

Fusarium species (section *Liseola*) occurrence and natural incidence of beauvericin, fusaproliferin and fumonisins in maize hybrids harvested in Mexico

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Received: 24 November 2010 / Revised: 1 April 2011 / Accepted: 4 April 2011 / Published online: 17 May 2011
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Abstract *Fusarium* species can produce fumonisins (FBs), fusaric acid, beauvericin (BEA), fusaproliferin (FUS) and moniliformin. Data on the natural occurrence of FBs have been widely reported, but information on BEA and FUS in maize is limited. The aims of this study were to establish the occurrence of *Fusarium* species in different maize hybrids in Mexico, to determine the ability of *Fusarium* spp. isolates to produce BEA, FUS and FBs and their natural occurrence in maize. Twenty-eight samples corresponding to seven different maize hybrids were analyzed for mycobiota and natural mycotoxin contamination by LC. *Fusarium verticillioides* was the dominant species (44–80%) followed by *F. subglutinans* (13–37%) and *F. proliferatum* (2–16%). Beauvericin was detected in three different hybrids with levels ranging from 300 to 400 ng g⁻¹, while only one hybrid was contaminated with FUS (200 ng g⁻¹). All

samples were positive for FB₁ and FB₂ contamination showing levels up to 606 and 277 ng g⁻¹, respectively. All *F. verticillioides* isolates were able to produce FB₁ (13.8–4,860 µg g⁻¹) and some also produced FB₂ and FUS. Beauvericin, FUS, FB₁ and FB₂ were produced by several isolates including *F. proliferatum* and *F. subglutinans* and co-production was observed. This is the first report on the co-occurrence of these toxins in maize samples from Mexico. The analysis of the presence of multiple mycotoxins in this substrate is necessary to understand the significance of these compounds in the human and animal food chains.

Keywords *Fusarium* · Beauvericin · Fusaproliferin · Fumonisins · Maize

Introduction

Fusarium species are widely distributed plant pathogens. *Fusarium verticillioides* is responsible for several diseases such as maize seedling blight, root, stalk and ear rot and can also infect vegetative and reproductive tissues without evident symptom development. Previous studies on maize harvested in Mexico showed that *Fusarium* species were the predominant fungi isolated (Cortez-Rocha et al. 2003; Desjardins et al. 1994; Morales-Rodriguez et al. 2007; Sánchez-Rangel et al. 2005). The dominant species isolated was *Fusarium verticillioides* (*Gibberella fujikuroi* mating population A; teleomorph, *Gibberella moniliformis* Wineland). However, *Fusarium proliferatum* (*G. fujikuroi* mating population D; teleomorph, *G. intermedia*) and *Fusarium subglutinans* (*G. fujikuroi* mating population E; teleomorph, *Gibberella subglutinans*) have also been infrequently reported from maize. These species can produce mycotoxins such as fumonisins (FBs), fusaric acid, beauvericin (BEA),

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fusaproliferin (FUS), and moniliformin (MON) (Bottalico 1998; Glenn 2007; Leslie and Summerell 2006; Kommedahl and Windels 1981).

BEA is a cyclodepsipeptide compound, a specific cholesterol acyltransferase inhibitor mainly produced by strains of *F. proliferatum* and *F. subglutinans* among other *Fusarium* species (Logrieco et al. 1998; Glenn 2007; Reynoso et al. 2004; Torres et al. 2001). Beauvericin has been found as a natural contaminant of maize in countries from Europe, Africa and America (Jestoi 2008; Krska et al. 1997; Logrieco et al. 1993; Munkvold et al. 1998; Ritieni et al. 1997; Shephard et al. 1999). The primary biological activity of BEA in vitro is considered to be related to their ionophoric properties. Accordingly, they are capable of promoting the transport of mono- and divalent cations through membranes leading to disturbances in their normal physiological concentrations (Jestoi 2008).

Fusaproliferin (FUS) is a toxic metabolite produced by several *Fusarium* spp. such as *F. proliferatum*, *F. subglutinans*, *F. gutiforme*, *F. pseudocircinatum*, *F. pseudonygamai*, *F. verticillioides* and *F. konzum* (Glenn 2007; Jestoi 2008; Leslie et al. 2004; Reynoso et al. 2004; Torres et al. 2001). Information about natural occurrence of FUS in maize is scarce; however, FUS has been detected in various crops and commodities such as moldy maize ears in Italy, maize and animal feed samples from USA, home-grown maize samples from South Africa and preharvest maize samples with ear rot symptoms in Slovakia (Munkvold et al. 1998; Ritieni et al. 1997; Shephard et al. 1999; Srobarova et al. 2002). This toxin is toxic to brine shrimp (*Artemia salina*) and mammalian cells and causes teratogenic effects on chicken embryos (Jestoi 2008; Logrieco et al. 1996; Ritieni et al. 1997).

