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Ear rot susceptibility and mycotoxin contamination of maize hybrids inoculated with *Fusarium* species under field conditions*

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Abstract

The development of new maize hybrids with resistance to *Fusarium* infection is an effective means of minimizing the risk of mycotoxin contamination. Several maize hybrids have been investigated for *Fusarium* ear rot and accumulation of fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), beauvericin (BEA) and fusaproliferin (FP) after artificial inoculation in the field with toxigenic strains of *Fusarium verticillioides* and *Fusarium proliferatum.* The year of inoculation had a significant influence on the disease severity and mycotoxin accumulation in maize kernels. Of all the hybrids tested, only Mona exhibited resistance to ear rot caused by *F. verticillioides* and produced low levels of fumonisins during three years of experiments. In *Fusarium*-damaged kernels (FDK), fumonisin B_1, fumonisin B_2, beauvericin and fusaproliferin were detected at concentrations much higher (up to 10–20 times) than in healthy-looking kernels (HLK). Animal and human exposure to these mycotoxins can be drastically reduced by removing mouldy and visibly damaged kernels from the commodity.

Introduction

Fusarium verticillioides (synonym *F. moniliforme*) and *F. proliferatum* are important maize pathogens causing stalk and ear rot. They can produce a variety of mycotoxins including fumonisins, beauvericin and fusaproliferin both in pre-harvested and in stored products (Bottalico et al., 1989; Chulze et al., 1998; Logrieco et al., 1995; Moretti et al., 1997; Shephard et al., 1999).

Fumonisins are a group of structurally related mycotoxins occurring in maize and maize-based products intended for animal and human consumption worldwide (Bullerman, 1996; Visconti, 1996). At present, at least fifteen fumonisin analogues have been characterized, but only three of these, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3), are abundant in naturally contaminated products. FB_1 generally occurs at levels higher than FB_2 or FB_3. Fumonisin B_1 (and to some extent FB_2) causes equine leukoencephalomalacia, porcine pulmonary oedema and liver or kidney cancer in experimental rats or mice. Moreover, fumonisins have been statistically associated with an increased risk of oesophageal cancer in humans consuming contaminated maize in some regions of South Africa (IPCS, 2000; Marasas, 1995; US NPT, 1999).

Beauvericin (BEA) is a mycotoxin produced by several *Fusarium* species, mainly by *F. proliferatum*, *F. semitectum* and *F. subglutinans* (Logrieco et al., 1998; Moretti et al., 1997; Shephard et al., 1999). It has insecticidal activity and is highly toxic towards *Artemia salina* larvae and murine and human cell lines, and induces apoptosis (Grove and Pople, 1980; Krska et al., 1997; Ojcius et al., 1991). It has been found as a natural contaminant of maize in Italy, Austria, Poland, South Africa and USA (Krska et al., 1997;

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Logrieco et al., 1993; Munkvold et al., 1998; Ritieni et al., 1997b; Shephard et al., 1999).

Fusaproliferin (FP), a recently characterized metabolite of *F. proliferatum* and *F. subglutinans* (Logrieco et al., 1996; Moretti et al., 1997; Shephard et al., 1999), exhibits toxicity to *Artemia salina*, to insect cells and human B lymphocytes (Logrieco et al., 1996), and was highly teratogenic on chicken embryos (Ritieni et al., 1997a).

The natural co-occurrence of fusaproliferin, beauvericin and fumonisins in pre-harvest maize samples contaminated by *Fusarium* species has been reported in Italy, South Africa and Iowa (Munkvold et al., 1998; Ritieni et al., 1997b; Shephard et al., 1999).

The production of maize hybrids with resistance to *Fusarium* infection and consequent mycotoxin accumulation is an efficient method for decreasing the risk of mycotoxin contamination. The results of studies carried out for several years jointly by the CNR Institute of Toxins and Mycotoxins, Bari (Italy) and the Institute of Plant Genetics, Polish Academy of Sciences, Poznan (Poland) on the susceptibility of different maize hybrids to *F. verticillioides* and *F. proliferatum* infection are summarized in this mini-review in relation to the accumulation of fumonisins, beauvericin and fusaproliferin in maize kernels after artificial infection in the field.

