similar study, Heiniger *et al.* (2002) compared daily rainfall, temperature and RH records for three locations, and showed that the most striking environmental event related to fumonisin development was the precipitation recorded on August 12 and 13 at all locations. These rainfall events were accompanied by increases in RH and short-term decreases in temperature. Immediately prior to these rainfall events, temperatures had increased dramatically, reaching their highest point for the summer in early August.

Based on our research, rainfall in August 2005 totalled 114.2 mm, but there was no rain in August 2006. Average moisture was lower in August 2006 than in August 2005. These results may explain why both fumonisin production and the FB_1 level were higher in 2005 than in 2006. In August 2005, rainfall was three times superior to the 30-yr average of 37.5 mm. Gong *et al.* (2009) demonstrated that the high level of FB_1 contamination of corn in the Sichuan and Guangxi provinces was correlated with the meteorological data of these provinces, including moderate mean temperature and high relative humidity and rainfall. Gamanya and Sibanda (2001) also found levels of fumonisin in Zimbabwe to be higher in regions with high rainfall and moderate annual temperature than in those with low rainfall. Hennigen *et al.* (2000) found fumonisin contamination in Argentina to differ markedly over two consecutive growing seasons. Such yearly variation may, among other things, be attributable to differences in environmental conditions. Desjardins *et al.* (1998) also reported that the frequency of infected kernels was higher in their 1993 field tests than in their 1994 ones. This difference may have been due to unusually heavy rainfall causing poor growing conditions during the spring and summer of 1993. In contrast, ears harvested in 1994 generally were of good quality, with few visibly moldy, discoloured, or chalky kernels.

Most of the samples collected for this study did not show any disease symptoms; therefore, no correlation was found between FB_1 and visibly diseased kernels. However, Desjardins *et al.* (1998) reported that fumonisin levels were low (< 1µg g^{-1}) in symptomless kernels, and higher (138 µg g^{-1}) in symptomatic kernels. Bush *et al.* (2004) found that the analysis of both symptomless and symptomatic kernels revealed a relatively high concentration of fumonisin. In other studies, individual ears of maize confirmed prior observations of a poor correlation between fumonisin levels and the incidence of *F. verticillioides* in bulked maize ears collected from farmlands in South Africa (Rheeder *et al.* 1992,1995).

Fusarium graminearum and *F. culmorum* were found only in 2006, but *F. culmorum* was found in different locations of a single field. Environmental factors may have affected spore germination and fungal growth on corn kernels. It has been reported that *F. graminearum* needs a minimum water potential (a_w) of 0.94 to 0.95 at 25°C to germinate (Sung and Cook 1981), whereas at approximately the same temperature, ascospores of *F. verticillioides* are reported to germinate down to 0.88 a_w (Marin *et al.* 1999). The concentration of deoxynivalenol in *F. graminearum*- and *F. culmorum*-infected grain was not detectable by HPLC analysis.

The present study shows that *F. verticillioides* is the predominant fungus in corn field populations in the Samsun province of Turkey. All *F. verticillioides*, *F. proliferatum* and *F. subglutinans* isolates were investigated and all of them produced FB_1. None of them produced FB_2. Fumonisin B_1 production varied from 0.11 to 8.48 mg kg^{-1} in naturally infected corn fields. Corn contamination with both *Fusarium* and FB_1 was found to be possible everywhere in the Samsun province, but was strongly dependent on the environmental and seasonal conditions observed during the study. No *Fusarium* contamination was found in some native white-type and popcorn-type corn cultivars during the 2005-2006 study period. Further investigation is needed in order to screen cultivars for resistance to *Fusarium* spp., FB_1 and FB_2, and to establish relationships between genotypes and environmental conditions. Additional studies are also needed to define fumonisin-producing *Fusarium* species and their molecular characterization in other parts of Turkey.

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REFERENCES

Aktaş, H., and B. Tunali. 1990. Untersuchungen zur Bestimmung und Methodik von Fungal-flora an Maissamten. J. Turk. Phytopathol. 19 : 71-79.

Aktas, H., B. Tunali, and I. Aktuna. 1994. Determination of the fungal agents in maize cultivation areas in Bolu and Zonguldak provinces and investigation of corn variety reactions against some important pathogens. Turk. J. Agric. For. 18 : 287-295.

Anonymous. 2007. Maize (plant). [http://tr.wikipedia.org/wiki/M%C4%B1s%C4%B1r_(bitki)15 June 2010].