Fumonisin are produced by *F. verticillioides*, *F. proliferatum*, and other *Fusarium* species (Gelderblom et al. 1988; Bolger et al. 2001; Glenn 2007). While these mycotoxins are found in other commodities, animal and human health problems related to these mycotoxins are almost exclusively associated with the consumption of contaminated maize or products made from maize (Bolger et al. 2001; Marasas 2001). The human health effects of fumonisins are uncertain. However, fumonisins are suspected risk factors for esophageal (Marasas 2001; Rheeder et al. 1992) and liver (Ueno et al. 1997), cancers, neural tube defects (Gelineau-van Waes et al. 2005; Missmer et al. 2006), and cardiovascular problems in populations consuming relatively large amounts of food made with contaminated maize (Fincham et al. 1992).

Consumption of moldy maize has long been a recognized cause of equine leukoencephalomalacia (ELEM) (Kellerman et al. 1990; Marasas et al. 1988) and porcine pulmonary edema (PPE) (Harrison et al. 1990; reviewed by Haschek et al. 2001).

Maize-based foods are consumed by very large numbers of people in Latin America and they form an important part on its economy. Therefore, the presence of *Fusarium* mycotoxins has impacts in animal and human health. Worldwide, data on the natural occurrence of FBs in maize have been widely reported (Cortez-Rocha et al. 2003; Doko et al. 1995; Ramirez et al. 1996), but information of other toxins produced by *Fusarium* species such as BEA and FUS on this substrate intended for human and animal consumption is limited (Ritieni et al. 1997).

At present, there is no information on FUS and BEA contamination in maize harvested in Mexico. In addition, there is no information about the toxigenic profile related to FUS and BEA of *Fusarium* species isolated from maize harvested in Mexico. Most of the information refers only to fumonisin production and natural contamination (Desjardins et al. 1994; Dombrink-Kurtzman and Dvorak 1999; Robledo and Marín 2001; Rosiles et al. 1998, 2007; Sánchez-Rangel et al. 2005).

The aims of the study were: (1) to evaluate the prevalence of *Fusarium* species in different maize hybrids cultivated in Mexico; (2) to assess by sexual crosses the mating populations of the isolated species; (3) to determine the natural occurrence of fumonisins, beauvericin and fusaproliferin in the samples; and (4) to determine ability of the strains to produce fumonisins, fusaproliferin and beauvericin.

Materials and methods

Sampling

A total of seven maize hybrids (Lucero 801, Lucero 807, Lucero 808, Lucero 901, Lince, Alsa 036 W and UDG 600) cultivated in experimental field trials of the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), Universidad de Guadalajara (Jalisco State, Mexico) during the 2003 harvest season were screened for *Fusarium* spp. contamination and toxin content. A complete randomized design with 4 repetitions was used. Each plot had 4 rows (8 m length) and 160 plants (5 plants per m). Sampling was done at physiological maturity stage (135 days). Forty-two ears (per sample) from each plot were collected, hand-shelled and pooled, and a subsample of 200 g was taken. These subsamples were immediately analyzed for fungal contamination and then stored at 4°C (up to 7 days) until mycotoxin analyses. All maize samples were asymptomatic (no evident kernel damage). Samples were stored at 4°C until mycotoxin analyses.

Mycological analyses

Each subsample of maize kernels (4 from each hybrid) was surface-disinfected with NaClO (1%) for 1 min, and then

rinsed with distilled water (three times). One hundred kernels were plated (10 kernels per Petri dish) on a medium containing pentachloronitrobenzene (PCNB) (Nash and Snyder 1962). The PCNB plates were incubated at 24°C for 7 days with 12/12 h photoperiod under cold white and black fluorescent lamps (Leslie and Summerell 2006). Single -spores of putative *Fusarium* colonies were transferred to carnation leaf agar and potato dextrose agar plates, incubated for 7 days in the same conditions as the PCNB plates described above and then identified by using morphological criteria (Nelson et al. 1993). The mycelium and conidia from each strain were lyophilized and stored at 4°C. Representative strains were deposited in the culture collection of Departamento de Salud Pública, Universidad de Guadalajara, Jalisco, Mexico (*Fusarium verticillioides* UDG-163-408, *F. proliferatum* UDG-259, 264, 265, 272, 275, 277 and *F. subglutinans* UDG-255, 266, 276, 374, 375).