Materials and methods

Twenty-nine maize hybrids were inoculated with toxigenic strains of *F. verticillioides* (KF 1795 and KF 1214) and *F. proliferatum* (ITEM 1752), in one to four seasons, under field conditions. In particular, 14 hybrids were inoculated with *F. verticillioides* during the years 1992–1995 and 15 hybrids with *F. proliferatum* during the years 1996, 1997 and 1999 (Table 1).

Maize hybrids were sown in May, in identical field trials, at Radzikow, 30 km west of Warsaw, Poland. Hybrids were selected for relatively similar maturity dates and different susceptibilities to *Fusarium* ear rot. One-hundred and sixty plants of each hybrid were sown in four rows (5 m long), of which 10 were inoculated with the fungal strains. Fungal inoculation was performed with toothpicks placed in the maize ear according to Hart et al. (1982), at the medium silking stage, one week after silk emergence. Ears from inoculated plants and from control plants (non-inoculated plants) were harvested during the third week of October and transported to the laboratory. Disease assessments were made on 10 inoculated ears and on 10 control ears. A five degree scale was used to evaluate the infection of each ear (Chelkowski et al., 1989). The *Fusarium* ear rot index (*Fi*), ranging from 0 to 500, was calculated using the following equation:

$$Fi = \left(\sum F \times 100\right) / n$$

where F is the disease score of individual ears (1–5) and n is the number of scored ears. Kernels from the same ears were separated in two fractions: *Fusarium*-damaged kernels (FDK, i.e. visibly mouldy kernels) and healthy-looking kernels (HLK, i.e. symptomless kernels). Separate analyses of FDK

Table 1. Maize hybrids tested in different years in *Fusarium* inoculation experiments

Fusarium verticillioides		*Fusarium proliferatum*	
Hybrid	Inoculation year	Hybrid	Inoculation year
KLG 2210	1992, 1993, 1994, 1995	BURAN	1996, 1997, 1999
SMOLIMAG	1992, 1993, 1994, 1995	ELA	1996, 1997, 1999
MONA	1993, 1994, 1995	EWA	1996, 1997, 1999
RUTEN	1992, 1993, 1995	KLG 2210	1996, 1997, 1999
ZENIT	1992, 1993, 1994	MILPA	1996, 1997, 1999
BETULISA	1992, 1995	MONA	1996, 1997, 1999
RAHBE 90102	1992, 1993	JANINA	1996, 1999
ANNA	1994	RAH 895	1996, 1997
BURAN	1994	RUTEN	1996, 1999
ELA	1994	KASIA	1999
MILPA	1994	OLENKA	1999
RAHBE 921573	1995	RAH595	1997
RAHBE 86101	1992	RAH996	1997
SMH 4792	1994	SMH8195	1997
		WILGA	1999

and HLK were performed for fumonisins, beauvericin and fusaproliferin (Pascale et al., 1999). Meteorological data (mean monthly temperatures and monthly precipitations) were obtained from the Meteorological Station located in the Radzikow experimental station.

Results

Results of disease assessments on maize hybrids inoculated with *F. verticillioides* and *F. proliferatum* under field conditions in different years are summarized in Tables 2 and 3.

Inoculation with F. verticillioides. The inoculation of hybrids with *F. verticillioides* resulted in different degrees of ear rot (from 0 to 350) (Table 2). Moreover, the artificial infection of the same hybrids for two or more years gave rise to significant differences in ear rot disease. Hybrids Ruten and Smolimag showed low susceptibility to infection in 1992 and 1993, but they exhibited higher disease scores in 1994 and 1995. Hybrids Ela, Zenit and RAHBE 90102

Table 2. *Fusarium* ear rot index (disease score) and percentage of *Fusarium*-damaged kernels (FDK) in maize hybrids inoculated with *Fusarium verticillioides* in different years in Poland