Anonymous. 2008. FAO Statistical Databases. [http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor].

Arici, M., O. Daglioglu, T. Gumus, and F. Daglioglu. 2004. Occurrence of fumonisin in processed and low processed corn-based products in Turkey. Acta Aliment. 33 (3) : 325-328.

Barnett, H.L., and B. Hunter. 1998. Illustrated Genera of Imperfect Fungi, 4th ed. APS Press, St. Paul, MN, USA. 218 p.

Booth, C. 1977. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, UK. 58 p.

Burgess, L.W., B.A. Summerrell, S. Bullock, K.P. Gott, and D. Backhouse. 1994. Laboratory Manual for *Fusarium* Research, 3rd ed. University of Sydney, Australia. Chapter 3, p. 12-16.

Bush, B.J., M.L. Carson, M.A. Cubeta, W.M. Hagler, and G.A. Payne. 2004. Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. Phytopathology 94 : 88-93.

Chang, H.L. 1984. Rapid determination of deoxynivalenol (Vomitoxin) by liquid chromatography using modified Romer Clean up column. J. Assoc. Off. Anal. Chem. 67 : 52-54.

Desjardins, A.E., R.D. Plattner, M. Lu, and L.E. Claflin. 1998. Distribution of fumonisins in maize ears infected with strains of *Fusarium moniliforme* that differ in fumonisin production. Plant Dis. 82 : 953-958

Ellis, M.B. 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, UK. 507 p.

Fandohan, P., K. Hell, W.F.O. Marasas, and M.J. Wingfield. 2003. Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. Afric. J. Biotechnol. 12 : 570-579.

Francis, R.O., and L.W. Burgess. 1975. Surveys of Fusaria and other fungi associated with stalk rot of maize in eastern Australia. Australas. J. Agric. Res. 26 : 801-807.

Gamanya, R., and L. Sibanda. 2001. Survey of *Fusarium moniliforme* (=*Fusarium verticillioides*) and production of fumonisin B_1 in cereal grains and oilseeds in Zimbabwe. Int. J. Food Microbiol. 71 : 141-149.

Gerlach, W., and H.I. Nirenberg. 1982. The Genus *Fusarium* – a Pictorial Atlas. Mitt. Bit. Bundesanst. For Stwirtsch. Berlin-Dahlem, Germany. 406 p.

Gong, H.Z., R. Ji, Y.X. Li, H.Y. Zhang, B. Li, Y. Zhao, L. Sun, F. Yu, and J. Yang. 2009. Occurrence of fumonisin B_1 in corn from the main corn-producing areas of China. Mycopathologia 167 : 31-36.

Heiniger, R.W., G.J. Vernon, and F.E. O'Neal. 2002. Fumonisin: Growing Problem in Corn. [http://www.ces.ncsu.edu/plymouth/cropsci/docs/fumonisin2002/fumonisin2002.html].

Hennigen, M.R., L.M. Valente Soares, S. Sanchez, N.M. Di Benedetto, A. Longhi, G. Eyherabide, J. Torroba, and M. Zanelli. 2000. Fumonisins in corn hybrids grown in Argentina for two consecutive seasons. Pages 331-339 *in* W.J De Koe, R.A Samson, H.P Van Egmond, J. Gilbert, and M. Sabino (eds.), Proceeding of the Xth International IUPAC Symposium on Mycotoxins and Phytotoxins, 21-25 May 2000, Guaruja, Brazil.

Kedera, C.J., R.D. Plattner, and A.E. Desjardins. 1999. Incidence of *Fusarium* spp. and levels of fumonisin B_1 in maize in western Kenya. Appl. Environ. Microbiol. 65 : 41-44.

Leslie, J.F., and B.A. Summerall. 2006. The Fusarium Laboratory Manual. Blackwell Publishing, IOWA, USA. 388 p.

Leslie, J.F., C.A.S. Pearson, P.E. Nelson, and T.A. Toussoun. 1990. *Fusarium* spp. from corn, sorghum and soybean fields in the Central and Eastern United States. Phytopathology 80 : 343-350.

Macdonald, M.V., and R. Chapman. 1997. The incidence of *Fusarium moniliforme* on maize from Central America, Africa and Asia during 1992-1995. Plant Pathol. 46 : 112-125.

Marasas, W.F.O. 2001. Discovery and occurrence of the fumonisins: a historical perspective. Environ. Health Perspect. 109 : 239-243.

