

## INVITED REVIEW

**FUSARIUM DISEASES OF CEREALS:  
SPECIES COMPLEX AND RELATED MYCOTOXIN PROFILES, IN EUROPE**

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## SUMMARY

Several *Fusarium* species occurring worldwide on cereals as causal agents of 'head blight' (scab) of small grain cereals and 'ear rot' of maize, are capable of accumulating, in infected kernels, several mycotoxins some of which of notable impact to human and animal health. The main groups of *Fusarium* toxins commonly recognized in grains are: trichothecenes, including T-2 toxin (T2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), fusarenone X (FUS), and nivalenol (NIV); zearalenones, essentially zearalenone (ZEN); and fumonisins, in particular fumonisin B<sub>1</sub> (FB<sub>1</sub>). In addition, moniliformin (MON), beauvericin (BEA), and fusaproliferin (FUP) were also found in *Fusarium* infected cereal ears, important as problems due to them are coming to light. The most frequently encountered mycotoxins in ear blight of wheat and other small grain cereals in Europe, proved to be DON and ZEN, produced by *F. graminearum* and *F. culmorum*, occurring from southern (warm) to northern (cold) European areas, respectively. In association with DON, NIV and FUS were found formed, in all climates by *F. graminearum*, *F. crookwellense*, *F. culmorum* and, in Nordic areas, also by *F. poae*. Moreover, in central to northeastern European countries, MON produced by *F. avenaceum*, and T-2 and DAS produced by *F. sporotrichioides* and *F. poae*, were found respectively. In maize red ear rot affected by *F. graminearum* ZEN, DON, NIV and FUS were found. In addition, the presence of *F. avenaceum* and *F. subglutinans* usually led to the accumulation of MON. Moreover, NIV and FUS were found associated with *F. crookwellense*, and DAS and T-2 with *F. poae* and *F. sporotrichioides*. In maize pink and random kernel rot caused by *F. moniliforme*, there is increasing evidence of the occurrence of FB<sub>1</sub>, especially in southern European areas where the same fusariosis was reinforced by the wide occurrence of *F. proliferatum* capable of producing FB<sub>1</sub>, MON, BEA and FUP.

## RIASSUNTO

**FUSARIOSI DEI CEREALI: PROFILI DELLE SPECIE ASSOCIATE E DELLE RELATIVE MICOTOSSINE, IN EUROPA.** Diverse specie di *Fusarium* agenti di fusariosi dei cereali e in particolare di "scabbia" del frumento e di "marciume della spiga" del mais, sono capaci di formare nelle spighe infette numerose micotossine alcune delle quali di notevole impatto sulla salute umana ed animale. I composti di maggiore importanza fanno parte dei seguenti gruppi principali: tricoteceni, comprendenti tossina T-2 (T2), diacetossiscirpenolo (DAS), deossinivalenolo (DON), fusarenone-X (FUS) e nivalenolo (NIV); zearalenoni, con lo zearalenone (ZEN); e, fumonisine, con la fumonisin B<sub>1</sub> (FB<sub>1</sub>). Tra le altre micotossine ritrovate nelle spighe infette meritano di essere ricordate per le relative problematiche emergenti: moniliformina (MON), beauvericina (BEA) e fusaproliferina (FUP). Tra le micotossine che più frequentemente sono state trovate nelle spighe di frumento e di altri cereali affetti da "scabbia" vi sono essenzialmente DON e ZEN, formati da *F. graminearum* e da *F. culmorum* che nell'ordine, si succedono in maniera predominante, dalle aree europee meridionali a quelle settentrionali. Assieme a DON, sono stati spesso trovati NIV e FUS formati un po' ovunque come conseguenza degli attacchi di *F. graminearum*, di *F. crookwellense* e di *F. culmorum* e, nelle aree più settentrionali, di quelli più rilevanti di *F. poae*. Inoltre, nelle aree europee centrali e nord-orientali sono stati trovati, spesso assieme con DON e ZEN, anche MON in seguito ad attacchi ricorrenti di *F. avenaceum*, nonché T2 e DAS in relazione ad attacchi sporadici rispettivamente di *F. sporotrichioides* e di *F. poae*. Nelle spighe di mais affette da "marciume rosso-vinoso", più ricorrente nelle aree centro-settentrionali e causato prevalentemente da *F. graminearum* e in minor misura da *F. culmorum*, sono stati frequentemente trovati DON, NIV, FUS e ZEN. Nelle stesse spighe, maggiori concentrazioni di NIV e FUS sono state trovate in concomitanza di attacchi di *F. crookwellense*, così come sono stati spesso trovati anche MON, T2 e DAS in conseguenza di attacchi rispettivamente di *F. avenaceum*, e di *F. sporotrichioides* e *F. poae*. Invece, nelle spighe di mais affette da "marciume rosato", più ricor-

