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Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe

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Abstract

Several *Fusarium* species occurring worldwide on maize as causal agents of ear rot, are capable of producing mycotoxins in infected kernels, some of which have a notable impact on human and animal health. The main groups of *Fusarium* toxins commonly found are: trichothecenes, zearalenones, fumonisins, and moniliformin. In addition, beauvericin and fusaproliferin have been found in *Fusarium*-infected maize ears. Zearalenone and deoxynivalenol are commonly found in maize red ear rot, which is essentially caused by species of the *Discolour* section, particularly *F. graminearum*. Moreover, nivalenol and fusarenone-X were often found associated with the occasional occurrence of *F. cerealis*, and diacetoxyscirpenol and T-2 toxin with the occurrence of *F. poae* and *F. sporotrichioides*, respectively. In addition, the occurrence of *F. avenaceum* and *F. subglutinans* usually led to the accumulation of moniliformin. In maize pink ear rot, which is mainly caused by *F. verticillioides*, there is increasing evidence of the wide occurrence of fumonisin B_1. This carcinogenic toxin is usually found in association with moniliformin, beauvericin, and fusaproliferin, both in central Europe due to the co-occurrence of *F. subglutinans*, and in southern Europe where the spread of *F. verticillioides* is reinforced by the widespread presence of *F. proliferatum* capable of producing fumonisin B_1, moniliformin, beauvericin, and fusaproliferin.

Abbreviations: AcDON – Mono-acetyldeoxynivalenols (3-AcDON, 15-AcDON); AcNIV – Mono-acetylnivalenol (15-AcNIV); BEA – Beauvericin; DiAcDON – Di-acetyldeoxynivalenol (3,15-AcDON); DAcNIV – Diacetylnivalenol (4,15-AcNIV); DAS – Diacetoxyscirpenol; DON – Deoxynivalenol (Vomitoxin); FB_1 – Fumonisin B_1; FB_2 – Fumonisin B_2; FB_3 – Fumonisin B_3; FUP – Fusaproliferin; FUS – Fusarenone-X (4-Acetyl-NIV); FUC – Fusarochromanone; HT2 – HT-2 toxin; MAS – Monoacetoxyscirpenol; MON – Moniliformin; NEO – Neosolaniol; NIV – Nivalenol; T2 – T-2 toxin; ZEN – Zearalenone; ZOH – zearalenols (α and β isomers).

Introduction

Several *Fusarium* species are widespread pathogens on maize in temperate and semi-tropical areas, including all European maize-growing areas. They cause root, stem and ear rot, with severe reductions in crop yield, often estimated at between 10% and 30%. In addition, certain strains are capable of producing mycotoxins which can be formed in pre-harvest infected plants, or in stored grains. The phase of maize fusariosis with the greatest mycotoxicological concern is the ear rot, but the formation of mycotoxins in rotted stalks (notably ZEN, ZOH and DON) (Bottalico et al., 1985; Lew et al., 1997), infected leaves (NIV) (Lew et al., 1997), and in whole plants (ZEN) (Oldenburg, 1993) could represent a significant risk for forage and silo maize. The occurrence of mycotoxins in maize kernels is of great concern worldwide, because their presence

in feeds and foods is often associated with chronic or acute mycotoxicoses in livestock and, to a lesser extent, in humans. It has been estimated that 25% of the world food crops is affected by mycotoxins (Charmley et al., 1995), but for some *Fusarium* toxins in maize, such as DON and FB_1, it is likely that this percentage is even higher (Eriksen and Alexander, 1998; Bullerman, 1996).

In this review, the most relevant aspects of the distribution of toxigenic *Fusarium* species and related mycotoxins in ear rot of maize in European countries are reviewed, with emphasis on the occurrence of trichothecenes, zearalenone, and fumonisins in infected plants standing in the field. Moreover, the relationship between the ecological distribution of *Fusarium* species and the relative mycotoxin profile is also stressed, because this may be useful for predicting the mycotoxins that are most likely to be formed in maize and those which may be affected by conducive factors such as tillage practices, host genotypes and environmental conditions.

Fusarium species involved and mycotoxins produced

The species of *Fusarium* causing maize ear rot are worldwide in distribution and are characterized by the co-occurrence or by the quick succession of different species. It is common to isolate up to nine different *Fusarium* species from a single fragment of infected tissue or kernel. Together with the restricted number of toxigenic species which are regarded as pathogenic, there are also several less pathogenic or opportunistic *Fusarium* species capable of producing considerable amounts of toxins. The toxigenic profile of a contaminated crop is therefore determined not only by the predominant pathogenic species, but also by the opportunistic species (Bottalico, 1997).