Hybrid	*Fusarium* ear rot index (Fi)*				*Fusarium*-damaged kernels (%)			
	1992	1993	1994	1995	1992	1993	1994	1995
SMOLIMAG	60	60	130	200	1	2	5	7
KLG 2210	40	130	110	80	<1	2	2	3
ZENIT	140	230	311	—	2	24	31	—
RUTEN	100	0	—	120	<1	0	—	2
MONA	—	30	110	60	—	2	3	1
RAHBE 90102	240	230	—	—	3	9	—	—
BETULISA	120	—	—	140	2	—	—	9
RAHBE 86101	140	—	—	—	1	—	—	—
RAHBE 921573	—	—	—	170	—	—	—	15
ELA	—	—	350	—	—	—	60	—
MILPA	—	—	220	—	—	—	26	—
ANNA	—	—	133	—	—	—	10	—
SMH 4792	—	—	125	—	—	—	5	—
BURAN	—	—	125	—	—	—	6	—

— not tested. *Fi = 0–500 (0 = healthy cob, 500 = totally rotted cob).

Table 3. *Fusarium* ear rot index (disease score) and percentage of *Fusarium*-damaged kernels (FDK) in maize hybrids inoculated with *Fusarium proliferatum* in different years in Poland

Hybrid	*Fusarium* ear rot index (Fi)*			*Fusarium*-damaged kernels (%)		
	1996	1997	1999	1996	1997	1999
MONA	170	280	360	11	17	39
BURAN	230	270	410	19	27	37
MILPA	270	250	425	26	17	31
ELA	280	370	450	24	52	54
KLG 2210	340	230	500	37	12	64
EWA	370	360	480	38	37	71
RAH 895	180	280	—	16	28	—
JANINA	210	—	400	25	—	65
RUTEN	240	—	437	24	—	56
SMH 8195	—	260	—	—	18	—
RAH 595	—	270	—	—	20	—
RAH 996	—	310	—	—	30	—
WILGA	—	—	300	—		27
KASIA	—	—	400	—		29
OLENKA	—	—	460	—		40

— not tested. *Fi = 0–500 (0 = healthy cob, 500 = totally rotted cob).

were very susceptible, with high disease scores (up to 350) and a high percentage of FDK (up to 60%). Hybrid Mona was found to be the only one of 14 tested to exhibit low susceptibility in all seasons with about 1.8% FDK in inoculated ears (Pascale et al., 1997).

Inoculation with F. proliferatum. Significant differences in the degree of *Fusarium* ear rot disease were observed in hybrids inoculated with *F. proliferatum* (Table 3). *Fusarium* ear rot index (Fi) ranged from 170 to 370 in 1996, from 250 to 370 in 1997 and from 300 to 500 in 1999; the percentage of *Fusarium*-damaged kernels (FDK) ranged from 11% to 38% in 1996, from 12% to 52% in 1997 and from 27% to 71% in 1999. The hybrids grown in 1999 showed a higher susceptibility to fungal infection compared to the other two years. Moreover, for the same hybrid, different disease scores were observed during two or three years of investigation. In particular, most hybrids (Mona, Buran, Ela, RAH 895, Janina and Ruten) showed higher susceptibility to *Fusarium* ear rot in 1997 and 1999 than in 1996, whereas the hybrids Milpa, Ewa and KLG 2210 were more susceptible to infection in 1996 and 1999 than 1997. In particular, hybrid KLG 2210 showed high disease scores in 1996 and in 1999 (340 and 500, respectively), but exhibited the lowest *Fusarium* ear rot index (230) in 1997 (Pascale et al., 2001).

Mycotoxin accumulation

Results for accumulation of fumonisin B_1 and B_2 in maize kernels after inoculation with *F. verticillioides* are reported in Table 4. The amounts of FB_1, FB_2, beauvericin (BEA) and fusaproliferin (FP) accumulated in maize kernels after inoculation with *F. proliferatum* are reported in Table 5.

