Fusarium moniliforme, F. subglutinans and Aspergillus flavus in maize products in Slovakia

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Ubiquitous microfungi *Fusarium moniliforme, F. subglutinans, F. proliferatum* represent frequent contaminants of maize products and can produce some mycotoxins: beauvericin, fusaproliferin and, the most important, fumonisins A_1, A_2, B_1–B_4, C_1 etc. Fumonisins are known to cause serious veterinary, and potentially human, mycotoxicosis. The aim of our study was to characterize the incidence of *F. moniliforme* and *F. subglutinans* in the presence of *Aspergillus flavus* in maize products produced in Slovakia during a four-years period. One hundred and forty samples of maize grain, groat, semolina, flour, and 28 samples of maize straw, cornhusk, corn silk and soil from the maize fields were mycologically evaluated for the named strains using potato-dextrose agar with 0.02 % chloramphenicol and 0.3 % of 0.2 % Botran and incubation in dark at 25 °C for 7–10 days. No *Fusarium* sp. and *A. flavus* strains were present in 40 % of the maize samples. The highest number of *F. moniliforme, F. subglutinans* and *A. flavus* isolates were encountered in flour samples in 1996 (4 264 cfu/g on average), in groat in 1998 (17 743.7 cfu/g on average), and in groat in 1996 (353 cfu/g on average). Twenty two per cent *A. flavus* isolates and 10 *F. moniliforme* strains were tested for their ability to produce aflatoxins, or fumonisin B_1, *in vitro*. No aflatoxin-producing *A. flavus* isolate was found, but all tested*F. moniliforme* strains produced fumonisin B_1 in amounts detectable by TLC. According to the results presented in this paper it is evident that strains of *F. moniliforme, F. subglutinans* and *A. flavus* were not very important contaminants of maize products from crops harvested in 1995–98 in Slovakia.

Key words: *Fusarium moniliforme, F. subglutinans, Aspergillus flavus*, mycotoxins, maize

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Celosvetovo rozšírené mikroskopické huby *Fusarium moniliforme, F. subglutinans, F. proliferatum*, často sa vyskytujúce v kukuričných produktoch, sú schopné produkovať viaceré mykotoxíny: beauvericín, fuzaproliferín, ako aj najviac sledované fumonizíny A_1, A_2, B_1–B_4, C_1 atď. Je známe, že fumonizíny spôsobujú vážne veterinárne, a pravdepodobne aj humánne, mykotoxikózy. Cieľom 4-ročnej štúdie bolo charakterizovať incidenciu *F. moniliforme* a *F. subglutinans*, spolu s *Aspergillus flavus*, v kukuričných produktoch slovenskej proveniencie. Po kultivácii na zemiakovo-dextrózovom agare s 0,02 % chloramfenikolu a 0,3 % z 0,2 % Botranu počas 7–10 dní pri 25 °C v tme sa mykologicky vyšetrilo 140 vzoriek kukuričného zrna, šrotu, krupice a múky a 28 vzoriek kukuričnej slamy, šúpolia, vlásia a pôdy z kukuričných polí. Zo 40 % kukuričných vzoriek sa neizoloval žiaden kmeň *Fusarium* sp. a *A. flavus*. Najviac izolátov *F. moniliforme, F. subglutinans* a *A. flavus* sa získalo zo vzoriek múky v r. 1996 (priemerne 4264 kolónie tvoriacich jednotiek – KTJ/g), šrotu v tom istom roku (priemerne 353 KTJ/g) a v r. 1998 (priemerne 17743 KTJ/g). Dvadsaťdva percent izolátov *A. flavus* a 10 kmeňov *F. moniliforme* sa testovalo na schopnosť produkovať aflatoxíny, resp. fumonizín B_1 *in vitro*. Nezistil sa ani jeden aflatoxín-produkčný kmeň *A. flavus*, ale všetky testované kmene *F. moniliforme* produkovali fumonizín B_1 v množtve detekovateľnom tenkovrstvovou chromatografiou. Na základe získaných výsledkov možno konstatovať, že kmene *F. moniliforme, F. subglutinans* a *A. flavus* významne nekontaminovali kukuričné produkty z úrody v r. 1995–98 na Slovensku.

