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Review

Reduction of mycotoxins and toxigenic fungi in the Mediterranean basin maize chain

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Summary. The main mycotoxigenic fungi found in maize in the Mediterranean basin include *Aspergillus* section *Flavi* and several *Fusarium* species, *Fusarium* section *Liseola* being the more widespread. While *Aspergillus* section *Flavi* species can develop in the field or in stored maize in Mediterranean countries, *Fusarium* species colonise maize ears in the field. As a consequence, fumonisins are the major contaminants in Mediterranean maize, together with aflatoxins. The prevalence of *Fusarium* section *Liseola* in the Mediterranean countries is closely linked to the activity of insects such as *Sesamia nonagrioides* and *Ostrinia nubilalis*. The incidence of the different mycotoxigenic fungi and mycotoxins across the Mediterranean countries is extensively reviewed in this work. Furthermore, both pre- and postharvest strategies to reduce the presence of such toxins in the maize chain are described. Finally, the incidence and levels of mycotoxins encountered in maize products intended for direct human consumption in the Mediterranean countries are also assessed; they are much lower than those in maize grain as a result of food processing technologies.

Key words: *Fusarium*, *Aspergillus*, preharvest, processing, fumonisins.

Introduction

Maize (*Zea mays*) belongs to the grass family (*Gramineae*). It is a tall annual plant with an extensive fibrous root system. Maize is one of mankind's earliest innovations, domesticated 5000 years ago from teosinte by selecting for improved yield and quality (Gewin, 2003). The earliest evidence of maize cultivation was found by archaeologists in Mexico, from where the crop was distributed to other regions by humans. At the end of the fifteenth century, after the exploration of the American continent by Christopher Columbus, maize was introduced into Europe through Spain.

As shown in Figure 1, the Mediterranean countries with highest maize production rates during 2009 were France, Italy, Egypt, Serbia, Spain and Turkey (FAOSTAT, 2009).

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Maize is commonly colonized by several spoilage fungi in pre- and post-harvest conditions, where the relative abundance of those species depends on several abiotic and biotic factors. Water activity and temperature are important factors that influence the development of moulds in cereals, and their capability to produce mycotoxins. Maize and maize kernels can be colonized competitively by species of genus *Aspergillus* mainly those in section *Flavi*, *Nigri* and *Circumdati*, by several species of *Penicillium* spp., or by several species of *Fusarium,* such as *F. verticillioides*, *F. proliferatum* and *F. graminearum* (Marin *et al*., 1995). These genera are considered to be the most prevalent toxin-producing fungi in northern temperate regions. Considering this wide range of moulds with ability to colonize maize kernels, a large variety of mycotoxins can be found under specific conditions in maize products and their derivatives. The most common mycotoxins that contaminate maize in Mediterranean countries are *Fusarium* toxins, produced mainly in the field and occasionally post-harvest when storage conditions are in-

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Figure 1. Production of maize and legislation of mycotoxins in maize in Mediterranean countries [Production in tones during 2009 (FAOSTAT, 2009)].

adequate (Marin *et al.*, 2004). The most frequently reported *Fusarium* toxins are the trichothecenes, zearalenone (ZEA) and fumonisins (FB), while little is known about other metabolites of *Fusarium* spp., the emerging mycotoxins fusaproliferin (FUS), beauvericin (BEA), enniatins (ENNs) and moniliformin (MON) (Jestoi, 2008).

In the Mediterranean countries, maize is more widely used for animal nutrition than to be processed into corn-flakes, corn snacks or sweet corn for human consumption. Some countries traditionally use maize in regional cooking such as broa (maize-based bread from Portugal) and polenta (from Italy). Therefore, *Fusarium* toxins, as well as aflatoxins (AFs) and other toxins, can be found in feeds, raw materials and processed foods with maize in their composition. Moreover, as a consequence of the use of maize grits in the brewing process, AFs and FBs can be also found in beer (Pietri *et al.*, 2009).

The European Commission has established maximum levels of the most important mycotoxins in feeds for animal nutrition and foods for human consumption in European countries. Commission Regulation 574/2011 of 16 June 2011 states the maximum levels of aflatoxin B_1 (AFB_1) in animal feed. Moreover, ZEA, ochratoxin A (OTA), deoxynivalenol (DON), T-2 and HT-2 toxins and FBs levels in feeds for animal nutrition were provided in the European Recommendation 2006/576/EC of 17 August 2006 (European Commission, 2006a). Levels of mycotoxins in raw materials and processed food for human consumption were stipulated in the European Commission Regulation 1881/2006 of 19 December 2006, and subsequently extended in the Commission Regulation 1126/2007 of 28 September 2007 where finally the maximum levels of the *Fusarium* toxins DON, ZEA and FBs were established in maize and maize products (European Commission, 2006b, 2007). These regulations have been adopted by several non EU countries as well as the EU candidate country, Croatia. For the other non-EU Mediterranean countries, several regulations or guidelines exist, which were reported by FAO (2004), with exception of Libya where no data was available. The main mycotoxins regulated in maize, until 2003, were total AFs whose maximum levels were from 4 μg kg^{-1} (Turkey) to 20 μg kg^{-1} (Algeria). In some countries, regulations for the maximum levels of OTA in cereals (Turkey, Israel and Morocco) and ZEA in maize and cereals (Morocco and Serbia) exist. None of the non-EU countries had specific regulation for the most frequent maize mycotoxins, the FBs (FAO 2004). As reported by Zinedine and Mañes (2009), no regulation is yet in force in Morocco, neither in other Mediterranean countries in Africa, leading to a disharmonized situation that impairs the commercial relationships between countries.

Maize mycobiota in Mediterranean countries

As previously mentioned, maize ears are naturally contaminated with different fungi including *Fusarium* spp., e.g. *F. verticillioides*, *Fusarium subglutinans*, *F. proliferatum*, *F. graminearum*, *Fusarium oxysporum* and *F. solani*, and their toxins. These species are generally classified as field pathogens, causing stalk and ear rot worldwide, including some Mediterranean countries. However, *F. verticillioides* is considered to be the most common *Fusarium* species occurring in tropical and subtropical climates, and the most prevalent fungus associated with maize in southern Europe. *F. proliferatum* is also important but less than *F. verticillioides* (Logrieco *et al*., 1995; Bakan *et al*., 2002; Butron *et al*., 2006). *F. verticillioides* is cosmopolitan and has been reported in all climatic regions of the world in association with a wide variety of crop plants. Maize infection with *F. verticillioides* and *F. proliferatum* causes reduction of yield and quality, due to grain deterioration and mycotoxin contamination. The growth and mycotoxin production by moulds in this important crop in southern Europe depends on the humidity and temperature (climatic conditions) during the maize flowering period. Another important factor is insect damage, due to both *Ostrinia nubilalis* and *Sesamia nonagrioides* (Munkvold *et al*., 1999). Agricultural practices (fertilisation, herbicide and insecticide control, irrigation), plant density (Blandino *et al*., 2008b), and susceptibility of cultivated hybrids are other important factors.

On the other hand, *Aspergillus* can occur in the field or as a storage mould in Mediterranean countries. The importance of maize moisture content at harvest has been stressed in relation to the risk of *A. flavus* contamination. Moisture content at harvest should be below 28% because harvesting at higher than this moisture content may cause significant damage to the grain. In order to obtain high quality maize, harvest moisture must be as low as 20–22%. Invasion of maize by *A. flavus* is prevented at moisture content below 13%. Moisture contents of fresh maize may range from 21–30%. Therefore, second crop maize must be dried until its moisture content drops to 13% in bins before storage. Optimum growth of *A. flavus* occurs on maize at 18% moisture. Fungal growth elevates respiration that releases heat and moisture into the surrounding environment in the stored grain mass. Increased moisture content and temperature of the surrounding maize results in a hot spot of increasingly mouldy grain. In order to minimize risk of mycotoxin contamination in maize, the grain should be stored at moisture content 13% or less (Alptekin *et al.,* 2009).

The importance of contamination by moulds must be considered in relation to their toxigenic potential. Table 1 summarises some studies carried out in Mediterranean countries about the toxigenicity of strains isolated from maize-based products. Special attention has been focused on the species of the *Fusarium* section *Liseola* (*F. verticillioides*, *F. proliferatum*, *F. subglutinans* and *F. anthopilum*) and their capacity to produce FBs. Strains isolated from maize in the Mediterranean countries showed a high-degree of variability in the amounts of FBs produced.

Several studies have addressed fungal prevalence in maize in the different Mediterranean countries; thus for example, *Penicillium* and *Fusarium* are the most frequent genera growing on maize in Croatia (Jurjevic *et al*., 1999). A study carried out with 15 maize grain samples collected during the autumn of 2002 in Croatia with a high percentage of samples contaminated by FBs (100%), ZEA (80%) and OTA (46.6%) showed a high infection by *Penicillium* spp. and *Fusarium* spp. *F. proliferatum* and *F. graminearum* were the most dominant *Fusarium* species (Domijan *et al*., 2005). Moreover, Ivic *et al.* (2009) studied the contamination of this cereal by different species of this genus in Croatia. In general, the percentage of

Table 1. Mycotoxin-producing ability of *Fusarium* and *Aspergillus* strains isolated from maize and milling fractions in Mediterranean countries.