Inoculation with F. verticillioides. Fumonisin concentrations in maize kernels ranged from 0.1 to 93.2 $\mu g\, g^{-1}$ for FB_1 and from 0.02 to 15.8 $\mu g\, g^{-1}$ for FB_2. FB_2 was detected at levels lower than FB_1 in all samples tested with the FB_2/FB_1 ratio ranging from 0.13 to 0.43 (0.29 average for all samples). Largest amounts of fumonisins accumulated in kernels of hybrid Zenit, examined for three seasons (up to 37.4 $\mu g\, g^{-1}$ of FB_1 and 8.0 $\mu g\, g^{-1}$ of FB_2), and in hybrid Ela (93.2 $\mu g\, g^{-1}$ of FB_1 and 15.8 $\mu g\, g^{-1}$ of FB_2) inoculated only in 1994. The hybrid Mona contained low levels of fumonisins during three consecutive seasons ranging from 0.1 to 1.7 $\mu g\, g^{-1}$ and from 0.02 to 0.4 $\mu g\, g^{-1}$ of FB_1 and FB_2, respectively. However, significantly higher amounts of fumonisins were detected in grains of hybrids inoculated in the 1994 and 1995 seasons, compared to grains of the same hybrids inoculated in the 1992 and 1993 seasons. Results of the analysis of control ears (non-inoculated) showed that the amount of FB_1 did not exceed 0.04 $\mu g\, g^{-1}$ and FB_2 was not detected.

Table 4. Fumonisin (FB_1 and FB_2) contamination in kernels of maize hybrids inoculated with *Fusarium verticillioides* in different years in Poland

Hybrid	Fumonisin content ($\mu g\, g^{-1}$)*							
	1992		1993		1994		1995	
	FB_1	FB_2	FB_1	FB_2	FB_1	FB_2	FB_1	FB_2
SMOLIMAG	0.2	0.1	0.9	0.2	6.4	1.8	9.6	3.1
KLG 2210	0.4	0.1	0.4	0.1	3.7	1.0	3.4	1.0
ZENIT	2.5	0.8	10.0	2.6	37.4	8.0	—	—
RUTEN	0.9	0.3	0.2	0.02	—	—	5.4	1.6
MONA	—	—	0.1	0.02	1.7	0.4	1.3	0.4
RAHBE 90102	4.3	1.6	6.8	2.4	—	—	—	—
BETULISA	2.2	0.9	—	—	—	—	4.2	1.3
RAHBE 86101	2.6	1.1	—	—	—	—	—	—
RAHBE 921573	—	—	—	—	—	—	14.9	4.6
ELA	—	—	—	—	93.2	15.8	—	—
MILPA	—	—	—	—	12.8	3.7	—	—
ANNA	—	—	—	—	8.1	2.0	—	—
SMH 4792	—	—	—	—	3.9	1.1	—	—
BURAN	—	—	—	—	2.5	0.8	—	—

— not tested. *Weighted averages derived from separate analyses of *Fusarium*-damaged kernels and healthy looking kernels.

Table 5. Fumonisins (FB_1, FB_2), beauvericin (BEA) and fusaproliferin (FP) contamination in maize hybrids inoculated with *Fusarium proliferatum* in different years in Poland