Fusarium species are responsible for at least two kinds of maize ear rot, roughly differentiated as red ear rot or red fusariosis, mainly caused by species of the *Discolour* section, and pink ear rot or pink fusariosis mainly caused by representatives of the *Liseola* section (Shurtleff, 1980; Chelkowski, 1989). The predominant species causing maize red ear rot are *F. graminearum* (teleomorph *Gibberella zeae*), *F. culmorum*, *F. cerealis* (syn. *F. crookwellense*), and *F. avenaceum* (teleomorph *G. avenacea*). The species frequently isolated from maize pink ear rot are essentially the widespread anamorphs of the rather rare *Gibberella fujikuroi*, namely, *F. verticillioides* (syn. *F. moniliforme*), *F. proliferatum*, and *F. subglutinans*. Among the other toxigenic *Fusarium* species less frequently isolated from both types of maize ear rot are *F. equiseti* (teleomorph *G. intricans*), *F. poae*, *F. sporotrichioides*, *F. acuminatum* (teleomorph *G. acuminata*), *F. semitectum* (syn. *F. pallidoroseum*, *F. incarnatum*), *F. solani* (teleomorph *Nectria haematococca*), and *F. oxysporum*. Finally, there are other species which are only sporadically isolated from maize, such as *F. anthophylum*, *F. chlamydosporum* (syn. *F. fusarioides*), *F. compactum*, *F. heterosporum* (syn. *F. reticulatum*, *F. graminum*), *F. lateritium*, *F. sambucinum*, *F. torulosum*, and *F. venenatum*. It is worth stressing that the complex species *G. fujikuroi* has been subdivided into seven distinct mating populations (biological species), indicated from A to G, and covering ten *Fusarium* anamorphs (Leslie, 1995). Those most frequently found on maize were *F. verticillioides* (A), *F. proliferatum* (D), and *F. subglutinans* (E), which can also be differentiated by their toxigenic capabilities (Moretti et al., 1997).

Mycotoxins produced

Fusarium species on maize can produce many mycotoxins, some of which are of notable importance. The naturally occurring *Fusarium* mycotoxins belong to the trichothecenes, zearalenones, and fumonisins. Moreover, MON, BEA and FUP have also been found in naturally infected maize kernels and are considered as emerging toxicological problems. The mycotoxins produced by *Fusarium* species from cereals are illustrated in Table 1.

Trichothecenes

Of several trichothecene derivatives produced by *Fusarium* species, only a few have been encountered as natural contaminants of maize products. The *Fusarium* trichothecenes have been divided into type A-trichothecenes, characterized by a functional group other than a ketone at C-8, and type B-trichothecenes with only the carbonyl at C-8. The type A-trichothecenes include: (a) T2 and HT2, mainly produced by strains of *F. sporotrichioides*, *F. acuminatum*, and *F. poae*; (b) DAS, and MAS, chiefly produced by strains of *F. poae*, *F. equiseti*,

Table 1. Mycotoxigenic *Fusarium* species associated with cereals and their mycotoxins

Fusarium species[a]	Mycotoxins[b]
F. acuminatum	**T2**, **MON**, HT2, DAS, MAS, NEO, BEA
F. anthophilum	BEA
F. avenaceum	**MON, BEA**
F. cerealis	**NIV**, **FUS**, **ZEN**, ZOH
F. chlamydosporum	**MON**
F. culmorum	**DON**, **ZEN**, **NIV**, FUS, ZOH, AcDON
F. equiseti	**ZEN**, **ZOH**, MAS, DAS, NIV, DAcNIV, FUS, FUC, BEA
F. graminearum	**DON**, **ZEN**, **NIV**, **FUS**, **AcDON**, DAcDON, DAcNIV
F. heterosporum	**ZEN**, ZOH
F. nygamai	BEA, FB_1, FB_2
F. oxysporum	**MON**, BEA
F. poae	**DAS**, **NIV**, **FUS**, MAS, T2, HT2, NEO, BEA
F. proliferatum	**FB_1**, **BEA**, **MON**, **FUP**, FB_2,
F. sambucinum	**DAS**, T2, NEO, ZEN, MAS, BEA
F. semitectum	**ZEN**, BEA
F. sporotrichioides	**T2**, **HT2**, **NEO**, MAS, DAS
F. subglutinans	**BEA**, **MON**, FUP
F. tricinctum	**MON**, BEA
F. verticillioides	**FB_1**, FB_2, FB_3

[a] *Fusarium* nomenclature according to Nelson et al. (1983). [b] Bold letters indicate the important mycotoxins. *Abbreviations*: AcDON – Mono-acetyldeoxynivalenols (3-AcDON, 15-AcDON); AcNIV – Mono-acetylnivalenol (15-AcNIV); BEA – Beauvericin; DiAcDON – Di-acetyldeoxynivalenol (3,15-AcDON); DAcNIV – Diacetylnivalenol (4,15-AcNIV); DAS – Diacetoxyscirpenol; DON – Deoxynivalenol (Vomitoxin); FB_1 – Fumonisin B_1; FB_2 – Fumonisin B_2; FB_3 – Fumonisin B_3; FUP – Fusaproliferin; FUS – Fusarenone-X (4-Acetyl-NIV); FUC – Fusarochromanone; HT2 – HT-2 toxin; MAS – Monoacetoxyscirpenol; MON – Moniliformin; NEO – Neosolaniol; NIV – Nivalenol; T2 – T-2 toxin; ZEN – Zearalenone; ZOH – zearalenols (α and β isomers).