INTRODUCTION

Ubiquitous worldwide-spread microfungi *Fusarium moniliforme, F. subglutinans* and *F. proliferatum* represent frequent contaminants of maize products. They are able to produce some mycotoxins: beauvericin, fusaproliferin and, the most important, fumonisins A_1, A_2, B_1–B_4, C_1 and their isoderivatives. *F. napiforme, F. nygamai* and *F. dlamini* which occurr more rarely in plants are also producers of fumonisins (Ritieni et al. 1997a, Sibanda et al. 1997, MacKenzie et al. 1998).

It is known that fumonisins cause serious veterinary mycotoxicosis, including fatal organ debilitites of laboratory animals. The consumption of contaminated maize grain and maize-based products has been statistically associated with an elevated incidence of human oesophageal cancer in some parts of the world (Nair 1998, Rosiles et al. 1998), and also has been reported to have caused accute toxicosis in consumers (Bhat et al. 1997). According to a study by Ueno et al. (1997) in some parts of China endemic for primary hepatocarcinoma, fumonisins B and deoxynivalenol represented risk factors for this disease while aflatoxin B_1 was postulated as the initiating factor for hepatocarcinogenesis. According to IARC fumonisins are potential human carcinogens (class 2B). Because of the ability of these highly heat- and fermentation-resistant mycotoxins presence in cereal products, usually contaminated with carcinogenic aflatoxins, the Joint FAO/WHO Expert Committee on Food Additives and Contaminants reccommends their study in any way (Shephard et al. 1996, Jackson et al. 1997, Torres et al. 1998). Their level should not exceed 100–200 μg/kg in contaminated maize. The maximum daily tolerated intake (MDTI) for humans is being concidered for 1 000 ng, and the maximum tolerated level of fumonisins A and B in foodstuffs, 1 mg/kg, is the current regulation in Switzerland (Diener 1997, de Nijs et al. 1998).

In contrast to neighbouring countries with similar climates (Austria, the Czech Republic, Hungary and Poland) there has been a lack of information on the incidence of *F. moniliforme* and *F. subglutinans* in maize products produced in Slovakia. The aim of our study was to characterize their colonisation by both fusaria strains in the presence of *A. flavus*.

MATERIAL AND METHODS

In 1995–98 one hundred and forty samples of maize products (43 samples of maize grain, 24 groat, 54 semolina and 19 flour, stored at a silo with relative air humidity 58–68 % and temperature approx. 2–3 °C less than outside ambient), 2 samples of maize straw, 5 samples of cornhusk, 1 sample of corn silk, and 20 samples of soil from maize fields, were taken in southern Slovakia where climatic conditions are suitable for corn cultivation. Climatological information on evaluated period are given in Table 1 (according to the Slovak Hydrometeorological Institute, Bratislava, Slovakia). June–August are months with the highest average temperatures, and December – February the coldest ones. Average month's air relative humidity is usually the highest in December and January while the lowest in July.

Table 1. Climatological characterization of southern region of Slovakia (measuring station Gabčíkovo) in 1995–98

Year	average month's T /C/	average max. month's T /C/	Range average min. month's T /C/	average month's air relat. humid. /%/	average month's precipitation /mm/
1995	−0.3–23.1	1.8–29.8	−2.9–15.6	63.9–90.0	1.2–90.4
1996	−3.0–19.7	−0.3–25.7	−7.9–14.2	64.4–89.7	11.4–136.2
1997	−2.5–20.4	−0.3–27.7	−5.5–14.3	66.9–92.2	7.1–175.1
1998	−1.5–20.7	1.0–27.5	−3.8–15.4	62.1–86.0	2.4–124.6

Note: T – temperature

Every product sample was mycologically evaluated for *F. moniliforme, F. subglutinans* and *A. flavus* strains by a dilution method (max. 4-times) in sterile saline on 10 plates of potato-dextrose agar (Difco) with 0.02 % of chloramphenicol and 0.3 % of 0.2 % Botran (0.2 ml of suspension per plate) or 50 grains from every seed sample were put onto the 10 agar plates. After incubation in the dark at 25 °C for 7–10 days the number of colony forming units (cfu) per 1 g of sample or relative number [%] of contaminated seeds was quantified. Every isolated colony of both fusaria and *A. flavus* was identified according to their macro- and micromorphology.