Country	Species	Product	Isolates	Toxigenic (%)	Mycotoxin	Reference
Croatia	*F. verticillioides*	Maize	66	66 (100)	Fumonisins	Segvic and Pepeljnjak, 2003
	F. verticillioides	Maize	27	18 (66.7)	Fumonisins	Cvetnic *et al.*, 2005
	F. verticillioides	Maize	27	3 (11.1)	Zearalenone	Cvetnic *et al.*, 2005
	F. graminearum	Maize	5	4 (80)	Zearalenone	Cvetnic *et al.*, 2005
Egypt	*F. verticillioides*	Maize	18	15 (83.3)	Fumonisins	Fadl-Alla, 1998
Italy	*F. proliferatum*	Infected maize ears	26	26 (100)	Fumonisins	Logrieco *et al.*, 1995
	F. proliferatum	Infected maize ears	26	22 (84.6)	Beuavericin	Logrieco *et al.*, 1995
	F. proliferatum	Infected maize ears	26	12 (46.2)	Moniliformin	Logrieco *et al.*, 1995
	Aspergillus section *Flavi*	Maize	70	49 (70)	Aflatoxins	Giorni *et al.*, 2007
	Aspergillus section *Flavi*	Maize	70	43 (61.4)	Cyclopiazonic acid	Giorni *et al.*, 2007
Libya	*Aspergillus* section *Flavi*	Maize	75	27 (36)	Aflatoxins	Yousseff, 2009
	Fusarium spp (*F. tricinctum*, *F. oxysporum*, *F. equiseti*)	Maize Maize	20 20	19 (95) 17 (85)	Trichothecenes Zearalenone	Yousseff, 2009 Yousseff, 2009
Spain	*F. verticillioides*	Maize, maize screenings, feeds	11	8 (72.7)	Fumonisins	Sanchis *et al.*, 1995a
	F. proliferatum	Maize, maize screenings, feeds	19	19 (100)	Fumonisins	Sanchis *et al.*, 1995a
	F. verticillioides	Maize,	112	40 (35.7)	Fumonisins	Sala *et al.*, 1997
	F. verticillioides	Maize, poultry feed	26	25 (96.2)	Fumonisins	Castella et al., 1996
	F. verticillioides	Conventional and organic maize	20	16 (80)	Fumonisins	Ariño *et al.*, 2007
	Aspergillus section *Flavi*	Maize	37	15 (40.5)	Aflatoxins	Sanchis *et al.*, 1984

seeds with *Fusarium* colonies, ranged from 25% to 100% in the 45 samples of maize grain of the study, where *F. verticillioides* was the most frequent species, accounting for as much as 83% of isolates from maize. Other species isolated were *F. graminearum*, *F. proliferatum*, *F. sporotrichioides* and *F. solani*. The dominance of *F. verticillioides* in the study is in accordance with other studies conducted in Croatia (Segvic and Pepeljnjak, 2003; Cvetnic *et al.*, 2005). Its toxigenic profile implies that the most common mycotoxins in maize should be FBs or other toxins produced by this species.

In Egypt, *F. verticillioides* was the predominant species isolated from 18 freshly harvested samples of maize ears collected from Minia. 15 out of 18 isolates of this species had capacity to produce FBs (Fadl-Alla, 1998). Similar results were obtained by El-Maghraby *et al.* (1995); they isolated four species of *Fusarium* from grains of white maize hybrids, and *F. verticillioides* was the dominant species. In a study carried out in Egypt by Abd Alla (1997) with 50 samples of Egyptian maize from the market they found a high incidence of *F. culmorum*, *F. graminearum*, *F. roseum* and *F. verticillioides*. Other species isolated were *F. oxyspo-*

rum, *F. solani* and *F. poae*. The high predominance of *F. culmorum* and *F. graminearum* in the samples was supported by mycotoxin results, because 15 of the 50 samples studied were contaminated by ZEA and a high percentage of the isolates of these two species showed capacity to produce ZEA on autoclaved rice.

In France, Garon *et al*. (2006) monitored the maize silage mycobiota during 9 months. The production of maize silage entails the use of the whole plant and very poor conditions (low pH and anaerobiosis) for the growth of most fungi. During monitoring they observed a limited fungal diversity with 20 different species, as well as the co-occurrence and the recurrence of some potential toxigenic strains. Among these fungi four species could be considered as major toxigenic: *A. parasiticus*, *A. fumigatus*, *F. verticillioides* and *Monascus ruber*. These fungi were considered as possible producers of AFs, gliotoxin, FBs and citrinin, respectively.

In Italy, Logrieco *et al*. (1995) reported the occurrence of *F. verticillioides* and *F. proliferatum* in preharvest maize ears (yellow hybrids) from 42 samples with ear rot, and they were the predominant species in infected ear kernels. The relative incidence of these two species was 54% and 34%, respectively. Less frequently isolated were *F. equiseti* (8%) and *F. graminearum* (2%), and to a much lesser extent, *F. chlamydosporum*, *F. culmorum*, *F. oxysporum*, *F. semitectum*, *F. solani*, *F. sporotrichioides*, and *F. subglutinans*. The predominance of *F. verticillioides* and *F. proliferatum* in preharvest maize ears was confirmed by the study carried out by Ritieni *et al*. (1997) and Blandino *et al*. (2008c). In the study by Ritieni *et al.* (1997), *F. proliferatum* was predominant in the samples contaminated by FUS. On the other hand, a high percentage (70%) of *Aspergillus* section *Flavi* strains isolated from maize samples in Northern Italy had ability to produce AFs.

The first Libyan report on fungal contamination of maize grain showed that *Aspergillus* section *Flavi* was the predominant species on AFs contaminated maize samples, and *Fusarium* species (*F. tricinctum*, *F. oxysporum* and *F. equiseti*) were dominant on trichothecene-contaminated maize samples. The isolates recovered from the samples had capacity to produce these mycotoxins (Table 1) (Youssef, 2009).

In Spain, a study carried out in Valencia (eastern Spain) in 116 freshly harvested maize samples, showed that *Fusarium* was the predominant genus in the samples (45.3% of infected grains). Infection by the genera *Penicillium* and *Aspergillus* was much lower, 8.7% and 4.0% of infected grains, respectively. *F. verticillioides* was the predominant species in the samples (Sanchis *et al*., 1984). These results were confirmed by Ariño *et al.* (2007) in a study with 30 postharvest maize samples. Moreover, *Alternaria* was another important genus in the samples. However, in another study carried out by the same research group in 20 stored maize samples, *Aspergillus* and *Penicillium* were predominant, and *Fusarium* presented a much lower incidence (Viñas *et al*., 1984). In Galicia, in northwest Spain, the most frequent fungi found in 309 stored maize samples were *F. verticillioides* and *F. subglutinans*; however *F. graminearum* was present in the samples contaminated with ZEA and DON (Muñoz *et al*., 1990). *F. verticillioides* was the *Fusarium* species dominant in 657 feed and seed samples from Spain, representing 92.2% of the total *Fusarium* strains isolated (Cantalejo *et al*., 1998). Species of the genus *Penicillium* were prevalent (62.8% in total). *Aspergillus* and *Fusarium* contaminated the samples at intermediate levels (between 21% and 53% of the samples). The prevalence of *F. verticillioides* as the predominant species isolated from maize kernels has been reported in other studies in Spain (Sala *et al*., 1994; Castella *et al*., 1999). The percentage of the isolates able to produce FBs is very high (Table 1).

Finally, in Turkey, a study carried out by Alptekin *et al*. (2009) in 30 postharvest maize samples from Kahramanmaras, in the southern part of the country, showed an incidence of *Penicillium* spp. significantly higher than *Fusarium* and *Aspergillus*. 43% of the samples were contaminated with AFB_1. However, a study carried out by Askun (2006) in 20 retail and bulk maize samples from Balikesir, in the western part of the country, showed fungal infection by *Aspergillus* spp. (25%), *Fusarium* spp. (21%), *Rhizopus* spp. (21%) and *Penicillium* spp. (13%). *Aspergillus tubingensis* (5.0%), *Aspergillus foetidus* var. *acidus* (5.0%), *A.flavus* (4.5%), *F. proliferatum* (17.1%). *Rhizopus oligosporus* (5,7%) and *Penicillium oxalicum* (7.6%) were the most frequently species isolated and are the predominant maize mycobiota under storage conditions in this part of Turkey.

Fusarium **toxins in freshly harvested maize in Mediterranean countries**

FBs are the mycotoxins which have been most widely assessed in maize in the Mediterranean coun-

Table 2. Occurrence of fumonisins[a] in freshly harvested maize from Mediterranean countries.

Location	Year[b]	No. samples	Positives (%)	Mean (positives) (μg kg^{-1})	Range (μg kg^{-1})	Reference
Croatia	1992	19	5	20	n.d.–70	Doko *et al.*, 1995
Croatia	2002	49	100	459.5 FB_1	196.3–1377.6 FB_1	Domijan *et al.*, 2005
France	1999	3	100	3.4 FB_1	0.4–9 FB_1	Bakan *et al.*, 2002
Italy	1989–1991	26	100	450	10–2850	Doko *et al.*, 1995
Italy	1994	22	91	67090 FB_1	n.d.–300000 FB_1	Ritieni *et al.*, 1997
Italy	2000	24	100	2600	10–19690	Avantaggiato *et al.*, 2003
Italy	2000–2002	12	100	3210	345–7361	Marocco *et al.*, 2008
Italy	2001–2003	180	--	5000 FB_1	10–19600 FB_1	Berardo *et al.*, 2005
Italy	2001–2004	14	100	--	822–4924 FB_1	Blandino *et al.*, 2008b
Italy	2002–2007	438	98	5095 FB_1	n.d.–27418 FB_1	Battilani *et al.*, 2008
Italy	2003–2005	111	100	--	n.d.–21000	Maiorano *et al.*, 2009
Italy	2004–2005	4	100	--	2013–20575	Blandino *et al.*, 2008c
Italy	2005–2007	36	100	--	618–26200	Blandino *et al.*, 2010
Italy	2006–2007	12	--	--	1733–12412	Blandino *et al.*, 2009
Portugal	1995	9	100	1930	90–4450	Doko *et al.*, 1995
Spain	2009	13	50	729	n.d.–2600	Ariño *et al.*, 2009

[a] All fumonisin levels refer to FB_1+FB_2, except where specifically noted.
[b] Year of sampling, if not available, publication year.
n.d., Not detected.

tries; in particular, Italian researchers invested much effort and from 1989 their studies have demonstrated both the high incidence and concentration levels. A few studies from other Mediterranean countries confirm the importance of this hazard, with close to 100% positive samples for FBs in most of the cases (Table 2).