Hybrid	Mycotoxin content ($\mu g\,g^{-1}$)*											
	1996				1997				1999			
	FB_1	FB_2	BEA	FP	FB_1	FB_2	BEA	FP	FB_1	FB_2	BEA	FP
MONA	18.0	1.6	0.8	0.3	66.4	5.3	3.0	1.3	99.6	11.1	9.0	2.0
BURAN	72.4	6.2	1.2	1.8	67.5	6.3	3.9	1.3	122.2	12.3	16.5	1.9
MILPA	54.5	4.3	2.5	0.5	41.1	3.6	4.3	0.4	76.0	7.4	5.5	0.8
ELA	46.3	4.1	1.0	1.1	125.3	11.1	7.4	3.9	173.8	15.9	12.2	4.1
KLG 2210	97.7	8.3	4.2	1.5	26.4	1.9	1.3	0.4	231.9	26.5	18.0	6.4
EWA	130.4	10.7	3.0	3.6	100.1	8.6	7.1	2.8	148.3	15.9	12.1	5.3
RAH 895	20.7	1.6	0.2	0.6	93.1	6.8	2.9	1.4	—	—	—	—
JANINA	38.2	0.4	0.4	0.5	—	—	—	—	210.4	25.3	19.6	4.9
RUTEN	11.4	1.6	1.6	1.1	—	—	—	—	138.7	13.4	15.1	2.8
SMH 8195	—	—	—	—	34.1	2.7	2.3	0.9	—	—	—	—
RAH 595	—	—	—	—	40.8	3.2	2.5	0.8	—	—	—	—
RAH 996	—	—	—	—	50.8	4.2	3.1	1.1	—	—	—	—
WILGA	—	—	—	—	—	—	—	—	29.6	2.8	1.7	0.7
KASIA	—	—	—	—	—	—	—	—	79.5	9.7	8.6	1.5
OLENKA	—	—		—	—	—	—	—	124.2	12.2	14.2	3.0

— not tested. *Weighted averages derived from separate analyses of *Fusarium*-damaged kernels and healthy looking kernels.

The fumonisin content in *Fusarium*-damaged kernels varied greatly, from 5.1 $\mu g\,g^{-1}$ FB_1 and 1.4 $\mu g\,g^{-1}$ FB_2 to 196.0 $\mu g\,g^{-1}$ FB_1 and 62.0 $\mu g\,g^{-1}$ FB_2. Sixteen samples (57%) contained fumonisins at levels higher than 100 $\mu g\,g^{-1}$. Healthy-looking kernels of inoculated ears contained the much lower amounts of fumonisins compared to the FDK fraction, ranging from 0.06 to 1.5 $\mu g\,g^{-1}$ of FB_1 and from 0.02 to 0.4 $\mu g\,g^{-1}$ of FB_2 (data not shown). This clearly indicates that damaged kernels account for almost all the fumonisin contamination.

The totality of data, including all hybrids tested from one to four seasons, led to a good correlation between the *Fusarium* ear rot index (Fi) and fumonisin contamination, with a coefficient of correlation (r) of 0.933 (Pascale et al., 1997).

Inoculation with F. proliferatum. Fumonisin concentrations in maize kernels ranged from 18.0 to 231.9 $\mu g\,g^{-1}$ for FB_1 and from 0.4 to 26.5 $\mu g\,g^{-1}$ for FB_2, from 0.2 to 19.6 $\mu g\,g^{-1}$ for BEA and from 0.3 to 6.4 $\mu g\,g^{-1}$ for FP. Similarly to the disease score, a considerable influence of the year of inoculation on mycotoxin accumulation in maize hybrids was observed. In particular, significantly higher amounts of mycotoxins were detected in kernels of hybrids inoculated in the 1999 season, compared to kernels of the same hybrids inoculated in the 1996 and 1997 seasons. Between hybrids, comparison showed that Mona and RAH 895 contained low amounts of fumonisins, beauvericin and fusaproliferin in 1996, but were highly contaminated in 1997, whereas the hybrid KLG 2210 accumulated high concentrations of all four mycotoxins in 1996 and the lowest concentrations in 1997.

The amount of fumonisins in *Fusarium*-damaged kernels varied from 115.7 to 361.5 $\mu g\,g^{-1}$ and from 8.3 to 41.1 $\mu g\,g^{-1}$ for FB_1 and FB_2, respectively. The fraction of healthy-looking kernels of inoculated ears contained much lower levels of fumonisins compared to the *Fusarium*-damaged kernels fraction, ranging from 1.6 to 26.0 $\mu g\,g^{-1}$ of FB_1 and from 0.1 to 2.3 $\mu g\,g^{-1}$ of FB_2. The contamination levels of beauvericin and fusaproliferin in *Fusarium*-damaged kernels were significantly lower than fumonisins, ranging from 1.1 to 44.3 $\mu g\,g^{-1}$ for BEA and from 1.7 to 10 $\mu g\,g^{-1}$ for FP. Also, these toxins accumulated mainly in *Fusarium*-damaged kernels, whereas only trace amounts were found in healthy-looking kernels (up to 0.2 $\mu g\,g^{-1}$ for BEA and 0.3 $\mu g\,g^{-1}$ for FP). The analysis of control ears (not inoculated) showed that only four samples out of 30 contained fumonisin B_1 at levels not exceeding 1.2 $\mu g\,g^{-1}$, whereas fumonisin B_2, beauvericin and fusaproliferin were not detected (data not shown).