One hundred and thirty six, i. e. 22 % of isolates of *A. flavus* were tested for their ability to produce aflatoxins *in vitro* using the method described by Abarca et al. (1988) in liquid medium with 20 % of sucrose and 2 % of yeast extract. The ability of 10 *F. moniliforme* strains to produce fumonisin B_1 *in vitro* was tested semi-quantitatively by TLC (silica gel GF 254, Merck; mobile phase acetonitrile: toluene: water – 95: 5: 3; v/v) after 20 d of cultivation on sterile corn with 40 % humidity (Pepeljnjak et al. 1998).

RESULTS

No strains of *A. flavus* were isolated from soil, straw, cornhusk and corn silk samples (all taken in 1998). No fusaria were present in 46 % of these samples while *F. moniliforme* was isolated from 50 % of the soil samples with 110.5 cfu/g on average, one straw and corn silk samples yielded 65 000, and 40 cfu/g, respectively, and 20 % of the soil samples and the other straw sample were contaminated with *F. subglutinans* yielding 22.3 cfu/g, and 205 000 cfu/g, respectively.

No searched moulds were present in 40 % of maize samples during our study. The only strains isolated from 6.4 % of the samples were *F. subglutinans,* while no sample was contaminated just with *F. moniliforme* strains. Both these fusaria occurred in 27 % of the samples. *A. flavus* strains, as well as fusaria together with *A. flavus,* occurred in 12 %of the samples investigated (Table 2).

Table 2. Occurrence of *Fusarium moniliforme* (FM), *A. subglutinans* (FS) and *Aspergillus flavus* (AF) strains in maize products in 1995–98.

Crop year	1995		1996		1997		1998	
	abs.	[%]	abs.	[%]	abs.	[%]	abs.	[%]
No. of negative samples	11	52	12	36	20	37	3	9
No. of samples with only FM	0	0	0	0	0	0	0	0
No. of samples with only FS	0	0	1	3	1	2	7	22
No. of samples with FM+FS	4	19	2	7	19	35	13	41
No. of samples with only AF	2	10	3	9	8	15	4	12.5
No. of samples with AF+FM	1	5	5	15	4	7	0	0
No. of samples with AF+FS	0	0	1	3	1	2	1	3
No. of samples with AF+FM+FS	3	14	9	27	1	2	4	12.5
Total	21	100	33	100	54	100	32	100

The incidence of *F. moniliforme* in grain was 3, 1, 0.8 or 0.3 % and that of *F. subglutinans* was 22, 1, 0.5 or 0.4 % in 1995, 1996, 1997 and 1998, respectively. *A. flavus* contaminated 1, 0.7, 2 or 1.4 % of tested grains in these years (Table 3).

Table 3. Contamination of maize grain with *Fusarium moniliforme* (FM), *F. subglutinans* (FS) and *Aspergillus flavus* (AF) (total 43 samples).

Year Total No. of samples/No. of contaminated samples/average percentage of contaminated grains	1995	1996	1997	1998
FM	8/1/3 %	7/5/1 %	20/9/0.8 %	8/8/0.3 %
FS	8/1/22 %	7/5/1 %	20/9/0.5 %	8/8/0.4 %
AF	8/1/1 %	7/5/0.7 %	20/9/2 %	8/8/1.4 %

In 1995 *F. moniliforme* had a higher incidence than that of *F. subglutinans* in similar samples, while *A. flavus* had the least contamination levels. None of the 6 *A. flavus* isolates produced aflatoxin B_1 *in vitro*. In 1996 *F. subglutinans* had a higher incidence in groat samples, while *F. moniliforme* remained high in semolina and flour. *A. flavus* on the other hand had a high incidence in groat samples and of the 46 (28 %) isolates tested none produced aflatoxin B_1 *in vitro*. *F. subglutinans* had a higher frequency of occurrence in 1997 than *F. moniliforme* with incidences of 2 638.5 and 53.7 cfu/g compared to 145 and 6.7 cfu/g in groat and semolina, respectively. *A. flavus* had consistently lower incidences, 103.5 and 0.4 cfu/g. None of 22 (63 %) evaluated *A. flavus* isolated strains produced aflatoxin B_1 *in vitro* under experimental conditions. In 1998 strains of *F. subglutinans* had a higher incidence in groat and flour than *F. moniliforme*, 17 743. 7, 1 195 and 948.7 compared to 185.9 and 220 cfu/g. However, incidences were comparable in semolina samples. *A. flavus* had an occurrence of 41.2, 30 and 177.7 cfu/g. None of the 62 (20 %) *A. flavus* isolates produced aflatoxin B_1 *in vitro* (Table 4–6).