Regarding ZEA, mean incidence was 58% in the existing studies in the Mediterranean countries, with mean values up to 128 μg kg^{-1}, and maximum values up to 969 μg kg^{-1} (Table 3). It must be taken into account that the reported incidence levels are a function of the LOD of each study, thus direct comparison is not possible. Regarding trichothecenes, positive reported samples ranged from 5 to 3430 μg kg^{-1} of DON, and 21–2440 μg kg^{-1} of nivalenol (NIV), with incidence levels similar to ZEA (Table 4).

Reducing *Fusarium* toxins in the maize chain: preharvest strategies in the Mediterranean basin

Numerous factors may influence the infection of maize kernels by *Fusarium*. It has been demonstrated that damage resulting from insect feeding provides preferential sites for the penetration of the fungi, and some insects can even operate as vectors of mycotoxigenic fungi (Munkvold *et al.*, 1997). It has also been shown that the environmental conditions found in the

Table 3. Occurrence of zearalenone in freshly harvested maize from Mediterranean countries.

Location	Year[a]	No. samples	Positives (%)	Mean (positives) (μg kg^{-1})	Range (μg kg^{-1})	Reference
Croatia	2002	49	84	3.84	0.43–39.12	Domijan *et al.*, 2005
Croatia	2002	15	80	1.7	0.62–3.22	Domijan *et al.*, 2005
Egypt	1993–1995	50	30	22.3	10.4–45.2	Abd Alla, 1997
France	1999	3	100	1.7	0.62–3.22	Bakan *et al.*, 2002
Italy	2001–2004	14	28.6	--	10–217	Blandino *et al.*, 2008b
Italy	2002	46	28.2	128.3	n.d.–969	Cavaliere *et al.*, 2005

[a]Year of sampling, if not available, publication year.
n.d., Not detected.

Table 4. Occurrence of deoxynivalenol in freshly harvested maize from Mediterranean countries.

Location	Year[a]	No. samples	Positives (%)	Mean (positives) (μg kg^{-1})	Range (μg kg^{-1})	Reference
France	1999	3	100	467.3	179–751	Bakan *et al.*, 2002
Italy	2002	46	43.5	436.4	5–3430	Cavaliere *et al.*, 2005
Italy	2001–2004	14	57.1	--	10–1195	Blandino *et al.*, 2008b
Italy	2005–2007	36	100	--	14–1238	Blandino *et al.*, 2010

[a]Year of sampling, if not available, publication year.

specific area of cultivation play an important role in the accumulation of mycotoxins in maize. Although no much information has been published on mycotoxins occurrence in freshly harvested maize, some researchers have focused their interest on those field factors which may be crucial to determine the mycotoxins present in harvested maize, in particular, Blandino and coworkers developed in Italy a series of field trials which account for most of the agronomic factors which may have an impact (Blandino *et al.*, 2007, 2008a, b, c, 2009). It is clear that climatic conditions encountered in Southern Europe are clearly conducive to FB accumulation in maize thus most studies in preharvest have dealt with *F. verticillioides* and FBs.

Impact of maize genotypes on FBs incidence

Differences among maize genotypes for FBs accumulation have been found. The natural occurrence of FB_1 and FB_2 has been investigated in 26 maize inbred lines grown in Italy, 19 maize hybrids grown in Croatia and 9 maize hybrids grown in Portugal. High contamination was reported in Italy (100%) and Portugal (100%) with FBs (FB_1+FB_2) levels up to 2850 and 4450 ng g^{-1}, respectively. Maize hybrids from Croatia showed very low levels of contamination (≤70 ng g^{-1}) with 50% incidence of positive samples. However, no single maize genotype was cultivated in different countries. When considering the overall samples from some different European and African countries a trend could be observed indicating low FB_1+FB_2 contamination in flint-type endosperm (very low level, 10 ng g^{-1}), compared to intermediate contamination in flint-dent or semident endosperm (mean-positive concentration 50 ng g^{-1}), and high contamination in dent endosperm (mean-positive concentration 290 ng g^{-1}) (Doko *et al.*, 1995).

No differences in fungal infection were found among white maize genotypes in Spain, but differences in FB_1+FB_2 contamination were significant and could be related, in part, to differences in husk tightness. Husk tightness could act as a barrier to fungus entrance and delay FBs contamination. However, genotypes that differed from the best hybrid for husk coverage showed FB_1+FB_2 levels as low as the least contaminated hybrid. Therefore, other mechanisms besides husk tightness should contribute to the lower level of FBs contamination in some genotypes (Butron *et al*., 2006). Waxy hybrids have showed in Italy a higher average contamination of FB_1 than normal hybrids with the same or similar genealogy, although they showed similar European corn borer incidence and *Fusarium* ear rot incidence and severity. No differences were observed for ZEA contamination. It is supposed that the presence of starch, almost exclusively as amylopectin, can stimulate a greater toxinogenesis of the fungi that produce this toxin, thus making the waxy hybrids more susceptible to FBs contamination (Blandino and Reyneri, 2007).

Breeding for ear rot resistance

Mid-late and late maturity maize varieties are predominantly used for grain production in Southern France, Hungary, Italy, Spain, and the Balkan states and are mostly infected by *F. verticillioides*. Here, additional kernel infection by insect attack plays a major role. In 2007 and 2008, three maturity groups (early, mid-late, late) each comprising about 150 inbred lines were tested in Germany, France, Italy, and Hungary according to their maturity group. In early maturing flints and dents, *F. graminearum* caused significantly higher ear rot severity than *F. verticillioides*. *F. verticillioides* inoculation in Southern Europe (mid-late, late) resulted in similar means between 10.3 and 14.0%. Correlation between *F. graminearum* and *F. verticillioides* severity was moderate in flints and dents (r= 0.59 and 0.49, respectively) but lines resistant to both fungi exist. It was concluded that chances for selecting improved European elite maize material within the existing germplasms is promising by multienvironmental inoculation trials (Löffler *et al*., 2010).

Impact of genetically engineered maize hybrids on *Fusarium* toxins incidence

Maize grain from Bt hybrids and near-isogenic traditional hybrids was collected in France and Spain from the 1999 crop, which was grown under natural conditions. The predominant *Fusarium* species isolated from Bt hybrids as well as from non-Bt hybrids were *F. verticillioides* and *F. proliferatum*. FB_1 grain concentrations ranged from 0.05 to 0.3 μg kg^{-1} for Bt maize and from 0.4 to 9 μg kg^{-1} for isogenic maize. Moderate to low concentrations of trichothecenes and ZEA were measured on transgenic as well as on non-transgenic maize. The protection of maize plants against insect damage (European corn borer – *O. nubilalis* - and pink stem borer – *S. nonagrioides*) through the use of Bt technology seems to be a way to reduce the contamination of maize by *Fusarium* species and the resultant FB_1 in maize grain (Bakan *et al*., 2002). Similarly, the mean percentages of *Fusarium*-infected kernels were significantly lower in Bt maize varieties (10–15%) than in conventional maize varieties (20–34%) in Spain (Alborch *et al*., 2010). Moroever, Bt maize and its isogenic non-Bt counterpart were cultivated in Southwestern France. The results showed that Bt maize decreased concentrations of FB_1+FB_2 by 90% and ZEA by 50%, whereas the concentration of DON was slightly increased. Those findings suggest a competition among *Fusarium* species that produce FBs or trichothecenes. They hypothesised that the control of insects limited the invasion of opportunistic fungi *F. verticillioides* or *F. proliferatum*, and, as a consequence, favoured the development and the activity of *F. graminearum*, which infested the plant regardless of insect damage (Folcher *et al*., 2010).

Agronomic management

No direct fungal control measures have been developed for maize yet, so natural infection depends on the climatic factors when maize hybrids are susceptible to the disease. Thus, the only effective solution seems to be prevention in the field by Good Agricultural Practices (GAP) that are able to guarantee less favourable conditions to fungal development and toxigenesis (Munkvold, 2003).

Impact of insecticides in Fusarium *toxin reduction in maize*

Fusarium verticillioides has been reported to be closely correlated to insect injuries, and this appears to be the most important infection pathway in temperate areas (Avantaggiato *et al*., 2003). *O. nubilalis* is the main maize pest in Central and South Europe.

Two generations of European corn borer (ECB) larvae usually occur per year in North Italy: the first generation attacks plants during the mid to late vegetative stages and the second generation attacks during the reproductive stages (from early milk stage to maturity). Second-generation larvae, in particular, play an important role in the epidemiology of *F. verticillioides* in maize (Sobek and Munkvold, 1999) and insect damage of ears can increase FBs contamination of kernels (Logrieco *et al*., 2003). In maize grain, ECB feeding activity is crucial in FB_1+FB_2 contamination: damaged ears suffer a 40 times higher contamination rate than healthy ears (Alma *et al*., 2005). Insecticide treatment against second-generation larvae of ECB plays an increasing role in maize crop practices, and several insecticides, mainly synthetic pyrethroids, are currently labelled for ECB control in maize and generally applied after the first generation flight peak of adults. Systemic infection through the seed or during anthesis is less relevant than insect-assisted infection during kernel ripeness.