F. sambucinum, and *F. sporotrichioides*; and (c) NEO, mainly produced by strains of *F. sporotrichioides*, *F. poae*, and *F. acuminatum.* The type B-trichothecenes include: (a) DON, and its derivatives mono- (3- and 15-AcDON), and diacetylated (3,15-AcDON) derivatives, produced by strains of *F. graminearum* and *F. culmorum*; and (b) NIV and FUS, and diacetylated derivatives (4,15-AcNIV), produced by strains of *F. cerealis*, *F. poae*, *F. graminearum* and *F. culmorum*. Trichothecenes cause a variety of toxic effects in laboratory and farm animals, including skin inflammation, digestive disorders, haemorrhages in several internal organs, haemolytic disorders and depletion of the bone marrow, impairment of both humoral and cellular immune responses, and nervous disorders (Rizzo et al., 1992; IARC, 1993; Rotter and Prelusky, 1996). Trichothecenes are commonly associated with several mycotoxicoses in livestock, including haemorrhagic and emetic syndromes; and have also been implicated in human toxicoses (Bhat et al., 1989; IARC, 1993; Wang et al., 1993; Beardall and Miller, 1994; Ehling et al., 1997).

Zearalenones

Zearalenone (ZEN) is mainly produced by *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti*, and *F. semitectum*, often associated with ZOH (α- and β-zearalenol isomers). ZEN is among the most widely distributed *Fusarium* mycotoxin in agricultural commodities, and has very often been encountered, even at very high concentrations, in maize. ZEN is an uterotrophic and estrogenic compound responsible for recurring toxicoses in livestock, characterized by hyper-estrogenism in swine, and infertility and poor performance in cattle and poultry (Kuiper-Goodman et al., 1987; IARC, 1993).

Fumonisins

Fumonisins were first isolated from *F. verticillioides*, and then found in cultures of *F. proliferatum* and in a few other less ecologically important *Fusarium* species (Nelson et al., 1992). Four series of fumonisins have been described, and named A, B, C, and P. The

B series includes the most active fumonisins, particularly FB_1 which is causing great mycotoxicological concern (Hopmans and Murphy, 1995; Musser et al., 1996; Abbas et al., 1998). Fumonisin B_1 occurs naturally at biologically significant levels in maize and in a variety of maize-based human foodstuffs and animal feeds worldwide (Rheeder et al., 1992; Sanchis et al., 1995; Logrieco et al., 1995; Shephard et al., 1996). Feeds contaminated with FB_1 are known to cause leukoencephalomalacia in horses (ELEM), pulmonary oedema and hepatic syndrome in swine (PPE), poor performance in poultry, and alteration in hepatic and immune function in cattle (Ross et al., 1990; Kellerman et al., 1990; Harrison et al., 1990; Osweiler, 1995). The first case of ELEM was reported in north Italy associated with the presence of FB_1 in maize-based feed (Caramelli et al., 1993). Moreover, home-grown corn contaminated with FB_1 has been statistically associated with high rates of human oesophageal cancer (HEC) in areas of southern Africa, China, and the southeastern U.S.A. (Marasas, 1995). A similar claim has been made for northeastern Italian areas (Franceschi et al., 1990), where FB_1 was found in maize flour (polenta) in concentrations up to 3.76 mg kg^{-1} (Pascale et al., 1995). The evidence that cultures of *F. verticillioides* and samples of FB_1 are capable of promoting liver cancer in rats (Gelderblom et al., 1996), together with other observations obtained from several genotoxic tests, led IARC (1993) to classify the toxins of *F. verticillioides* as carcinogenic to animals and possibly as carcinogenic to humans (Group 2B).

Moniliformin

Moniliformin has been purified from cultures of several *Fusarium* species, including: *F. subglutinans*, *F. proliferatum*, *F. avenaceum*, *F. tricinctum* and several others of less ecological importance (Marasas et al., 1986; Chelkowski et al., 1990; Schütt et al., 1998). Diets containing culture material naturally contaminated with MON, or amended with purified MON, were responsible for reduced performance, haematological disorders, myocardial hypertrophy, and mortality in rodents, chicks, ducklings, and pigs (Ledoux et al., 1995; Harvey et al., 1997).