Mating type

Sexual crosses to confirm mating population and to identify mating types were made by triplicate on carrot agar by using the protocol of Klittich and Leslie (1988) with standard tester strains A-00149 (*MATA-1*), A-00999 (*MATA-2*), D-04853 (*MATD-2*), D-04854 (*MATD-1*), E-03693 (*MATE-2*) and E-03696 (*MATE-1*) as female parents and the uncharacterized field isolates as male parents. A cross was scored positive only if we observed perithecia oozing a cirrus of ascospores (Leslie and Summerell 2006).

Natural occurrence of toxins

Each subsample was analyzed for the presence of FB₁ and FB₂ using the method of Shephard et al. (1999), as described by Doko et al. (1995). Residues were redissolved in methanol, and an aliquot was derivatized with *o*-phthaldialdehyde (OPA) prior to separation on a reversed-phase LC system using fluorescence detection. The standard solution was obtained by dissolving pure fumonisins FB₁ and FB₂ (Sigma, St. Louis, MO, USA) in acetonitrile:water (1:1, v/v) at concentrations of 100 µl ml⁻¹ for FB₁ and 50 µl ml⁻¹ for FB₂. Fumonisins were quantified by comparing peak areas from samples with a calibration curve of standards. The detection limit of the analytical method for the two fumonisins was 0.02 µg g⁻¹. All analyses were run in duplicate and the mean values are reported. Calculated standard deviation was always lower than 5%.

The amount of beauvericin and fusaproliferin was determined by LC as previously described by Munkvold et al. (1998). Standard of BEA was purchased from Sigma, while the standard FUS was kindly supply by Dr Ritieni,

Department of Scienza degli Alimenti, Naples, Italy. Mycotoxins were quantified by comparing peak areas from samples with a calibration curve of standards. The detection limit was 100 ng g⁻¹ for BEA and 50 ng g⁻¹ for FUS. All analyses were run in duplicate, and the mean values are reported. Calculated standard deviation was always lower than 5%.

Toxigenic ability of *Fusarium* isolates

The ability of 217 isolates to produce BEA, FUS and FBs was determined by growing the strains on maize kernels as follows. Yellow maize kernels (100 g) incubated overnight at about 45% moisture in 500-ml Erlenmeyer flasks, were autoclaved twice for 30 min at 120°C. After cooling, maize kernels were inoculated with 1 ml of an aqueous suspension containing approximately 10⁷ conidia ml⁻¹ and incubated at 25°C in the darkness for 4 weeks. Harvested culture material was dried at 60°C for 48 h, finely ground with a laboratory mill, and stored at 4°C until mycotoxin analyses. The same conidial suspension volume was replaced by water in non-inoculated control samples and treated in the same way.

Beauvericin and FUS analyses were done as follows. First, 10 g of inoculated maize were homogenized in an orbital shaker for 30 min with 15 ml of methanol. Samples were filtered through Whatman No. 4 paper and methanol was removed under reduced pressure. This extraction procedure yielded 1.5 g of raw organic extract that was used to quantify BEA and FUS (Munkvold et al. 1998). Then 500 µl of methanolic extracts, corresponding to 500 mg of starting material, were filtered through 0.22-µm filter paper before LC injection (20 µl). Both toxins were analyzed using a reversed phase LC fluorescence detection system

Fumonisins analyses were done as follows: from each maize culture, a 10-g sample was extracted in an orbital shaker with 50 ml acetonitrile:water (1:1, v/v) for 30 min and filtered through Whatman No. 4 filter paper. An aliquot (50 µl) of the extract was derivatized with 200 µl *O*-phthaldialdehyde (OPA) solution. Fumonisins OPA derivates (20 µl solution) were analyzed using a reversed phase LC fluorescence detection system. Toxin quantification was done following the same methodology described above.