In Northern Italy, two insecticide application treatments with lambda–cyhalothrin (at 7 and 14 days after the ECB flight peak) were compared with the untreated control. Insecticide applications to plants resulted in lower ear rot severity (up to 29% reduction), and significantly lower grain FB_1+FB_2 concentration (45% reduction) than the untreated control. No links were detected between *O. nubilalis* presence and ZEA contamination (Blandino *et al*., 2008c). There is evidence that *O. nubilalis* feeding activity is not correlated to ZEA contamination of kernels: that could be the consequence of biological competition between *F. verticillioides* and *F. graminearum* (Reid *et al*., 1999). Similarly, in north-western Italy (2000–2006), *Fusarium* ear rot and FB_1+FB_2 contamination were affected by second generation ECB control, including deltamethrin, lambda-cyhalothrin, alpha-cypermethrin, chlorpyrifos and cypermethrin, and indoxacarb insecticides. The ear damage was reduced, on average, by 44.1%. The occurrence of FB_1+FB_2 was significantly reduced, on average by 68% (Saladini *et al*., 2008).

Field trials were carried out in nine areas located in France during 2004–2006 to study the control of Lepidoptera caterpillars (*O. nubilalis* and *S. nonagrioides*) by agrochemical treatments involving either an insecticide (deltamethrin) or an insecticide-fungicide (deltamethrin-tebuconazole) association. *Fusarium* spp. mycobiota was not significantly affected by treatments; the control had a mean of 24.11% of contaminated grains with fungi that produced FB_1+FB_2, while fungal contamination of 15.67% and 21.56%, respectively, was observed for insecticide and insecticide plus fungicide treatments. The levels of contamination of trichothecene-producing fungi were, respectively, 12.11% for control, 7.56% for insecticide treatment and 5.44% for insecticide plus fungicide (again, the treatment effect was not statistically different). The mycotoxin levels for the control were significantly greater than those for the pesticide treatments for FBs, trichothecenes and for ZEA. This efficacy was, respectively, evaluated at 89.96% for FB_1+FB_2 with the insecticide treatment and 89.97% with insecticide+fungicide. For trichothecenes, the efficacy was 73.50% with the insecticide treatment and 84.17% with insecticide+fungicide. For ZEA, there was a reduction of 85.40% of contamination with the insecticide and 82.10% with insecticide+fungicide (Folcher *et al*., 2009). This last point seems to contradict the above results by Blandino *et al*. (2008c).

Taking into account ECB dynamics population, the timing of insecticides application may be crucial. Increasing the number of insecticide applications is not practicable because it is not economically favourable and could have a higher impact on non-target biota, in particular the natural enemies of ECB, and could lead to an increase in other pests. ECB larvae on maize plants are in fact difficult to combat because they are exposed to spraying for only a short period before they bore into the plant. The objective should be to obtain the longest period of protection of the plant against the pest and, indirectly, against *Fusarium* development. Correct application is crucial in reaching the larvae during the few days between hatching and when they enter into the stalk and ear (Mason *et al*., 1996).

Field experiments were performed in 2005 to 2007 in NW Italy with different insecticide (lambda-cyhalothrin) application timings, from maize flowering to approximately 15 days after the flight peak of adult ECB. The treatment approximately 7–10 days before the ECB flight peak had a significantly lower ear rot incidence (29–48%) than treatments at the end of maize flowering and approximately at the ECB flight peak. The efficacy of the best timing of insecticide application (13–17 days after silking, 7–10 days before the ECB adult flight peak) in controlling FB_1+FB_2 contamination was, on average, 76–93% compared to the untreated control. Contamination levels of these mycotoxins increased with either an earlier or later treatment (Blandino *et al*., 2009, 2010). Since pyrethroids, which have an approximately 15-day residual activity (Rinkleff *et al*., 1995), are only effective when the ECB is in its larval state and has not yet penetrated the stalk or ear, the correct treatment window for their application ranges between the initial capture of 1st generation adults and the flight peak. If the treatment is delayed until after the adult flight peak, larvae from the eggs deposited early in the laying period enter the plant and are not controlled effectively by the insecticide, causing ear damage and FBs contamination. Thus, the higher efficacy of early pyrethroid application is probably due to the control of larvae that feed earlier during the maize-ripening stages and have greater potential to cause greater *Fusarium* development and FBs contamination than those hatched at the end of adult flight, which feed on harder and drier kernels (Mason *et al*., 1996).

Sesamia nonagrioides is a major pest of maize in Mediterranean countries. The infestations tend to be worse in areas where summer and spring crops of maize coexist in the immediate vicinity. An investigation of *Sesamia* attacks and FB_1+FB_2 accumulation on 25 maize hybrids sown as a second crop after wheat was performed under field conditions in Central Italy in 2000 (Avantaggiato *et al*., 2003). FB_1+FB_2 analysis of healthy-looking and insect damaged ear samples of each hybrid showed 100% incidence of positive samples, with FB_1+FB_2 contents ranging from 0.01 to 20 $\mu g\ kg^{-1}$ for healthy-looking ears and from 27 to 287 $\mu g\ kg^{-1}$ for insect-damaged ears. The poor efficacy of chemical control, due to the entirely endophytic life of *Sesamia*, has been shown in some experimental works.

Sowing time and insecticide application

Pilcher and Rice (2001) reported that later sowing times let to a higher incidence of ECB larvae damage in the United States. Furthermore, with the delay of sowing, the ECB infestation occurs in a physiological stage that is more attractive for the insects and injuries caused by 2nd-generation larvae on the ears result to be higher.

In Italy and other Southern European countries, favourable climatic conditions allow high yields of maize as a second crop after the harvest of winter small cereals (barley and soft wheat). Early-maturing maize hybrids have been found to be the most appropriate. However, the cultivation of maize as a second crop is limited by attacks of stem borer, especially the pink stem borer *S. nonagrioides*. In summer-sown maize, attacks of second and third-generation *Sesamia* (and even fourth generation in some areas) are particularly strong (Quaranta *et al*., 1989).

Two sowing dates (at about 25–30 day intervals) and two insecticide application treatments with lambda-cyhalothrin (at 7 and 14 days after the ECB flight peak) were compared with the untreated control in North Italy (2004–2005). Earlier sowing dates resulted in ear rot incidence and severity reductions of 13% and 16%, respectively, compared to later sowing dates. Maize sown earlier and treated with insecticide 7 days after the ECB flight peak resulted in a significantly lower grain FB_1+FB_2 concentration (up to 67% reduction), compared to those of untreated and late sowed maize. In Mediterranean areas, the choice of an early sowing date and appropriate treatments with pyrethroid insecticides against ECB might represent a useful tool to limit raw maize FBs concentration (Blandino *et al*., 2008c). An early sowing date could significantly reduce ECB injury because the first part of the grain filling, the most sensitive stage, takes place before the occurrence of the 2nd generation larvae peak (Pilcher and Rice, 2001).

Fertilisation

In Northern Italy (2000–2002), conventional tillage and no-till treatments had no significant effect on the levels of FB_1+FB_2 (Marocco *et al*., 2008). This finding suggests that above-ground residues infected by *Fusarium* would not lead to an increase in FBs incidence. *Fusarium* spp. are known to overwinter in host residues. However, survival of *F. verticillioides* was, in some reports, lower in maize stubble on the soil surface than at 30 cm depth, whereas, in other studies, *F. verticillioides* was recovered in equal quantities from buried and surface maize residues (Flett and Wehner, 1991). Maize ear rot fusaria are seed-borne and have a wide range of alternative hosts from which they can be disseminated to adjacent maize fields by wind or insects, thereby masking tillage effects (Marocco *et al*., 2008). Also, dispersal of spores from maize residue in neighbouring fields is possible.

Contradictory results regarding N fertilisation can be found in the literature. N fertilisation significantly increased FB_1+FB_2 levels in Italy, by 70–99%. This may result from a suitably wet microclimate and mechanically weaker, more susceptible leaf tissues (Marocco *et al*., 2008). In contrast, fungal ear rot incidence and severity was generally higher in ears from plants fertilized with insufficient N (Blandino *et al*., 2008a). The distribution of slow-release fertilizer, instead of the use of urea, led to a significant increase in the *Fusarium* spp. infection. High N fertilizer application (>300 kg N ha^{-1}) significantly increased the ZEA content, while for FB_1+FB_2 the highest contamination was related to N deficiencies (+80%). A balanced N fertilizer application (200 kg ha^{-1}) generally seems to ensure lower mycotoxin contamination and is usually the best solution for low mycotoxin contamination (Blandino *et al*., 2008a).

Plant density

Field experiments were conducted from 2001 to 2004 in North West Italy to determine the effects of plant density on the susceptibility of medium and medium-late maturity maize hybrids to ear rot and to mycotoxin contamination under conditions of natural infection. Plots with the highest plant populations had higher incidence of kernel *Fusarium* infection (+24%) and higher fungal ear rot severity (+43%) than plots with lower plant densities. Natural occurrence of mycotoxins was always significantly higher in crops with a higher plant density. The plant density, however, did not influence the ECB infestation. This result suggests that the increased fungal ear rot symptoms that were observed were probably directly related to the effect of plant density on the microclimatic conditions inside the crop (Blandino *et* al., 2008b) or because of plant stress related to the high plant density, leading to higher susceptibility.