Beauvericin

Beauvericin is a well known cyclic hexadepsipeptide, first reported to be produced by some entomopathogenic fungi (*Beauveria* spp.) and then found in cultures of strains of *F. semitectum*, *F. subglutinans* and *F. proliferatum*, isolated from maize and maize-based feed for swine (Plattner and Nelson, 1994; Moretti et al., 1994; 1995). In addition, BEA was found in cultures of several other *Fusarium* species, including *F. avenaceum*, *F. acuminatum*, *F. equiseti*, *F. poae* and *F. oxysporum* (Logrieco, 2000). BEA was detected for the first time in maize ear rot in Poland (Logrieco et al., 1993), and then found as a toxic contaminant of maize in Italy (Bottalico et al., 1995; Logrieco et al., 1995). Besides its high toxicity to insects (Gupta et al., 1991), BEA is also cytotoxic to mammalian cell tissues, and was reported to cause apoptosis in both murine and human cell lines (Macchia et al., 1995). Moreover, BEA showed toxic effects on the contractility of guinea pig smooth muscle (Krska et al., 1997).

Fusaproliferin

Fusaproliferin is a novel sesterterpene first purified from a culture of *F. proliferatum* from maize ear rot in northern Italy (Ritieni et al., 1995), and then found both in cultures of several strains of *F. proliferatum* and *F. subglutinans* (Moretti et al., 1997), and in naturally infected maize (Ritieni et al., 1997a). Investigations on the toxicity of FUP indicated that this toxin is lethal to larvae of *Artemia salina*, and cytotoxic to the SF-9 insect cell line and to IARC/CL 171 human B lymphocyte culture (Logrieco et al., 1996). Moreover, high mortality in broiler chicks fed with maize cultures of *F. proliferatum* was reported by Javed et al. (1993) and Ramakrishnan and Wu (1994), and severe teratogenic effects were observed in chick embryo bioassays by Ritieni et al. (1997b).

Ear rot of maize in Europe

Fusarium *species in ear rot of maize*

The relative incidence of *Fusarium* species and related mycotoxins found in maize ear rot diseases in Europe are tentatively grouped into red fusariosis and pink fusariosis, and summarized in Table 2. The distribution and the prevalence of different *Fusarium* species causing the two kinds of ear rot disease are largely governed by environmental conditions, primarily temperature, as well as by many other factors including agro-technical practices (Arino and Bullermann, 1994). In general, red

Table 2. Toxigenic *Fusarium* species and associated mycotoxins from maize ear rot in Europe

Species[a]	Incidence		Mycotoxin found[b]
	North/Centre	South	
Red ear rot or Red fusariosis			
F. graminearum	+++	+	**DON**, AcDON, **NIV**, FUS, **ZEN**
F. subglutinans	++	±	**MON**, BEA, FUP
F. avenaceum	++	±	**MON**
F. cerealis	+	±	**NIV**, FUS, **ZEN**, ZOH
F. culmorum	+	−	**DON**, NIV, **ZEN**, ZOH
F. sporotrichioides	+	−	**T2**, **HT2**, NOS
F. poae	+	−	**DAS**, **NIV**
F. equiseti	+	±	DAS, ZEN, ZOH
F. acuminatum	+	±	T2, NEO
F. verticillioides	+	+	—
F. proliferatum	+	+	—
Pink ear rot or Pink fusariosis			
F. verticillioides	+	+++	**FB_1**, **FB_2**, FB_3
F. proliferatum	±	+++	**FB_1**, FB_2, **FUP**, **MON**, **BEA**
F. subglutinans	+++	+	**MON**, **BEA**, FUP
F. graminearum	+	±	—
F. culmorum	+	±	—
F. equiseti	+	±	—
F. solani	±	+	—
F. semitectum	±	+	—
F. cerealis	±	±	—
F. sporotrichioides	±	−	—
F. oxysporum	−	+	—

For footnotes refer Table 1.

fusariosis is particularly severe in years and locations characterized by frequent rainfall and low temperatures during the summer and early fall (Ellend et al., 1997; Bočarov-Stančić et al., 1997), while pink fusariosis prevails in drier and warmer climates of southern areas (Bottalico et al., 1986; 1995; Bottalico and Logrieco, 1988). In the last ten years, the most frequently reported dominant species was *F. verticillioides* associated with *F. subglutinans*, and followed only to a slightly lesser extent by *F. graminearum*. Furthermore, the emerging trend of *F. proliferatum* moving from southern to northern maize-growing areas is well documented (Bottalico, 1997; 1998).