Results

Fusarium species occurrence

Table 1 shows the occurrence of *Fusarium* spp. with special emphasis on Section *Liseola* in maize hybrids samples

Table 1 Occurrence of *Fusarium* in maize hybrids samples collected in 2003 in Mexico (Jalisco State)

Hybrid	% of <i>Fusarium</i> -infected kernels	Distribution of the species (%)			
		<i>F. verticillioides</i>	<i>F. proliferatum</i>	<i>F. subglutinans</i>	Others ^a
Alsa 036 W	63	65	14	16	5
Lince	60	80	2	13	5
Lucero 801	60	74	11	15	n.d.
Lucero 807	40	63	11	22	4
Lucero 808	42	44	16	37	3
Lucero 901	63	59	9	26	6
UDG 600	39	68	2	23	8

^a *F. graminearum*, *F. semitectum*, *F. oxysporum*.

collected in Mexico. *Fusarium verticillioides* was the dominant *Fusarium* species present in all evaluated maize hybrids at percentages ranging from 44 to 80%. *Fusarium subglutinans* and *F. proliferatum* also appeared in ranges from 13 to 37 and from 2 to 16%, respectively. Other species were occasionally isolated such as *F. graminearum* from three hybrids (UDG 600, Lucero 901, and Alsa 036 W) at levels ranging from 3 to 8%, *Fusarium oxysporum* was isolated from three samples (3–4%) and *F. semitectum* was isolated from only one sample (4%).

The identity of *Fusarium* section *Liseola* isolates was confirmed by sexual crosses with standard tester strains. Totals of 164 strains of *F. verticillioides*, 15 of *F. proliferatum*, and 40 of *F. subglutinans* were checked, all the strains being fertile and belonging to *G. fujikuroi* mating population A (= *G. moniliformis*); mating population D (= *G. intermedia*) and mating population E (= *G. subglutinans*), respectively.

Toxin contamination of maize samples

Table 2 shows the toxin contamination in the different maize hybrids samples collected in Mexico. Beauvericin

was detected in three different hybrids with levels ranging from 300 to 400 ng g⁻¹, while only one hybrid was contaminated with FUS (200 ng g⁻¹). All maize samples were contaminated with FB₁ and FB₂ (up to 606 and 277 ng g⁻¹, respectively). Only Lince maize hybrid was contaminated with all of the four analyzed toxins.

Toxins production

Table 3 shows the production of FUS and FBs by *F. verticillioides* isolated from maize hybrids. Fusaproliferin was produced by 17 out of 164 strains of *F. verticillioides* (15.5–44.7 μg g⁻¹). All isolates of *F. verticillioides* produced FB₁ (13.8–4,860 μg g⁻¹) and 147 out of 162 isolates of this species produced FB₂ (3–2,330 μg g⁻¹). None of the isolates of *F. verticillioides* produced BEA.

Table 4 shows the production of BEA, FUS and FBs by *F. proliferatum*. Beauvericin was produced by 6 out of 15 isolates of *F. proliferatum* (15–43 μg g⁻¹); FUS was produced by 11 out of 15 isolates tested (16.6–55.6 μg g⁻¹). All *F. proliferatum* isolates produced FBs (248–6,050 μg g⁻¹).

Table 2 Beauvericin, Fusaproliferin and fumonisin contamination in maize hybrids samples collected in 2003 in Mexico (Jalisco State)

Hybrid	Toxin contamination (ng g ⁻¹) (mean±SD)				
	BEA	FUS	FB ₁	FB ₂	FBs
Alsa 036 W	n.d.	n.d.	81±15	19±10	100±25
Lince	300±10	200±12	423±38	129±5	552±43
Lucero 801	300±18	n.d.	44±20	18±10	62±30
Lucero 807	n.d.	n.d.	40±7	13±3	53±10
Lucero 808	n.d.	n.d.	17±2	4±0.4	21±2.3
Lucero 901	n.d.	n.d.	38±3	9±0.8	47±3.8
UDG 600	400±23	n.d.	606±220	277±118	883±338

BEA beauvericin, FUS fusaproliferin, FB₁ fumonisin B₁, FB₂ fumonisin B₂, FBs total fumonisins, n.d. not detected

Table 3 Fusaproliferin and fumonisins produced by *Fusarium verticillioides* (*G. moniliformis*) isolated from maize hybrids