Mycotoxins in postharvest maize in Mediterranean countries

Mycotoxins in stored maize

It has been demonstrated that postharvest maize from different countries around the Mediterranean basin is frequently contaminated with mycotoxins originating both from the field and storage. The main problem when interpreting results is that many references do not indicate if the sampled maize was destined for human or animal consumption, which can influence in determining whether it was fit for consumption, according to its final destination.

In a multi-mycotoxin study that included samples from different Southern European countries (Greece/Cyprus, Spain, Italy and Portugal) it was observed that maize, maize meal and maize silage were contaminated with DON/acetyl-DON (ADON), T-2/HT-2 toxins, ZEA, FBs, AFs and OTA with mean values of 233, 35, 27, 2195, 5 and 0.3 μg kg^{-1}, respectively, when analysed by HPLC using different detectors (Griessler *et al*., 2010). Out of 85 analysed samples, 79% were positive for type B trichothecenes, whereas contamination by AFs, ZEA and OTA was detected in 29%, 18% and 14% of cases, respectively. Fifty out of 111 maize samples were subjected to FBs analysis, with 68% samples being positive.

Aflatoxin contamination in postharvest maize is very variable (Table 5). In Italy, 2 samples contaminated with up to 109 or 158 μg AFB_1 kg^{-1} were found, although mean values during five consecutive years (1995–1999) were in the range of 0.3–4.10 μg AFB_1 kg^{-1} (Pietri *et al*., 2004). High levels of AFs have been also observed in Turkey, with samples containing up to 133 μg kg^{-1} (Nizamlýoðlu and Oguzz, 2003) or 120.3 μg kg^{-1} (Giray *et al*., 2009). However, most of the samples showed contamination below detection limits (54% and 53%, respectively). There is little information on AFs in maize in Middle East countries, but it has been reported that maize sampled in Syria between 2003 and 2005 contained AFs at levels higher than the 20 μg kg^{-1} limit set by the legislation of that country (Majid, 2007). Hybrid maize sampled in Egypt showed a mean aflatoxin contamination of 30 μg kg^{-1} (Abdelhamid, 1990).

Co-occurrence of AFs with other mycotoxins has also been described. In Tunisia, simultaneous contamination of maize with OTA and AFs (19.4% of samples) or ZEA (4.7%) has been described (Ghali *et al*., 2008), whereas in Croatia, in a survey of cereals including maize, wheat, barley and oat, the most frequent two-toxin combinations were AFs+ZEA (17%), followed by AFs+OTA, OTA+ZEA and ZEA+FBs (12.5% each) (Šegvić *et al*., 2009).

In the same way as AFs, OTA contamination of maize shows highly variable incidence (Table 5), and mean levels are usually below 50 μg kg^{-1}. However, a unique situation occurs in the Balkans, where the OTA-related disease known as Balkan Endemic Nephropathy occurs. Thus, in a survey developed in 1975 in the former Yugoslavia, 26% of maize samples showed OTA contamination, with an average of 490 μg kg^{-1} and a range of 45–5100 μg kg^{-1}. Similarly, in a study conducted in Croatia in 1996 and 1997, OTA contamination levels of maize showed values as high as 224 μg kg^{-1} and 614 μg kg^{-1}, respectively (Jurjevic *et al*.,

Table 5. Occurrence of aflatoxins and ochratoxin A in postharvest maize and maize-based products from Mediterranean countries.

Toxin/ Location	Year [a]	No. samples	Positives (%)	Mean (positives) (μg kg^{-1})	Range (μg kg^{-1})	Reference
Aflatoxins [b]						
Croatia	2007	12	33.3	3.4	2.7–4.5	Šegvić *et al*., 2009
Egypt	1990	5	60	30	--	Abdelhamid, 1990
Italy	1995–1996	503	42.9	1.7 AFB_1	<0.05–158 AFB_1	Pietri *et al*., 2004
Morocco	2007	20	80	1.57 AFB_1	0.23–11.2 AFB_1	Zinedine *et al*., 2007
Spain	1984–1986	154	0	--	--	Muñoz *et al*., 1990
Tunisia	2004–2005	21	42.8	7.6	2.9–12.5	Ghali *et al*., 2008
Turkey	2002	26	57.7	n.a.	1.5–133	Nizamlýoðlu and Oguzz, 2003
Turkey	2002–2003	19	100	10.94	0.01–32.30	Oruc *et al*., 2006
Turkey	2009	47	47	n.a.	1.75-120.3	Giray *et al*., 2009
Yugoslavia	1975	191	0	--	--	Balzer *et al*., 1977
Several countries [c]	2005–2009	15 [d] 16 [e]	20 37.5	5 3	1–6 1–39	Griessler *et al*., 2010
Ochratoxin A						
Croatia	1996	105	9.52	37.87	0.36–224	Jurjevic *et al*., 2002
Croatia	1997	104	34.6	57.13	0.26–614	Jurjevic *et al*., 2002
Croatia	1999–2000	51	33.3	0.4-20	0.02–40	Puntarić *et al*., 2001
Croatia	2007	12	25	12.7	2.5–31.7	Šegvić *et al*., 2009
Egypt	1990	6	16.7	12	--	Abdelhamid, 1990
Italy	2002	70	27.1	n.a.	0.9–5.2	Palermo *et al*., 2002
Italy	2004	80	30	1.8	0.1–5.2	Muscarella *et al*., 2004
Tunisia	2004–2005	21	1.9	3.3	0.92–6.7	Ghali *et al*., 2008
Turkey	2009	47	57	n.a.	1.08–8.57	Giray *et al*., 2009
Yugoslavia	1975	191	26	490	45–5100	Balzer *et al*., 1977
Several countries [c]	2005–2009	7 [d] 7 [d]	28.6 0	0.3 --	n.a. --	Griessler *et al*., 2010

[a] Year of sampling, if not available, publication year.
[b] All aflatoxin levels refer to the total values, except where specifically noted.
n.a., Data not available.
[c] Greece/Cyprus, Italy, Spain and Portugal.
[d] Analysed by HPLC.
[e] Analysed by ELISA.

2002). Nevertheless, in a recent study conducted in Croatia, although the percentage of positive samples was still high (25%), the highest OTA amount found was 31.7 μg kg^{-1}, though it must be noted that the number of samples was relatively small (n= 12) (Šegvić *et al*., 2009).

With regard to *Fusarium* mycotoxins, FBs are the most frequent contaminants, with contamination incidence very often near 100% and mean values frequently in the range of μg kg^{-1} (Table 6). A study conducted in Northern Italy during five years (1995–1999) with a high number of samples (n= 503) showed a 100% contamination of samples, with mean values as high as 3064 μg FB_1 kg^{-1}, but with only 16.9% of samples over 5000 μg FB_1 kg^{-1} (and a sample containing 51690 μg FB_1 kg^{-1}) (Pietri *et al*., 2004). In a similar way 100% samples of maize grown in Turkey showed contamination with FBs, with mean levels even much higher (88240 μg kg^{-1}) (Oruc *et al*., 2006).

In Croatia, samples collected in 1996 and 1997 showed 97.1% and 93.3% FB_1+FB_2 contamination (mean ranging from 134 to 645 μg kg^{-1}), respectively (Jurjevic *et al*., 2002); in a study carried out a decade later only 25% of samples were contaminated, but with mean contamination levels much higher (7630 μg kg^{-1}) (Šegvić *et al*., 2009). Similar results were found by Scudamore and Patel (2009) when studying French maize imported between 2004 to 2007 into the UK, as 54 samples from 56 consignments of maize (96.4%) displayed FB_1+FB_2 contamination above 10 μg kg^{-1}, whereas mean values from different mills ranged between 403 to 696 μg kg^{-1}.

In Egypt, a study with yellow maize, white maize and maize meal showed FB_1 contamination of 80%, 33.3% and 53.8% of cases, respectively, although maize starch was found to be FB_1-free (Abd Alla *et al.*, 2003). Similarly, in Spain 87.3% of maize samples showed FB_1+FB_2 contamination (mean 5600 μg kg^{-1}), whereas barley and wheat contamination and levels were much lower (72.4% and 47.1%, and 1900 and 2900 μg kg^{-1}, respectively) (Castellá *et al.*, 1999).

Regarding ZEA contamination of postharvest maize, in the last 20 years several surveys have been carried out in Mediterranean countries, showing incidences between 2.6 to 100% of samples, with mean contamination levels from 5.4 to 316.5 μg kg^{-1} (Table 6). For instance, in Italy, ZEA contaminated 44% of maize samples during years 1995–1999, but only 14% of samples had concentrations higher than 200 μg kg^{-1}. Interestingly, during 1996, the percentage of samples above 200 μg kg^{-1} increased to 53.8% (91% of samples with detectable amounts of ZEA), with a mean contamination of 453 μg kg^{-1} and one sample containing 2531 μg kg^{-1} (Pietri *et al.*, 2004). The unique weather conditions during 1996 (unusually rainy and humid conditions, that delayed the date of harvest) favoured the growth of ZEA-producing fungi. A similar trend was observed regarding DON contamination.