Maize red ear rot

In maize red ear rot, the pathogen, notably *F. graminearum*, usually starts from the tip of the ear and develops a reddish mould covering the ear extensively. The brownish perithecia of the teleomorph *G. zeae* are commonly observed on the infected husks and ear shanks. A comprehensive evaluation of reports from Austria (Krska et al., 1996; 1997; Lew et al., 1991), Slovenia (Milevoj, 1997), Yugoslavia (Bočarov-Stančić et al., 1997; Lević et al., 1997), Poland (Chelkowski, 1989; Lew et al., 1996); Czech Republic (Nedelnik, 2000), and România (Nagy and Bâgiu, 2000), clearly indicates that *F. graminearum* is increasingly distributed from central to northern European areas, and is occasionally associated with many additional *Fusarium* species, the occurrence and prevalence of which changes from region to region, and year to year, depending mainly on the climatic conditions (temperature and rain) and tillage practices (crop rotation, fertilization and planting area). In this context a very relevant role is played by host genotypes (maturity class, *Fusarium*-susceptibility) (Doko et al., 1985; Visconti, 1996). There are some other *Fusarium* species associated with *F. graminearum*, namely *F. subglutinans*, which predominates over *F. graminearum* in some Austrian locations (Lew et al., 1991), *F. culmorum* and *F. cerealis*, which were found to be more common in central European areas, and *F. avenaceum,* the relative presence of which seems to increase from central to northern European areas. Besides the more representative species, including *F. graminearum*, *F. subglutinans*, *F. cerealis*, *F. culmorum* and *F. avenaceum*, which represent almost

90–95% of all species isolated, *F. sporotrichioides*, *F. poae*, *F. equiseti*, and *F. acuminatum*, and to a lesser extent *F. verticillioides* and *F. proliferatum*, were also isolated.

The *Fusarium* species profile, and consequently mycotoxin accumulation can change drastically with insect damage. Lew et al. (1991) noted a striking influence of European corn borer injuries on the *Fusarium* species profile on maize in Austria in 1988–89. On maize ears not damaged by the corn-borer, *F. graminearum* and *F. subglutinans* were evenly represented (54.5% and 48.7%, respectively), while on ears damaged by the corn-borers there were many more species belonging to the *Liseola* section (*F. subglutinans*, *F. verticillioides*) (up to 80%) than to the *Discolor* section (*F. graminearum*, *F. culmorum*, *F. cerealis*) (less than 15%). Thus, control of the European corn-borer can lead to a reduction of MON and FB_1 produced by *Liseola* representatives, but does not affect the occurrence of ZEN, DON and NIV which are produced by *Fusarium* species belonging to the *Discolor* section.

Maize pink ear rot

In maize pink ear rot, the pathogens colonize the ear from the tip by external infections, but the kernels may also be infected through the silk at the silk stage to make ear colonization complex. In fact, the most important pathway for *F. verticillioides* to achieve seed-borne kernel infection is through silk infections at the silk stage (Munkwold et al., 1997). Thus, in association with a more generalized colonization originating from airborne inoculum colonizing the ears from the tip, a random kernel rot phase of the disease can also occur, appearing as randomly scattered individuals or groups of kernels, usually tan to brown, which develop pink mycelium under wet conditions. In this case, the risk of mycotoxin (fumonisin) accumulation in infected kernels may begin early in maize ear development and increases as the kernels reach physiological maturity (Warfield and Gilchrist, 1999). Maize pink ear rot is commonly observed from southern to central European areas, and the species most frequently isolated is *F. verticillioides*, associated with *F. subglutinans* and to a lesser extent with *F. proliferatum*. *Fusarium proliferatum* is more common in southern European areas, but it is displaced by *F. subglutinans* in central areas where the latter predominates as the maize ear rot agent and is usually isolated in much higher amounts than *F. verticillioides*. *Fusarium proliferatum* was commonly reported together with *F. verticillioides* in Italy (Logrieco et al., 1995), but in Austria (Krüger, 1989; Krska et al., 1997) Croatia (Jurjević et al., 1997), Slovak Republic (Piecková and Jesenská, 1997; Srobárová, 1997), Hungary (Szécsi, 1994), and especially in Poland (Kostechi et al., 1995) the occurrence of *F. proliferatum* was rarely recorded. However, it seems that unusually drier and warmer summers, like those prevailing in the 1990s led to an increase of *F. proliferatum* in central Europe, as reported both for the Slovak Republic in 1996 (Srobárová et al., 2000), and in Austria where the overall number of *F. proliferatum* infections rose from less than 1% in the 1980s to 2–11% towards the end of the 1990s, leading to an expected increase of fumonisin in contaminated samples (Adler et al., 2001). Associated with *F. verticillioides*, *F. subglutinans* and *F. proliferatum*, many other species were commonly isolated from maize pink ear rot. The species incidence reported in Yugoslavia by Lević et al. (1997) during a three-year survey (1994–96) of freshly harvested maize ears, included *F. verticillioides* (63%), *F. subglutinans* (51%), *F. graminearum* (12%), *F. proliferatum* (10%), *F. oxysporum* (6%), and *F. solani* (2%). Among the other less frequently isolated species were *F. equiseti*, *F. sporotrichioides*, *F. chlamydosporum*, *F. cerealis*, and *F. semitectum*. In Italy, where environmental conditions are often conducive to a high incidence of maize pink ear rot, *F. verticillioides* predominates (in almost 100% of the infected kernels), with a profuse co-occurrence of *F. proliferatum* in southern areas (in over 60% of the infected kernels), then decreasing from central to northern areas (in about 54% and 34% of kernels) (Logrieco et al., 1995; Ritieni et al., 1997a). The higher occurrence of *F. proliferatum* in Italy has focussed attention on this species, which is usually confused with other closely related species of the *Liseola* section, and led to a more correct evaluation of its pathogenic and toxigenic capabilities (Logrieco et al., 1995). A spreading trend of *F. proliferatum* similar to that observed in Italy, was reported for nearby European countries (Lević et al., 1997; Srobárová, 1997).