A good correlation between the *Fusarium* ear rot index (Fi) and mycotoxin contamination (FB_1, FB_2, BEA and FP) was observed (correlation coefficient (r) = 0.895). The hybrids Buran (in 1996) and Janina (in 1999) were not considered in the analysis. The high

Table 6. Meteorological data (mean monthly temperatures and monthly precipitations) for 1992–1997 and 1999 at Radzikow and long-term averages

Month	Mean temperatures (°C)								Precipitation sums (mm)							
	1992	1993	1994	1995	1996	1997	1999	Long-term 1966–1995	1992	1993	1994	1995	1996	1997	1999	Long-term 1966–1995
May	14.2	18.2	13.8	13.8	16.4	14.8	15.7	13.8	17.6	36.3	64.5	41.6	69.4	56.0	39.3	47.6
June	18.3	17.4	17.6	18.6	18.2	18.1	19.7	16.7	47.3	20.0	8.3	73.1	66.8	84.3	155.1	62.1
July	20.2	18.2	23.9	22.1	17.5	19.1	23.1	18.4	55.8	65.5	22.8	63.3	132.6	227.7	30.7	67.8
August	21.6	18.1	19.4	19.8	20.2	20.9	20.6	17.8	24.2	49.5	71.9	66.1	39.7	31.9	5.0	54.8
September	12.7	12.6	15.4	14.1	11.2	13.9	18.3	13.3	78.6	46.5	53.6	138.7	55.6	18.6	5.4	45.4
October	5.9	8.5	6.9	11.1	9.8	6.8	8.9	8.4	39.8	27.6	74.2	8.7	25.3	26.6	18.8	35.8

levels of mycotoxins found in kernels of these hybrids were not in agreement with the external symptoms of infection observed; this could be explained by a possible fungal infection acting mainly inside the kernels (Pascale et al., 2001).

Discussion

The results showed that *F. verticillioides* and *F. proliferatum* were highly pathogenic to maize ears under field conditions. These species may contribute to significant contamination of maize with at least four toxic metabolites (i.e. FB_1, FB_2, BEA and FP). Damaged kernels accounted for almost all the mycotoxin contamination. Consequently, it is possible to drastically reduce the exposure of animals and humans to mycotoxins by segregating mouldy and visibly damaged kernels from healthy kernels.

A good correlation between the *Fusarium* ear rot index (Fi) and mycotoxin contamination was observed, both in *F. verticillioides* and *F. proliferatum* inoculation experiments. The severity of ear infection has been also found to be a good indicator of ear contamination by mycotoxins (Schaafsma et al., 1993).

Poor reproducibility of the results was observed between the different years of investigation. Low disease scores and low content of mycotoxins, observed in one or two seasons, did not necessarily produce low susceptibility of a given hybrid in other seasons. The highest disease scores and accumulation of mycotoxins in the maize hybrids were observed in the years 1994 and 1995 for the *F. verticillioides* inoculation experiments, and in the year 1999 for the *F. proliferatum* inoculation experiments. These seasons were characterized by high temperatures at the pollination period (July), but low precipitation was observed only in 1994 and 1999, not in 1995 (see Table 6). High temperatures, low moisture and dry weather at or just prior to pollination have been reported to be important factors in fumonisin production (Shelby et al., 1994). In the USA, fumonisin contamination in warm, dry years was reported to be greater than in cooler years (Murphy et al., 1996).