In Egypt, ZEA showed a higher occurrence in maize than in rice or wheat. Contamination ranged from 100% (maize) to 0–50% (wheat, wheat bran and wheat soya meal) and 0–50% (rice germ, rice bran), with mean ZEA values from 16 (white maize) to 42 μg kg^{-1} (yellow maize) (Abdelhamid, 1990). In the same way, Abd Alla (1997) found a higher occurrence in maize (30%) than in rice (8.9%) or wheat (12.5%), with mean values of 22.3 μg kg^{-1}. Similar mean values were found in samples from France (26 μg kg^{-1}) and in a multi-mycotoxin survey conducted in Greece/Cyprus, Spain, Italy and Portugal (27 μg kg^{-1}, analysed by HPLC) (Scudamore and Patel, 2009; Griessler *et al*., 2010). Unusual higher values were described in an older study reported from the former Yugoslavia (5100 μg kg^{-1}) (Balzer *et al*., 1977).

With regard to trichothecenes, DON has been frequently found in postharvest maize samples from the Mediterranean basin, although the percentage of positive samples has a fairly wide range (Table 6), with mean values ranging from 200 to 860 μg kg^{-1}, and occasional maximum values over 9000 μg kg^{-1} (Pietri *et al.*, 2004). Contamination of maize with T-2 and HT-2 toxins, DAS and NIV has also been described (Table 6), but more studies are needed in this geographical area to draw accurate conclusions.

Concerning emerging mycotoxins, in Italy, FUS was identified (up to 500 μg kg^{-1}) in 41% of maize samples mostly infected by *F. proliferatum.* FUS was often found to be associated with FB_1 (up to 300 μg kg^{-1}) and beauvericin (up to 520 μg kg^{-1}) (Ritieni *et al*., 1997). Moreover, the presence of beauvericin in maize samples has been described in samples from Croatia and Morocco, but levels found were very different (means from 3.93 to 696 μg g^{-1} in Croatia, and 17000 μg kg^{-1} in Morocco) (Jurjevic *et al*., 2002; Zinedine *et al*., 2011). In samples from Morocco occurrence of ENNs (42% occurrence, mean level 127500 μg kg^{-1}) and FUS (3.2 % occurrence, 600 μg kg^{-1}) has been recently described (Zinedine *et al*., 2011). Presence of ENNs in maize (48% of samples), as the sum of ENNA, ENNA1, ENNB and ENNB1, was slightly higher than in wheat (42%), but barley samples showed higher occurrence (87.5%). However, the maximum level of ENNs described (445,000 μg kg^{-1}) was found in a maize sample. ENNA1 was the more frequently ENN found in positive samples, with higher contamination levels (mean in maize samples, 207,000 μg kg^{-1}).

Table 6. Occurrence of *Fusarium* toxins in postharvest maize and maize-based products from Mediterranean countries.

Toxin/ Location	Year[a]	No. samples	Positives (%)	Mean (positives) (µg kg^{-1})	Range (µg kg^{-1})	Reference
Fumonisins[b]						
Croatia	1996	105	97.1	645	12–11661	Jurjevic *et al*., 2002
Croatia	1997	104	93.3	134	12–2524	Jurjevic *et al*., 2002
Croatia	2007	12	25	7630 $FB_1+B_2+B_3$	200–20700 $FB_1+B_2+B_3$	Šegvić *et al*., 2009
Egypt	2003	57	n.d. –80	n.a. FB_1	10–780 FB_1	Abd Alla 2003
France	2004–2007	56	96.4	538	<10–3401	Scudamore and Patel, 2009
Italy	1995–1999	503	100	3064 FB_1	53–51690 FB_1	Pietri *et al*., 2004
Spain	1994–1996	55	87.3	5600	200–24900	Castellá *et al*., 1999
Turkey	2002–2003	19	100	88240	800–356800	Oruc *et al*., 2006
Several countries[c]	2005–2009	21[d] 29[e]	95.2 62.1	2195 6306	92–7714 584–36390	Griessler *et al*., 2010
Zearalenone						
Croatia	2007	12	100	316.5	27.7–1182	Šegvić *et al*., 2009
Egypt	1990	4	100	16	4–30	Abdelhamid, 1990
Egypt	1990	4	100	42	2–79	Abdelhamid, 1990
Egypt	1993–1995	50	30	22.3	10.4–45.2	Abd Alla, 1997
France	2004–2007	56	58.9	26	<10–165	Scudamore and Patel, 2009
Italy	1995–1999	503	44	124.2	<20–2531	Pietri *et al*., 2004
Spain	1984–1986	154	11.7	n.a.	0.7–9.6	Muñoz *et al*., 1990
Tunisia	2004–2005	21	9	5.4	1.9–11.4	Ghali *et al*., 2008
Yugoslavia	1975	191	2.6	5100	43–10000	Balzer *et al*., 1977
Several countries[c]	2005–2009	12[d] 66[e]	33.3 15.5	27 22	20–178 48–258	Griessler *et al*., 2010
Deoxynivalenol						
Egypt	1990	4	100	345	70–700	Abdelhamid, 1990
Egypt	1990	4	100	178	100–222	Abdelhamid, 1990
Egypt	2003	57	n.d. –14.3	n.a.	10.1–29.8	Abd Alla 2003
France	2004–2007	56	98.2	200	<10–932	Scudamore and Patel, 2009
Italy	1995–1999	503	93	860	<20–9357	Pietri *et al*., 2004
Spain	1984–1986	154	5.8	n.a.	43–315	Muñoz *et al*., 1990
Several countries[c]	2005–2009	14[d] 71[e]	50 84.5	233 547	68–1687 253–3025	Griessler *et al*., 2010

(*Continued*)

Table 6. *Continues.*

Toxin/ Location	Year[a]	No. samples	Positives (%)	Mean (positives) (μg kg^{-1})	Range (μg kg^{-1})	Reference
Other *Fusarium* toxins						
Croatia	1996	105	17.1	3.93	13–1864	Beauvericin Jurjevic *et al*., 2002
Croatia	1997	104	1	696	--	Beauvericin Jurjevic *et al*., 2002
Egypt	2003	57	n.d.–5 n.d. –14.3	n.a. n.a.	n.d. –12.7 9.8–22.1	T-2 toxin DAS Abd Alla, 2003
France	2004–2007	56	73.2	35	<10–170	Nivalenol Scudamore and Patel, 2009
France	2004–2007	56	60.7 39.3	9.6–17.1 18.4–24.6	<10–107 <10–149	T-2 toxin HT-2 toxin Scudamore *et al*., 2009
Morocco	2011	31	48 19.4 3.23	127500 17000 600	n.d. –445000 n.d. –59000 600	Enniatins Beauvericin Fusaproliferin Zinedine *et al*., 2011
Spain	1984–1986	154	0 0	-- --	-- --	T-2 toxin DAS Muñoz *et al*., 1990
Several countries[c]	2005–2009	9[d] 4[e]	11.1 25	35 20	-- --	T-2/HT-2 toxins T-2/HT-2 toxins Griessler *et al*., 2010

[a] Year of sampling, if not available, publication year.
[b] All fumonisins levels refer to FB_1+FB_2, except where specifically noted.
[c] Greece/Cyprus, Italy, Spain and Portugal.
[d] Analysed by HPLC.
[e] Analysed by ELISA.
n.d., Not detected.
n.a., Data not available.

Mycotoxins in feeds

As a result of the frequent contamination of cereal grains, feedstuffs are often contaminated by mycotoxins. Although there is enough literature about mycotoxin contamination of feeds, very often there is no description of the sample composition, or the final destination of each feed. Consequently, it is frequently difficult to discern which feeds certainly contain maize in their composition, although this may be the most frequent situation. Thus, in this review we have only included those references that specifically indicated that the feed contained maize in its composition.

Table 7 shows aflatoxin contamination of animal feeds in different Mediterranean countries. As previously described with stored maize, a recent study including finished compound feed samples from Greece/Cyprus, Spain, Italy and Portugal was conducted from 2005–2009. The occurrence of feed contamination with type B trichothecenes, FBs, ZEA, OTA, and AFs was 73%, 40%, 37%, 35.3% and 15.2%, respectively. No contamination with type A trichothecenes was observed, and 22% of the samples were contaminated with more than one mycotoxin. With regard to different commodities in feedstuffs (maize, wheat, barley, rye, sorghum, soy and others), data show that maize was positive for all analysed mycotoxin categories and was the most affected feed material. Occasionally, high levels of FBs (3228 μg kg^{-1}), DON/ADON (3036 μg kg^{-1}) or ZEA (189 μg kg^{-1}) were found (Griessler *et al*., 2010) but aflatoxin contamination never exceeded 4 μg kg^{-1}. In the European Union, AF contamination of feeds is regulated by the Commission Regulation No. 574/2011 of 16 June 2011 on undesirable substances in animal feed. This Directive states that the maximum content of AFB_1, relative to a feeding stuff with a moisture content of 12%, varies from 5 to 20 μg kg^{-1}, so samples in the study by Griessler *et al*. (2010) were always within legal limits (mean level obtained in this study was 0.3 μg kg^{-1}).

Sabatini *et al.* (2007) showed that mixed rations sampled in Italy showed AF contamination ranging between 0.03–7.76 μg kg^{-1}, but with regard to whole maize and maize fractions (broken maize, grits and flour), the broken fraction was responsible for providing most of the AFs, as contamination observed was up to 6.4 μg kg^{-1}, while in the other fractions contamination do not exceed 3 μg kg^{-1}.

On the other hand, reports from France showed samples of maize silage with up to 30 μg AFB_1 kg^{-1}, although this contamination decreased and stabilized to values near 10 μg AFB_1 kg^{-1} after 2 months of storage. Interestingly, in the same sample, citrinin was detected at the beginning of the silage at levels below 5 μg kg^{-1}, but after some months of storage this concentration increased to 25 μg kg^{-1} and, finally, it was stabilized at approximately 15 μg kg^{-1} (Garon *et al*., 2006). Similarly, Richard *et al.* (2009) found AFB_1 mean contamination in French maize silage around 28 μg kg^{-1}.