Occurrence of zearalenone and deoxynivalenol

Epidemics of maize red ear rot, induced by *F. graminearum* and *F. culmorum* usually lead to the

occurrence of ZEN, DON, and 3AcDON, as reported in Poland (Chelkowski, 1989; Grabarkiewicz-Szczesna et al., 1996), and Yugoslavia (Bočarov-Stančić et al., 1997). In relation to the severity of the disease, it is possible to find unexpectedly high concentrations of toxins. In maize ear samples collected in 1988–89 in Austria, mostly infected by *F. graminearum*, *F. culmorum* and *F. cerealis*, Lew et al. (1991) found very high amounts of ZEN (40 mg kg^{-1}), DON (500 mg kg^{-1}) and NIV (10 mg kg^{-1}). More recently, a survey of 85 freshly harvested maize ear samples, collected in 1996 in the eastern part of Austria, mostly contaminated by *F. graminearum*, contained DON in 95% of samples, 15-AcDON in 54%, 3-AcDON in 3.5% and ZEN in 70% (Ellend et al., 1997). A similar situation, but with much greater amounts of DON, 15-AcDON, 3-AcDON, and ZEN, was found for samples of maize ears infected by *F. graminearum*, collected from 1988 to 1991 in Poland (Visconti et al., 1990; Grabarkiewicz-Szczesna et al., 1996). Additional observations carried out in Poland on the distribution of mycotoxins in maize ears infected by *F. graminearum*, revealed that the levels of DON, ZEN, 3-AcDON, and 15-AcDON in whole ears were not evenly distributed, but were greater in the axial stems than in the kernels (Perkowski et al., 1991). In a survey carried out in 1977 in southern Italy (Metapontum), ZEN was found in *F. graminearum*-infected ears of maize sown as a second crop and harvested at the end of November (Bottalico, 1979), while high concentrations of DON were found in almost all the selected *F. graminearum*-infected maize ear samples (95%) collected in 1987 in northern Italy (Lombardy) (Bottalico et al., 1989).

Occurrence of nivalenol and fusarenone

Nivalenol and fusarenone have often been reported in maize red ear rot all over the European maize growing areas. Their formation in infected ears may be due to NIV-chemotypes of *F. graminearum*, especially when found together with DON and ZEN, and in the absence or scant presence of *F. cerealis*, as reported for Romania (Moldavia) (Ciudin and Bazgan, 1991), Italy (Logrieco et al., 1992), and Hungary (Szécsi and Bartok, 1995). But, the occurrence of NIV and FUS in European areas appeared to be related more to the spread of *F. cerealis* than that of *F. graminearum*. In fact, strains of *F. cerealis* from red ear rot of maize were essentially able to produce NIV and FUS associated with ZEN, but not DON (Sydenham et al., 1991), and this capability was confirmed for several strains collected from Finland, Germany, Yugoslavia, Italy, Austria and Poland (Golinski et al., 1988; Bottalico et al., 1990). In 1988–89 surveys of Austrian *Fusarium*-infected ears, in spite of the wide presence of *F. graminearum*, Lew et al. (1991) ascribed the occurrence of NIV to the presence of *F. cerealis*. Moreover, in the 1988 Polish survey of maize red ear rot, almost all samples predominantly colonized by *F. cerealis* were found to be highly contaminated by NIV and FUS (Visconti et al., 1990). Other Polish surveys (1990–91) of maize ear rot highly infected with *F. graminearum* and *F. cerealis* found DON, 15-AcDON and ZEN in ears mostly colonized by *F. graminearum*, while high concentrations of NIV, FUS and ZEN were found in ears predominantly infected by *F. cerealis* (Grabarkiewicz-Szczesna et al., 1996). Finally, in Polish samples of maize ears affected by pink ear rot caused by *F. poae,* NIV was found, associated with FUS, both in grains and in cobs (Chelkowski et al., 1994a).

Occurrence of T-2 and HT-2 toxins

Epidemics of *F. sporotrichioides* usually lead to the accumulation of T-2 derivatives. Polish surveys of maize ears in 1984–85 showed *F. sporotrichioides* as the predominant fungus in approximately 2% of the samples. In hand-selected heavily damaged kernels, up to 1715 mg kg^{-1} of total type A trichothecenes were found. These included T2, and HT2 (992 and 642 mg kg^{-1}, respectively), and lesser amounts of NOS, T-2 triol and T-2 tetraol (Chelkowski et al., 1987; 1989).