Fumonisin levels in maize kernels after inoculation with *F. proliferatum* were much higher than those found in experiments carried out with *F. verticillioides*. Beauvericin levels in 1996 and 1997 were similar to those found in kernels of maize hybrids artificially inoculated with *F. subglutinans* (Krska et al., 1996), a known producer of beauvericin, whereas in 1999 they were much higher. No previous inoculation experiments under field conditions have been performed with respect to fusaproliferin accumulation, although high levels of the toxin have been found in pre-harvest maize ear rot (Ritieni et al., 1997b).

The ability of maize hybrids to accumulate only low amounts of mycotoxins is a highly desired trait for farmers. In our experiments, a generally high susceptibility to fungal infection and high accumulation of mycotoxins in kernels was observed. However, some promising traits were found in the hybrid Mona resulting in a low *F. verticillioides* ear rot index and low fumonisin contamination. These results suggest that the possibilty of finding selected hybrids with different susceptibility to *Fusarium* ear rot and different tendencies to accumulate mycotoxins in the field exists, but further long-term experiments are necessary before maize hybrids resistant to infection by *Fusarium* species and consequent mycotoxin accumulation are available commercially.

References

Bottalico A, Logrieco A and Visconti A (1989) *Fusarium* species and their mycotoxins in infected cereals in field and in stored grains. In: Chelkowski J (ed) *Fusarium*-Mycotoxins, Taxonomy and Pathogenicity. Topics in Secondary Metabolites (pp 85–119) Elsevier, Amsterdam, the Netherlands

Bullerman LB (1996) Occurrence of *Fusarium* and fumonisins in food grains and in foods. In: Jackson LS, De Vries JW

and Bullermann LB (eds) Advances Experimental Medicine and Biology – Fumonisins in Food (pp 27–38) Plenum Press, New York, USA

Chelkowski J (1989) Mycotoxins associated with corn cob fusariosis. In: Chelkowski J (ed) *Fusarium*-Mycotoxins, Taxonomy, Pathogenicity. Topics in Secondary Metabolism (pp 53–62) Elsevier, Amsterdam, the Netherlands

Chulze SN, Ramirez ML, Pascale M and Visconti A (1998) Fumonisin production by, and mating population of, *Fusarium* section *Liseola* isolates from maize in Argentina. Mycological Research 102: 141–144

Grove JF and Pople M (1980) The insecticidal activity of beauvericin and the enniatin complex. Mycopathologia 70: 103–105

Hart LP, Braselton WE and Stebbins TC (1982) Production of zearalenone and deoxynivalenol in commercial sweet corn. Plant Disease 66: 1133–1135

IPCS (2000) Environmental Health Criteria 219 – Fumonisin B_1. International Programme on Chemical Safety: World Health Organization (WHO), Geneva

Krska R, Lemmens M, Schuchmacher R, Grasserbauer M, Pronczuk M, Wisniewska H and Chelkowski J (1996) Accumulation of the mycotoxin beauvericin in kernels of corn hybrids inoculated with *Fusarium subglutinans.* Journal of Agricultural and Food Chemistry 44: 3665–3667

Krska R, Schuchmacher R, Grasserbauer M, Lemmens M, Lemmens-Gruber R, Adler A and Lew H (1997) Effects of beauvericin to mammalian tissue and its production by Austrian isolates of *Fusarium proliferatum* and *Fusarium subglutinans*. Mycotoxin Research 13: 11–16

Logrieco A, Moretti A, Ritieni A, Chelkowski J, Altomare C, Bottalico A and Randazzo G (1993) Natural occurrence of beauvericin in pre-harvest *Fusarium subglutinans* infected corn ears in Poland. Journal of Agricultural and Food Chemistry 41: 2149–2152

Logrieco A, Moretti A, Ritieni A, Bottalico A and Corda P (1995) Occurrence and toxigenicity of *Fusarium proliferatum* from pre-harvest maize ear rot, and associated mycotoxins in Italy. Plant Disease 79: 727–731

Logrieco A, Moretti A, Fornelli F, Fogliano V, Ritieni A, Caiaffa M, Randazzo G, Bottalico A and Macchia L (1996) Fusaproliferin production by *Fusarium subglutinans* and its toxicity to *Artemia salina*, SF-9 insect cells and IARC/LCL 171 human B lymphocytes. Applied and Environmental Microbiology 62: 3378–3384