Hybrid	FUS		FB ₁		FB ₂		Total FBs	
	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)
Alsa 036 W	4/29	31.3 (15.8–44.7)	27/27	1,400 (59.3–4,000)	25/27	424 (26.5–1,090)	27/27	1,790 (108–4,960)
Lince	6/35	23.9 (15.8–37.7)	35/35	770 (13.8–3,770)	28/35	237 (3–1,530)	35/35	958 (83.8–3,860)
Lucero 801	3/35	16.1 (15.5–17.2)	35/35	988 (116–3,060)	34/35	197 (16.8–1,050)	35/35	1,180 (239–3,100)
Lucero 807	2/9	16.6 (15.6–17.5)	9/9	1,563 (59.5–4,860)	9/9	664 (65–2,330)	9/9	2,230 (24.5–7,190)
Lucero 808	0/14	n.d. ^b	14/14	966 (388–1,850)	13/14	327 (121–890)	14/14	1,270 (522–3,230)
Lucero 901	2/38	21.4 (16.5–26.2)	38/38	1,130 (154–3,810)	35/38	324 (8.5–1,210)	38/38	1,430 (176–4,610)
UDG 600	0/4	n.d. ^b	4/4	1,010 (874–1,260)	3/4	245 (135–385)	4/4	1,200 (1,010–1,650)

n.d. Not detected

^a Producers isolates/total number of isolates

Table 5 shows the production of BEA, FUS and FBs by *F. subglutinans*. Beauvericin was produced by 5 out of 40 isolates of *F. subglutinans* ($3.8\text{--}12.9 \mu\text{g g}^{-1}$) and FUS was produced by 33 of the isolates tested ($15.9\text{--}62.9 \mu\text{g g}^{-1}$). Thirty-five out of 40 *F. subglutinans* isolates were fumonisin producers. Most of these isolates produced only FB₁. The levels produced were low in comparison with the other two *Fusarium* species varying from 68.2 to $539 \mu\text{g g}^{-1}$, except one isolate that produced $1,000 \mu\text{g g}^{-1}$.

Co-production of BEA, FUS and FBs occurred in an important number of *Fusarium* isolates. Seventeen out of 162 isolates (10.5%) of *F. verticillioides* produced FUS and FBs (Table 3). As observed in Table 4, 6/15 isolates (49%) of *F. proliferatum* produced BEA, FUS and FBs and 5/15 isolates (33.3%) produced FUS and FBs. Table 5 shows that 2/40 isolates (5%) of *F. subglutinans* produced BEA, FUS and FBs, 3/40 isolates (7.5%) produced BEA and FUS and 24/40 (60%) isolates were able to produce FUS and FBs.

Discussion

The prevalence of *Fusarium* species and their mycotoxins in different maize hybrids harvested in Jalisco (Mexico) have been evaluated in this study due to the importance of this crop as the main staple food. Data reported in this study showed that *F. verticillioides* (= *G. moniliformis*) is a relevant contaminant of maize, although *F. subglutinans* (= *G. subglutinans*) occurred at significant frequencies in some samples. Occurrence and prevalence of *Fusarium* species in maize ears from different regions and years mainly depended on the temperature and rainfall (Bottalico 1998). Our results agree with previous reports from Mexico showing *F. verticillioides* as the main species isolated from maize harvested in different areas of the country (Cortez-Rocha et al. 2003; Desjardins et al. 1994, 2000; Morales-Rodriguez et al. 2007; Sánchez-Rangel et al. 2005).

Our results indicate that FUS and BEA can occur together with FBs in *Fusarium*-contaminated maize

Table 4 Beauvericin, fusaproliferin and fumonisins produced by *Fusarium proliferatum* (*G. intermedia*) isolates isolated from maize hybrids

Hybrid	BEA		FUS		Total FBs	
	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)
Alsa 036 W	4/8	24.3 (15–35)	6/8	42.3 (32–53.9)	8/8	3,010 (787–6,050)
Lucero 901	2/4	42.4 (41.8–43)	3/4	44.0 (40.5–55.6)	4/4	1,490 (248–2,250)
UDG 600	0/1	n.d.	1/1	53.9	1/1	1,040
Lucero 801	0/1	n.d.	1/1	16.6	1/1	2,700
Lucero 807	0/1	n.d.	0/1	n.d.	1/1	2,110

n.d. Not detected

^a Producers isolates/total number of isolates

Table 5 Beauvericin, fusaproliferin and fumonisins produced by *Fusarium subglutinans* (*G. subglutinans*) isolates isolated from maize hybrids