Occurrence of mono- and di-acetoxyscirpenol

Sporadic epidemics of *F. poae* can be responsible for the accumulation, in infected ears, of DAS and MAS, usually associated with NIV and FUS. In maize samples from Austria, NIV and FUS, and DON and ZEN, all probably produced by a NIV-chemotype of *F. graminearum* and *F. culmorum*, were found together with DAS presumably formed by *F. poae* and *F. cerealis* (Bottalico et al., 1983). In Poland, where *F. poae* is a widespread agent of maize pink ear rot, DAS, and MAS, together with NIV and FUS, were produced significantly by almost all the strains (11/14) collected from different locations (Chelkowski et al., 1994a).

Occurrence of fumonisins

There is increasing evidence of the occurrence of FB_1 in maize and maize-based food and feed all over the world (WHO, 2000), including Europe (Sanchis et al., 1994; Pestka et al., 1994; Doko and Vizconti, 1993; Visconti et al., 1996; Patel et al., 1997). Investigations carried out in Italy revealed that *F. verticillioides* was the most frequently isolated fungus from infected maize plants and from commercial maize kernels associated with FB_1 at levels of up to 5.31 mg kg^{-1} (Pietri et al., 1995). In general, the occurrence of FB_1 appears to be important in southern European areas, that is Portugal, France (Le Bars and Le Bars, 1995; Dragoni et al., 1996), Spain (Rapior et al., 1993), Croatia (Jurjević et al., 1999), and Italy (Doko and Visconti, 1994; Visconti et al., 1996). Fumonisins are primarily formed in plants infected with *F. verticillioides* and *F. proliferatum*, when they are frequently found in pre-harvested maize ears. In Italy, high levels of fumonisins were often found (250 mg kg^{-1}, in 6/6 selected samples) in association with more severe pink ear rot than that reported for other European countries, (Bottalico et al., 1995; Bottalico, 1998). On the contrary, FB_1 levels seem significantly lower in central to north-eastern European areas, including Austria (15 mg kg^{-1}) (Lew et al., 1991; Krska et al., 1997), Switzerland (Pittet et al., 1992), Germany (Usleber et al., 1994), the Czech Republic (Ostrý and Ruprich, 1997), Croatia (Jurjević et al., 1999), and Poland (Chelkowski et al., 1994b). Surveys carried out in Croatia on freshly harvested samples of maize kernels collected in 1996–97 from 14 counties showed a scant presence of *F. verticillioides* (9.4%) and *F. subglutinans* (12.2%) associated with a micro-contamination of $FB_1 + FB_2$ (11.66 mg kg^{-1} in 93% of positive samples), together with a predominant occurrence of *Penicillium* spp. (98.5% of samples). However, it seems that in some more northern areas, under very favourable conditions, the fumonisins could reach significant levels. This was reported for the Slovak Republic where the level of fumonisins are usually recorded as negligible (Piecková and Jesenská, 1997). The 1998 season was characterized by severe epidemics of *F. verticillioides* (up to 100% of infected ears) reinforced by the occurrence of *F. proliferatum* (up to 94%). In particular, in pre-harvest infected maize ears, not only were high concentrations of FB_1 (26.9 mg kg^{-1}) and FB_2 found (6.3 mg kg^{-1}), but the formation of FUP was marked and traces of BEA were also found (Srobárová et al., 2000).

In southern Europe, *F. proliferatum*, which represents an additional FB_1 source, is frequently found with *F. verticillioides*. Logrieco et al. (1995) and Bottalico et al. (1995) reported that almost all strains of *F. proliferatum* collected in Italy produced FB_1. Moreover, selected maize ears mainly infected by *F. verticillioides* were found to be contaminated with FB_1, together with BEA and MON (Logrieco et al., 1995). In an additional survey, FB_1 was found in 9 out of 12 selected maize ears, mainly infected by *F. proliferatum* (Ritieni et al., 1997a). Therefore, as a result of the co-occurrence of both the principal fungal sources of FB_1, *F. verticillioides* and *F. proliferatum*, the probability of finding these carcinogenic toxins in maize is higher in southern than in central or northern Europe.