Logrieco A, Moretti A, Castella G, Kostecki M, Golinski P, Ritieni A and Chelkowski J (1998) Beauvericin production by *Fusarium* species. Applied and Environmental Microbiology 64: 3084–3088

Marasas WFO (1995) Fumonisins: their implications for human and animal health. Natural Toxins 3: 193–198

Moretti A, Logrieco A, Bottalico A, Ritieni A, Fogliano A and Randazzo G (1997) Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Sydownia 48: 45–56

Munkvold G, Stahr HM, Logrieco A, Moretti A and Ritieni A (1998) Occurrence of fusaproliferin and beauvericin in *Fusarium*-contaminated livestock feed in Iowa. Applied and Environmental Microbiology 64: 3923–3926

Murphy PA, Hendrich S, Hopmans EC, Hauck CC, Lu Z, Buseman G and Munkvold G (1996) Effect of processing on fumonisins content of corn. In: Jackson LS, De Vries JW and Bullermann LB (eds) Advances Experimental Medicine and Biology – Fumonisins in Food (pp 232–334) New York, USA

Ojcius DM, Zychlinsky A, Zheng LM and Young JD (1991) Ionophore-induced apoptosis: Role of DNA fragmentation and calcium fluxes. Experimental Cell Research 197: 43–49

Pascale M, Visconti A, Pronczuk M, Wisniewska H and Chelkowski J (1997) Accumulation of fumonisins in maize hybrids inoculated under field conditions with *Fusarium moniliforme* Sheldon. Journal of the Science of Food and Agriculture 74: 1–6

Pascale M, Visconti A, Avantaggiato A, Pronczuk M and Chelkowski J (1999) Mycotoxin contamination of maize hybrids after infection with *Fusarium proliferatum*. Journal of the Science of Food and Agriculture 79: 2094–2098

Pascale M, Visconti A, Pronczuk M. Wisniewska H and Chelkowski J (2001) Accumulation of fumonisins, beauvericin and fusaproliferin in maize hybrids inoculated under field conditions with *Fusarium proliferatum*. Mycological Research (submitted)

Ritieni A, Monti SM, Randazzo G, Logrieco A, Moretti A, Peluso G and Fogliano V (1997a) Teratogenic effects of fusaproliferin on chicken embryos. Journal of Agricultural and Food Chemistry 45: 3039–3043

Ritieni A, Moretti A, Logrieco A, Bottalico A, Randazzo G, Monti SM, Ferracane R and Fogliano V (1997b) Occurrence of fusaproliferin, fumonisin B_1 and beauvericin in maize from Italy. Journal of Agricultural and Food Chemistry 45: 4011–4016

Schaafsma AW, Miller JD, Savard ME and Ewing RJ (1993) Ear rot development and mycotoxin production in corn in relation to inoculation method, corn hybrid and species of *Fusarium*. Canadian Journal of Plant Pathology 15: 185–192

Shelby RA, White DG and Basuke EM (1994) Differential fumonisin production in maize hybrids. Plant Disease 78: 582–584

Shephard GS, Sewram V, Nieuwoudt TW, Marasas WFO and Ritieni A (1999) Production of the mycotoxins fusaproliferin and beauvericin by South African isolates in the *Fusarium* section *Liseola*. Journal of Agricultural and Food Chemistry 47: 5111–5115

US NPT (1999) NPT Technical Report on the 'Toxicology and carcinogenesis studies of fumonisin B_1 (CAS No. 116355-83-0) in F344/N rats and B6C3F_1 mice (feed studies)'. NPT TR 496, NIH Publication No. 99-3955, US Department of Health and Human Services.

Visconti A (1996) Fumonisins in maize genotypes grown in various geographic areas. In: Jackson LS, De Vries JW and Bullermann LB (eds) Advances Experimental Medicine and Biology – Fumonisins in Food (pp 193–204) Plenum Press, New York, USA