AF contamination of maize-based feeds has been also observed in other Mediterranean countries such as Egypt, Croatia, Morocco or Turkey (Abdelhamid, 1990; Nizamlýoðlu and Oguzz, 2003; Zinedine *et al*., 2007; Šegvić *et al*., 2009).

OTA contamination of feeds has been less often described in maize-based feedstuffs in Mediterranean countries (Table 7), although OTA contamination of feeds is probably much more frequently described in papers in which no specific reference to the composition of the feed is made. Of particular relevance is the data of feed contamination by OTA in Croatia, a country affected by the Balkan Endemic Nephropathy, where despite having found OTA-contaminated maize above the Croatian legal limits (5 μg kg^{-1}), no feed sample exceeded the 250 μg kg^{-1} level set as the upper OTA limit in feeds in this country (Šegvić *et al*., 2009).

Maize-based feedstuff contamination with *Fusarium* toxins has been widely described (Table 8). Among the toxins reported, FBs are frequently found in maize feeds, often at contamination percentages above 50%. Data from different countries indicate mean FBs contamination above 500 μg kg^{-1}, and, in some occasions, heavily contaminated samples have been found, such as the 23600 μg FB_1+FB_2 kg^{-1} found in one horse feed sample from Spain (Castellá *et al*., 1999).

Co-occurrence of different mycotoxins in feed samples has been also reported, as described in Croatian (ZEA+FBs, 54%; AFs+ZEA, 30%; OTA+ZEA, 15% samples) (Šegvić *et al*., 2009), as well as in Italian feed samples (AFs+FBs 100%) (Sabatini *et al*., 2007).

Reducing mycotoxins in the maize chain: processing to human food

Effect of food processing on mycotoxins

Although maize-based foods are not widely established in the Mediterranean diet, maize kernels used for human consumption are mainly processed to obtain maize flour, ground maize, canned sweet corn, corn snacks or corn flakes. Therefore, the most important industrial processes that can affect mycotoxins should be expected to be milling and extrusion. Industrial milling process of maize fractions affects mycotoxin concentration, showing a variable distribution depending on final distribution in the milled fractions. Commonly, a significant reduction of AFB_1, FB_1 and ZEA was observed in flour for human consumption and wide concentrations were found in animal feeds. The bran and the germ were the fractions with highest concentration levels, and processing to obtain polenta did not affect the mycotoxin levels (Brera *et al.*, 2004, Pietri *et al*., 2009). Corn flakes processing affected levels of AFs and FBs, reducing the final concentration (Castells *et al*. 2008). A study on the effect of four industrial brewing processes on AFB_1 and FB_1 concentrations showed clear reductions for both mycotoxins with transfer rates to bottled beer of 10.5 and 60.1 %, respectively (Pietri *et al*., 2010).

Occurrence of FBs in processed maize-food for human consumption

FBs are the mycotoxin group most surveyed in maize-based food for human consumption from

Table 7. Occurrence of aflatoxins and ochratoxin A in maize-based feeds from Mediterranean countries.

Toxin/ Location	Year[a]	No. samples	Positives (%)	Mean (positives) (μg kg^{-1})	Range (μg kg^{-1})	Feed/Reference
Aflatoxins[b]						
Croatia	2007	13	30.8	6.9	4.2–10.3	Feed Šegvić *et al.*, 2009
Egypt	1990	5	40	10	--	Broiler mixed feed Abdelhamid, 1990
France	2004–2005	1	100	10–30 AFB_1	5–30 AFB_1	Maize silage Garon *et al.*, 2006
France	2006	1	100	27.9 AFB_1	15.68–40.29 AFB_1	Maize silage Richard 2009
Italy	2005	45 45	100 n.a.	n.a. n.a.	0.03–7.76 0.94–6.41	Mixed rations Maize or maize fractions Sabatini *et al.*, 2007
Morocco	2007	21	66.6	0.84–1.26 AFB_1	0.05–5.38 AFB_1	Poultry feeds Zinedine *et al.*, 2007
Turkey	2002	52	71.11	n.a.	1.5–46.8	Layer feeds Nizamlýoðlu and Oguzz, 2003
Several countries[c]	2005–2009	66	15.2	0.3	0.5–4	Compound feeds Griessler *et al.*, 2010
Ochratoxin A						
Egypt	1990	3	33.3	13	--	Broiler mixed feed Abdelhamid, 1990
Croatia	2007	13	15.4	9.2	5.4–12.9	Feeds Šegvić *et al.*, 2009
France	2006	1	0	--	--	Maize silage Richard 2009
Italy	2005	45 45	0 0	-- --	-- --	Mixed rations Maize or maize fractions Sabatini *et al.*, 2007
Several countries[c]	2005–2009	17	35.3	7	2–54	Compound feeds Griessler *et al.*, 2010

[a] Year of sampling, if not available, publication year.
[b] All aflatoxin levels refer to the total values, except where specifically noted.
[c] Greece / Cyprus, Italy, Spain and Portugal.
n.a., Data not available.

Mediterranean country markets, and the main studies have been summarized in Table 9. Maize flour was the commodity most surveyed, but several regional foodstuffs were reported in Italy (polenta) and Portugal (maize bread).

FB_1 was found in 50% of maize samples from Morocco (n = 20), with mean and maximum levels of 1930 and 5960 μg kg^{-1}, respectively (Zinedine *et al.*, 2006). Moderate percentages of samples contaminated with FB_1 were reported in Italy, where percentages from 30 to 92% were found in maize-based food for human consumption (Doko and Visconti, 1994; Cirillo *et al.*, 2003; Faberi *et al.*, 2005). Differences between organic and conventional maize-based food-

Table 8. Occurrence of *Fusarium* toxins in maize-based feeds from Mediterranean countries.

Toxin/ Location	Year[a]	No. samples	Positives (%)	Mean (positives) (µg kg^{-1})	Range (µg kg^{-1})	Reference
Fumonisins[b]						
Croatia	2007	13	53.8	2300 $FB_1+B_2+B_3$	200–5000 $FB_1+B_2+B_3$	Feeds Šegvić *et al.*, 2009
Italy	2005	45 45	100 0	n.a. --	560–3970 --	Mixed rations Maize or maize fractions Sabatini *et al.*, 2007
Spain	1991–1992	66	0	--	--	Poultry feeds Castellá *et al.*, 1996
Spain	1994–1996	47 20 2	89.4 70 100	2400 3600 1300	400–11600 400–23600 1200–1400	Swine feeds Horse feeds Poultry feeds Castellá *et al.*, 1999
Tunisia	2005–2006	15	86.6	557.6	55–2800	Ruminant and poultry feeds Ghali *et al.*, 2009
Several countries[c]	2005–2009	15	40	622	100–3228	Griessler *et al.*, 2010
Zearalenone						
Croatia	2007	13	100	626.6	49.7–1168	Feeds Šegvić *et al.*, 2009
Egypt	1990	4	100	143	3–426	Broiler mixed feed Abdelhamid, 1990
France	2004–2005	9	100	n.a.	23–41	Maize silage Garon *et al.*, 2006
France	2006	1	0	--	--	Maize silage Richard *et al.*, 2009
Italy	2005	45 45	0 0	-- --	-- --	Mixed rations Maize or maize fractions Sabatini *et al.*, 2007
Several countries[c]	2005–2009	122	36.9	16	10–189	Compound feeds Griessler *et al.*, 2010
Deoxynivalenol						
Egypt	1990	4	50	820	680–960	Broiler mixed feed Abdelhamid, 1990
France	2004–2005	9	100	n.a.	5–25	Maize silage Garon *et al.*, 2006
France	2006	1	100	145.79	--	Maize silage Richard *et al.*, 2009
Italy	2005	45 45	0 0	-- --	-- --	Mixed rations Maize or maize fractions Sabatini *et al.*, 2007

(*Continued*)

Table 8. *Continues.*

Toxin/ Location	Year[a]	No. samples	Positives (%)	Mean (positives) (μg kg^{-1})	Range (μg kg^{-1})	Reference
Several countries[c]	2005–2009	145	72.4	290	52–3036	Compound feeds Griessler *et al*., 2010
Other mycotoxins						
France	2004–2005	1	100	n.a.	5–25	Citrinin Maize silage Garon *et al*., 2006
France	2006	1	100	11.91	--	Citrinin Maize silage Richard 2009
Several countries[c]	2005–2009	5	0	--	--	T-2 toxin Compound feeds Griessler *et al*., 2010

[a]Year of sampling, if not available, publication year.
[b]All fumonisins levels refer to FB_1+FB_2, except where specifically noted.
[c] Greece / Cyprus, Italy, Spain and Portugal.
n.d., Not detected.
n.a., Data not available.

stuffs were elucidated by Cirillo *et al*. (2003) in Italy and D'Arco *et al.* (2007) in Spain. Overall, the three studies concluded that lower levels of FBs may be expected in organic than conventional maize products. Maize flour, maize grits and polenta from Italy showed ranges of contamination with FB_1 of 420–3760 μg kg^{-1} and 80–910 μg kg^{-1} with FB_2 (Doko and Visconti 1994). In the same line, FB_1 was present in 54% samples of Egyptian maize meal, but levels were slightly lower, with values of 25.2 and 455.0 μg kg^{-1} for mean and maximum estimation levels, respectively (El-Sayed *et al*., 2003). In Spain, few samples of maize flour and maize grits were contaminated with FB_1, with mean levels between 60–362 μg kg^{-1} and maximum values from 70 to 937 μg kg^{-1} (Sanchis *et al*., 1994; Velluti *et al*., 2001). Corn snacks and corn flakes were the most common processed maize-based food analyzed but large surveys concerning FBs have not yet been published. The presence of FB_1 was low in corn snacks, with percentages of occurrence of 18.2–36% in Spain. Mean levels were from 46 to 130 μg kg^{-1} and maximum levels reached concentrations of 200 μg kg^{-1} (Sanchis *et al*., 1994; Velluti *et al.*, 2001). FB_1 was present in a low percentage of corn flakes samples from Spain (8–17%), but most commonly detected in breakfast cereals samples from France (87%) (Sanchis *et al*., 1994; Velluti *et al*., 2001; Molinié *et al*., 2005).