Occurrence of moniliformin

In infected maize ears, the main MON-producing *Fusarium* species are *F. subglutinans*, *F. proliferatum* and *F. avenaceum*. While *F. subglutinans* is widespread in central to northern Europe, and sometimes in part replaces *F. verticillioides*, *F. proliferatum* is increasingly reported from south to central European maize-growing areas as reinforcing the occurrence of *F. verticillioides* (Logrieco et al., 1995). In Italy, high levels of MON were found in maize ears with a widespread presence of *F. proliferatum* in association with *F. verticillioides* (Logrieco et al., 1995). In addition, MON was commonly found in maize ears infected with *F. subglutinans* from central and northern European countries, including Austria (Lew et al., 1991), and Poland (Kostechi et al., 1995; 1997). In particular, very high amounts of MON were found in all infected maize ears in Poland during years with severe epidemics of *F. subglutinans* (Lew et al., 1996). The high occurrence of MON in maize ear rot from Austria and Poland seemed to be related not only to the spread of *F. subglutinans*, but also to the frequent occurrence of *F. avenaceum* (Lew et al., 1991; 1996). In nature, *F. subglutinans* appears to be a higher MON producer than *F. avenaceum*, and levels up to 399.3 mg kg^{-1} were associated with its occurrence in infected kernels from ear rot. This level compares with very much lower MON concentrations associated with the presence of *F. avenaceum* (Sharman et al., 1991). The toxigenicity of *F. proliferatum* seems comparable with that of *F. subglutinans* and Logrieco et al. (1995) reported strains of *F. proliferatum* were able to produce very

large amounts of MON on autoclaved maize. However, the co-occurrence of *F. avenaceum* with *F. subglutinans* on maize in central–northern Europe increases the risk of MON accumulation in infected ears. Therefore, it appears that MON could be one of the more expected mycotoxins in maize ear rot in Europe (Schütt et al., 1998).

Occurrence of beauvericin

In maize ears infected with *F. verticillioides*, *F. subglutinans* and *F. proliferatum*, the occurrence of BEA was found in several European maize-growing areas together with the formation of FB_1 by *F. verticillioides* and *F. proliferatum*, and/or MON by *F. subglutinans* and *F. proliferatum*. In particular, BEA was reported in Italy (Moretti et al., 1994; Bottalico et al., 1995; Ritieni et al., 1997a), Poland (Logrieco et al., 1993; Kostechi et al., 1995), Austria (Krska et al., 1996), and the Slovak Republic (Srobarovà et al., 2000). However, it can be presumed that BEA is more widespread than recorded to date. In effect, BEA is produced not only by *F. proliferatum* and *F. subglutinans*, which are widespread from south–central to central–northern areas respectively (Krska et al., 1997; Logrieco, 2000), but also by several other *Fusarium* species occurring less frequently even in epidemic form, including *F. avenaceum*, *F. acuminatum*, *F. equiseti*, *F. poae* and *F. oxysporum* (Logrieco et al., 2000). It is worth noting that Austrian strains of *F. subglutinans* and *F. proliferatum* produced high levels of BEA on autoclaved maize (Krska et al., 1997). Similar results were obtained by Bottalico et al. (1995) with strains of *F. proliferatum* from Sardinia.

Occurrence of fusaproliferin

An important emerging toxicological problem seems to be connected with the occurrence of FUP. This novel fusariotoxin is produced, mostly together with BEA, by strains of *F. proliferatum* and *F. subglutinans* from maize and maize-based food and feed (Moretti et al., 1995; 1997; Logrieco et al., 1996). Since its isolation and characterization, FUP has been increasingly found in maize pink ear rot associated with *F. proliferatum*, especially in Italy (Ritieni et al., 1997a), and in the Slovak Republic (Srobarovà et al., 2000). In particular, 15 of 39 samples of pre-harvest maize ears collected throughout Italy during 1993–94, and predominantly infected by *F. proliferatum,* were contaminated with FUP, in association with FB_1 and BEA (Ritieni et al., 1997a).

Future perspectives

The many reports from Europe on maize contamination by mycotoxigenic *Fusarium* species show that the colonization of this important crop plant could be determined by organisms with a broad range of mycotoxin production and different environmental niches. Therefore, the evident biodiversity of these fungal species calls not only for a complex approach from plant pathologists, but requires particular attention to different toxicological problems related to the specific *Fusarium* contamination. The correct identification of the *Fusarium* species contaminating maize in different areas is evidence for a need not only for studying the levels of the interaction between *Fusarium* pathogens and maize, but also to obtain a precise picture of the toxicological risks related to the maize consumption by humans and animals. Developing modern, easy and fast tools for correct identification of toxigenic *Fusarium* species is therefore one of the future challenges for *Fusarium* workers. Moreover, as the profile of maize contaminants seems to be related to different environmental conditions, a better evaluation of each *Fusarium* species should be extremely useful for developing risk assessment models using data collected from a large range of sites in Europe with contrasting climatic conditions. Therefore, constant monitoring of maize throughout Europe in order to collect data on the *Fusarium* species and mycotoxins that are present in the maize is needed for establishing a European data base. Finally, the occurrence in maize of several mycotoxins with specific chemical traits and modes of action is a serious problem because of their additive and/or synergistic effects. Investigations of these possible effects and of the risks related to multiple mycotoxin contamination of maize will be a major goal for scientists involved in studies of *Fusarium* in the whole Europe.

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