Table 4. Contamination of maize groat with *Fusarium moniliforme* (FM), *F. subglutinans* (FS) and *Aspergillus flavus* (AF) (total 24 samples).

Year Total No. of samples/No. of contaminated samples/average No. of isolates [cfu/g]	1995	1996	1997	1998
FM	1/1/200	5/3/166	10/3/145	8/8/185.9
FS	1/1/0	5/3/1326	10/3/2638.5	8/8/17743.7
AF	1/1/0	5/3/353	10/3/103.5	8/8/41.2

Table 5. Contamination of maize semolina with *Fusarium moniliforme* (FM), *F. subglutinans* (FS) and *Aspergillus flavus* (AF) (total 54 samples).

Year Total No. of samples/No. of contaminated samples/average No. of isolates [cfu/g]	1995	1996	1997	1998
FM	9/6/61.1	13/8/416.9	24/22/6.7	8/8/1408.7
FS	9/6/15.5	13/8/3.1	24/22/53.7	8/8/1195
AF	9/6/4.4	13/8/4.8	24/22/0.4	8/8/30

Table 6. Contamination of maize flour with *Fusarium moniliforme* (FM), *F. subglutinans* (FS) and *Aspergillus flavus* (AF) (total 19).

Year Total No. of samples/No. of contaminated samples/average No. of isolates [cfu/g]	1995	1996	1997	1998
FM	3/2/1850	8/5/4264.1	0/0/0	8/5/220
FS	3/2/50	8/5/46.9	0/0/0	8/5/648.7
AF	3/2/1.7	13/5/12.5	0/0/0	8/5/177.7

Two (20 %) *F. moniliforme* strains isolated from grain in 1996 produced 125–185 mg/kg of biomass and 8 strains (80 %) with the same origin 232–417 mg/kg of fumonisin B_1 detected by TLC (Pepeljnjak et al. 1998).

DISCUSSION

According to Schlechter et al. (1998) American maize products in 1991–92 were contaminated with 10^5 cfu of *F. moniliforme* and max. 3 605 ng of total fumonisins per 1 g and products originated in South Africa 10^3 cfu of *F. moniliforme* and 465 ng of total fumonisins per g. *F. moniliforme* and *F. proliferatum* strains represented the most frequent contaminants of Italian maize in which also fumonisin B1, beauvericin and fusaproliferin, all together, were present (Ritieni et al. 1997b). Logrieco et al. (1998) regarded *F. subglutinans, F. proliferatum* and *F. semitectum* as natural contaminants of maize in Europe, South America and South Africa. They can produce beauvericin which has been already isolated from maize in named parts of the world as well as in North America (Logrieco et al. 1997). Czech cereal products from corn harvested in 1995 (71 samples) contained 278 ng of fumonisins B_1–B_3 and from 1996 harvest (76 samples) 131 ng of the same fumonisins per g (Ostrý and Ruprich 1997). The same authors evaluated also maize products by ELISA, from which 89 % were contaminated with fumonisins (180 ng/g on average), but 4 % with more than 1 000 ng/g (Ostrý and Ruprich 1998). Dutch authors (de Nijs et al. 1998) found that 93 % of tested samples of maize, imported to the Netherlands from 18 countries, were contaminated with fumonisin B_1 in average amount 1359 ng/g.

From our results of investigation of maize products from crop harvested in 1995–98 in Slovakia, it can be seen that more than one third of tested samples did not contain *F. moniliforme* or *F. subglutinans*. From the rest of the samples, both fusaria were hardly isolated in counts higher than 10^3 cfu/g, however, they were usually found in the presence of *A. flavus* strains. The occurrence of propagules of micromycetes in evaluated materials was not apparent in this study.

The *A. flavus* strains tested for *in vitro* production of aflatoxin B_1 were all negative, and *F. moniliforme* were able to produce only small quantities (10^2 mg/kg of biomass) of fumonisin B_1 *in vitro*.

CONCLUSION

Probably good conditions of farming technology during harvest and storage of raw maize and maize-based products in observed period were resulted in their low contamination with *F. moniliforme, F. subglutinans* and *A. flavus* moulds. Their ability to produce fumonisin B_1, or aflatoxin B_1 *in vitro* was not significant, too.

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