Hybrid	BEA		FUS		Total FBs	
	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)	Positive/total isolates ^a	Mean ($\mu\text{g g}^{-1}$) (range)
Alsa 036 W	0/5	n.d.	5/5	33.8 (15.9–52.5)	3/5	369 (132–440)
Lince	0/2	n.d.	2/2	40.3 (39–41.6)	2/2	244 (195–294)
Lucero 808	2/5	8.2 (3.5–12.9)	4/5	36.1 (28.6–40.6)	35	111 (78.8–130)
Lucero 901	3/21	4.2 (3.8–4.8)	18/21	36.6 (15.9–62.9)	20/21	363 (75–539)
UDG 600	0/7	n.d.	4/7	32.0 (16.3–53.8)	7/7	334 (68.2–1,000)

n.d. Not detected

^a Producers strains/total number of strains

samples in Jalisco State and probably in other areas of Mexico. The co-occurrence of *Fusarium* toxins was reported in different regions of the world (Logrieco et al. 1996; Munkvold et al. 1998; Ritieni et al. 1997; Shephard et al. 1999; Srobarova et al. 2002). However, this study is the first to report the co-occurrence of BEA, FUS and FBs in Mexico, and provides a contribution to the knowledge of the toxigenic potential of the *Fusarium* species isolated from maize.

The high infection by *Fusarium* species of all maize samples explained the contamination of all with at least one of the toxins analyzed. However, our data indicated that FB₁ levels in maize are generally lower than expected based on the high incidence of *F. verticillioides*. Others studies carried out in Mexico showed levels of FBs higher than those reported in this study. The levels found in Oaxaca (southern Mexico) and Sonora (northern Mexico) states ranged from 0.67 to 13.3 and 0.5 to 6.8 $\mu\text{g g}^{-1}$, respectively (Cortez-Rocha et al. 2003; Rosiles et al. 1998). These differences could be explained by the environmental conditions of the different growing areas, which seem to play an important role in the formation of FBs in maize, also the maize variety could have some relevance in the levels as have been observed in others countries (Doko et al. 1995; Ramirez et al. 1996).

Our results indicate that the ability to produce FUS is common in Mexican isolates of *F. proliferatum* and *F. subglutinans* although the levels produced were low (mean=42.5 and 35.8 $\mu\text{g g}^{-1}$, respectively). Previous studies have reported maximum production of FUS at levels ranging from 1,500 to 1,725 $\mu\text{g g}^{-1}$ by *F. proliferatum* (Moretti et al. 1996; Shephard et al. 1999) and from 1,600 to 2,630 $\mu\text{g g}^{-1}$ by *F. subglutinans* (Reynoso et al. 2004; Shephard et al. 1999). Some of the isolates of *F. verticillioides* produced FUS (10%). In general, this species is not considered to produce FUS, but there is one report by Srobarova et al. (2002) in which

three *F. verticillioides* isolates from maize samples collected in Slovakia produced FUS in levels ranged between 10 and 35 $\mu\text{g g}^{-1}$.

In other countries, both the frequency of isolation of *F. subglutinans* (= *Gibberella subglutinans*) and their ability to produce BEA are related to the climatic conditions of the area. Colder regions could be more favorable to the natural occurrence of BEA than temperate areas (Torres et al. 2001; Reynoso et al. 2004). Since BEA was produced at low levels by few of the tested isolates, mainly *F. proliferatum*, we believe that the maize contamination of maize in Mexico with this toxin could be low. Further data are needed to support this observation.

The effect of environmental conditions (moisture, temperature) on Mexican *Fusarium* strains needs to be examined to clarify their influence on mycotoxins production.

Fusarium section *Liseola* species from Mexico are capable of producing emerging mycotoxins, fusaproliferin and beauvericin. The results indicate that maize could be contaminated with these emerging mycotoxins, although in minute concentrations. This is the first report on the co-occurrence of BEA, FUS and FBs in maize samples from Mexico. Nevertheless, this is a limited survey of BEA and FUS and therefore additional surveys are needed to determine the prevalence of these compounds in Mexican maize.

The existing (mainly in vitro) data on biological activity of FUS and BEA clearly indicate the possible toxicity of these fungal metabolites (Jestoi et al 2009; Logrieco et al 2002). However, there is a clear lack in the in vivo toxicity data and studies on the chronic effects. In addition, the possible interactions of emerging mycotoxins with co-occurring mycotoxins should be explored and more data on the occurrence, concentration levels, and toxicity of FUS and BEA to understand the significance of these compounds in the human and animal food chains.

Acknowledgements This study was supported by grants from Propesti Project 2004–2005, México. M.M. Reynoso, A.M. Torres and S.N. Chulze are members of the Research Career of CONICET. Portions of this work were carried out during the stay of the Dr. M. M. Reynoso as invited Research Professor at Centro Universitario de Ciencias Biológicas y Agropecuarias, Departamento de Salud Pública, Universidad de Guadalajara, México.

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