Marasas, W.F.O., N.P.J. Kriek, J.E. Ficham, and S.J. Van Rensburg. 1984. Primary liver and esophageal basal cell hyperplasia in rats caused by *Fusarium moniliforme*. Int. J. Cancer 34 : 383-387.

Marin, S., N. Magan, N. Belli, A.J. Ramos, R. Canela, and V. Sanchis. 1999. Two dimentional profiles of fumonisin B_1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize. Int. J. Food Microbiol. 51 : 159-167.

Miller, J.D., M.E. Savard, A. Sibilia, S. Rapior, A.D. Hocking, and J.J. Pitt. 1993. Production of fumonisins and fusarins by *Fusarium moniliforme* from Southeast Asia. Mycologia 85 : 385-391.

Munkvold, G.P., and A.E. Desjardins. 1997. Fumonisin in maize. Can we reduce their occurrence? Plant Dis. 81 : 556-564.

Njuguna, J.G.M, C.J. Kedera, L.M. Muriithi, W. Songa, and B.O. Odhiambo. 1990. Overview of maize diseases in Kenya. Pages 52-61 *in* Proceedings of a Workshop on Review of the National Maize Program. Kenya Agricultural Research Institute, Nairobi.

Omurtag, G.Z. 2001. Determination of fumonisin B_1 and FB_2 in corn and corn-based products by HPLC. J. Food Prot. 64 : 1072-1075.

Oruc, H.H., M. Cengiz, and O. Kalkanli. 2006. Comparison of aflatoxin and fumonisin levels in maize grown in Turkey and imported from the USA. Anim. Feed Sci. Technol. 128 : 337-341.

Rheeder, J.P., W.F.O. Marasas, P.G. Thiel, E.W. Sydenham, G.S Shephard, and D.J. van Schalkwyk. 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82 : 353-357.

Rheeder, J.P., E.W, Sydenham, W.F.O. Marasas, P.G Thiel, G.S. Shephard, S. Stockenstrom, D.W. Cronje, and J.H. Viljoen. 1995. Fungal infestation and mycotoxin contamination of South African commercial maize harvested in 1989 and 1990. S. Afr. J. Sci. 91 : 127-131.

Rheeder, J.P., W.F.O. Mararas, and H.F. Vismer. 2002. Production of fumonisin analogs by *Fusarium* species. Appl. Environ. Microbiol. 68 : 2101-2105.

Riley, R.T., W.P. Norred, and C.W. Bacon. 1993. Fungal toxins in foods: recent concerns. Annu. Rev. Nutr. 13 : 167-189.

Ross, P.F., P.E. Nelson, J.L. Richard, G.D. Osweiler, L.G. Rice, R.D. Plattner, and T.M. Wilson. 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and pulmonary enema syndrome in swine. Appl. Environ. Microbiol. 56 : 3225-3226.

Ross, P.F., R.A. Rice, G.D. Osweiler, P.E. Nelson, J.L. Richard, and T.M. Wilson. 1992. A review and update of animal toxicosis associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia 17 : 109-114.

Shephard, G.S., E.W. Sydenham, P.G. Thiel, and C.A. Gelderblom. 1990. Quantitative determination of fumonisins B_1 and B_2 by high-performance liquid chromatography with fluorescence detection. J. Liq. Chromatogr. 13 : 2077-2087.

Shephard, G.S., P.G. Thiel, S. Stockenstrom, and E.W. Sydenham. 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. J. AOAC Int. 79 : 671-687.

Sung, J., and R.J. Cook. 1981. Effect of water potential on reproduction and spore germination by *Fusarium roseum* "Graminearum", "Culmorum" and "Avenaceum". Phytopathology 71 : 499-504.

Sydenham, E.W., G.S. Shephard, P.G. Thiel, W.F.O. Marasas, A. Visconti, and M.B. Doko. 1994. Survey of fumonisin production by *Fusarium* isolated from cereals in Europe. J. AOAC Int. 77 : 546-550.

Visconti, A., and M.B. Doko. 1994. Survey of fumonisin production by *Fusarium* isolated from cereals in Europe. J. AOAC Int. 77 : 540-550.

Windels, C.E., P.M. Burns, and T. Kommendahl. 1988. Five-year preservation of *Fusarium* species on silica-gel and soil. Phytopathology 78 : 107-110.