Occurrence of other mycotoxins in processed maize-food for human consumption

OTA was commonly found in maize bread from Portugal (60–80% of contaminated samples), where mean values ranged from 0.28 to 0.44 μg kg^{-1} (Juan *et al*., 2007, 2008b; Duarte *et al.*, 2009). A lower percentage of contaminated samples was found in breakfast cereals from France (41%) and Morocco (10%) with maximum values of 4.6 and 15.7 μg kg^{-1}, respectively (Molinié *et al*., 2005; Mahnine *et al*., 2011). Two organic maize-based food samples from Spain and Portugal were contaminated with OTA (n=5), with contamination between 0.80 and 1.90 μg kg^{-1} (Juan *et al*., 2008a).

AFB_1 and AFG_1 were found in several samples of corn flakes (14%) from Egypt, ranging between 5 and 35 μg kg^{-1} (El-Sayed *et al*., 2003), but not detected in corn-based food intended for human consumption from Spain (Sanchis *et al*., 1995b). Several studies reported the incidence of DON in maize-based commodities from this region. No contaminated samples of maize meal were found in Egypt (El-Sayed *et al*.,

Table 9. Occurrence of fumonisins in maize-based food from Mediterranean countries intended for human consumption.

Country	Commodity	Mycotoxin	Positives/total (%)	Range (μg kg^{-1}) (mean of positives)	Reference
Egypt	Maize meal	FBs	(54)	98–455 (25)	El-Sayed *et al.*, 2003
	Maize starch	FBs	2/7 (29)	29–134 (82)	El-Sayed *et al.*, 2003
	Pop corn	FBs	0	0	El-Sayed *et al.*, 2003
France	Maize flour imported to UK	FBs	--	10–1993 (456)	Scudamore and Patel, 2009
	Breakfast cereals	FBs	28/32 (87)	7–1113 (79)	Molinié *et al.*, 2005
Italy	Maize grits, maize flour, polenta	FB_1	--	420–3760	Doko and Visconti, 1994
		FB_2	--	80–910	Doko and Visconti, 1994
	Polenta	FB_1	2/2	140 (135)	JECFA, 2001
	Sweet corn	FB_1	--	420–790	Doko and Visconti, 1994
		FB_2	0	0	Doko and Visconti, 1994
	Corn flakes	FB_1	--	10	Doko and Visconti, 1994
	Pop corn	FB_1	--	60	Doko and Visconti, 1994
		FB_2	--	20	Doko and Visconti, 1994
	Tortilla chips	FB_1	--	60	Doko and Visconti, 1994
		FB_2	--	10	Doko and Visconti, 1994
	Maize based products	FB_1	24/26 (92)	7–2400 (21–1500)	Faberi *et al.*, 2005
		FB_2	18/26 (69)	n.d. –260 (13–180)	Faberi *et al.*, 2005
	Conventional food	FB_1	16/54 (30)	27–2160 (345)	Cirillo *et al.*, 2003
		FB_2	12/54 (22)	10–400 (20)	Cirillo *et al.*, 2003
	Organic food	FB_1	24/54 (44)	10–600 (185)	Cirillo *et al.*, 2003
		FB_2	17/54 (32)	30–150 (120)	Cirillo *et al.*, 2003
Morocco	Maize food	FB_1	10/20 (50)	n.d.–5960 (1930)	Zinedine *et al.*, 2006
Portugal	Maize meal	FB_1	41/41 (100)	50–1300 (474)	Martins *et al.*, 2008
		FB_2	29/41 (71)	100–450 (180)	Martins *et al.*, 2008
	Maize flour	FBs	2/3 (67)	n.d.–2026 (995)	Lino *et al.*, 2006
	Maize semolina	FBs	2/3 (67)	n.d.–183 (118)	Lino *et al.*, 2006
	Yellow maize	FBs	6/9 (67)	n.d.–1061 (421)	Lino *et al.*, 2006
	White maize	FBs	2/2 (100)	113–1162 (638)	Lino *et al.*, 2006
	Maize bread	FB_1	24 (80)	n.d.–448 (197)	Lino *et al.*, 2006
		FB_2	25 (83)	n.d.–207 (77)	Lino *et al.*, 2006
	Sweet corn	FB_1	36 (73)	50–400 (154)	Martins *et al.*, 2008
		FB_2	29 (71)	100–450 (180)	Martins *et al.*, 2008
	Corn flakes	FB_1	0	0	Martins *et al.*, 2008

(*Continued*)

Table 9. *Continues.*

Country	Commodity	Mycotoxin	Positives/total (%)	Range ($\mu g\ kg^{-1}$) (mean of positives)	Reference
Spain	Maize flour	FB_1	1/3 (33)	n.d.–70 (70)	Sanchis *et al.*,1982
		FB_1	8/24 (33)	n.d.–937 (362)	Velluti *et al.*, 2001
	Conventional maize-based food	FB_1	12/73 (16)	4–81 (30)	D'Arco *et al.*, 2008
		FB_2	--	3–53 (23)	D'Arco *et al.*, 2008
	Organic maize-based food	FB_1	7/9 (78)	2–235 (51)	D'Arco *et al.*, 2008
		FB_2	--	4–70 (35)	D'Arco *et al.*, 2008
	Corn snacks	FB_1	2/11 (18)	n.d.–200 (130)	Sanchis *et al.*, 1994
			14/39 (36)	n.d.–136 (46)	Velluti *et al.*, 2001
	Corn flakes	FB_1	2/12 (17)	n.d.–100 (60)	Sanchis *et al.*, 1994
			3/39 (8)	n.d.–18 (17)	Velluti *et al.*, 2001
	Sweet corn	FB_1	11/39 (28)	n.d.–119 (56)	Velluti *et al.*, 2001
	Pop corn	FB_1	12/51 (24)	n.d.–211 (48)	Velluti *et al.*, 2001
	Beer	FB_1	14/32 (44)	4.76–86	Torres *et al.*, 1998
Turkey	Corn Snacks	FB_1	2/15 (13)	350–370	Omurtag *et al.*, 2001

n.d., Not detected.

2003), neither in corn snacks from Turkey (Omurtag and Beyoglu, 2003), however 57% and 9% of Turkish flour and dried maize samples, respectively, were positive. The range of contamination of flour was 140–2670 $\mu g\ kg^{-1}$. DON was present in several samples of corn snacks and corn flakes from Spain; percentages of occurrence ranged from 20 to 79% for corn snacks with means from 11 to 109 $\mu g\ kg^{-1}$; while a range of 40–75% was found in breakfast cereals or corn flakes, the mean values being among 109 and 154 $\mu g\ kg^{-1}$ (Cerveró *et al*., 2007; Castillo *et al*., 2008; Cano-Sancho *et al*., 2011).

Other *Fusarium* mycotoxins have been poorly studied in maize-based commodities. T-2 toxin was found only in 3 samples out of 208 analyzed composites from Spanish market, mainly sweet-corn and corn snacks with a range of contamination of 70–256 $\mu g\ kg^{-1}$ (Cano-Sancho *et al*., 2011) and it was not detected in maize based food from Egypt (El-Sayed *et al*., 2003). NIV was also found in few samples of breakfast cereals (11%) and only one sample of corn snacks (n=120). The maximum values ranged between 56 and 121 $\mu g\ kg^{-1}$ (Castillo *et al*., 2008).

Recent studies have reported the presence of several emerging mycotoxins such as BEA, ENNs or FUS in maize based food for human consumption from Morocco and Spain. ENNs were present in 41% of corn flakes samples from Rabat (Morocco) with values of ENNA1 and ENNB1 up to 423.6 and 20 $\mu g\ g^{-1}$, respectively (Mahnine *et al*., 2011). These mycotoxins were also widely present in maize from Spain (89%), ENNA1 being the most important toxin with mean and maximum values of 168 and 813 $\mu g\ kg^{-1}$, respectively. BEA was present in 21% of Spanish maize samples (mean of 5.72 $\mu g\ g^{-1}$) and FUS was only found in one sample (2.47 $\mu g\ kg^{-1}$) (Meca *et al*., 2010).

As a conclusion, mycotoxins, mainly *Fusarium* mycotoxins are widely present in maize produced and consumed in the Mediterranean basin. Several strategies exist at the field level to reduce such contamination, although some contamination is unavoidable. Processing of maize to food products certainly reduces the initial mycotoxin presence in the raw maize, leading to final concentrations in food which lead to mycotoxin intakes which are safe for the general population, the infants and individuals with ethnic dietary patterns (based on maize) being the most exposed population groups (Cano-Sancho *et al*., 2011). Further research on field strategies as well as an in-depth knowledge of the food processing effects, other than milling, on mycotoxins would be required.

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