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rente nelle aree europee centro-meridionali e causato essenzialmente da *F. moniliforme*, è stata trovata FB<sub>1</sub>. Concentrazioni più elevate di FB<sub>1</sub> sono state trovate in alcune aree europee più meridionali, dove a *F. moniliforme* si associa *F. proliferatum*, produttore oltre che di FB<sub>1</sub>, anche di MON, BEA e FUP. Nelle aree centro-settentrionali dove *F. proliferatum* viene sostituito da *F. subglutinans*, nelle spighe affette da "marciume rosato" sono stati trovati MON e BEA.

*Key words:* *Fusarium*-mycotoxins, grain contamination, deoxynivalenol, fumonisins.

## INTRODUCTION

Several *Fusarium* species are widespread pathogens on cereals in both temperate and semitropical areas, including all European cereal growing areas. They can infect small grain cereals (soft and durum wheat, barley, oats, rye, triticale, rice, sorghum, millet) and maize, causing root, stem, and ear rot, with severe reductions in crop yield, often estimated at between 10 and 30 percent. In addition, certain strains are also capable of producing mycotoxins which can be formed in preharvest infected plants still standing in the fields, or in stored grains. Maize, wheat and barley which constitute almost two-thirds of the world production of cereals and almost 80% of the European grain production, are causing great concern both because of the extent of infections and the contamination by mycotoxins. However, also the other less cultivated small grains have been reported to contain *Fusarium* mycotoxins, though these crops appear, in general, to be less susceptible to *Fusarium* head blight and consequently to pre-harvest toxin contamination.

The occurrence of mycotoxins in cereal grains 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, also in humans. It was estimated that 25% of the world food crops are affected by mycotoxins (Charmley *et al.*, 1995), but for some *Fusarium* toxins in cereal grains such as deoxynivalenol (DON) and fumonisin B<sub>1</sub> (FB<sub>1</sub>) (Bullerman, 1996, Eriksen and Alexander, 1998), it is likely that this percentage is even higher.

A number of reviews and books on *Fusarium* toxins in cereal grains dealing with the several biologic aspects of the *Fusarium* species involved, and with the natural occurrence, toxicology and risk assessment for animal and human health of the critical compounds, have recently been published (Chelkowski, 1989a, 1991; IARC, 1993; Miller and Trenholm, 1994; Smith *et al.*, 1994;

Miller, 1995; Jackson *et al.*, 1996; Bottalico, 1997; Eriksen and Alexander, 1998). Moreover, many European aspects of cereal fusariosis have been discussed during the five European Seminars so far dedicated to *Fusarium* taxonomy, pathogenicity and mycotoxins (Logrieco *et al.*, 1997; Mesterházy, 1997), and in a joint special project (Nirenberg, 1995).

In this paper, the most relevant aspects of the distribution of toxigenic *Fusarium* species and related mycotoxins in ear rot of cereals in European countries are reviewed, with emphasis on the occurrence of trichothecenes, zearalenone, and fumonisins in infected plants standing in the field. The relationship between the *Fusarium* species complex and the relative mycotoxin profile, besides underlining the toxicological risk of the cereal fusarioses, may be useful for predicting the mycotoxins that are most likely to be formed in grains, with reference to the most conducive factors, including, predominant *Fusarium* species, geographical area, and environmental conditions.

## **FUSARIUM SPECIES INVOLVED AND MYCOTOXIN PRODUCED**

**FUSARIUM SPECIES INVOLVED.** The species of *Fusarium* (teleomorph *Gibberella*) causing fusariosis of cereals are worldwide in distribution and can cause several diseases generally recognized, according to the host, as *Gibberella* seedling blight, foot rot, and head blight (scab) of small grain cereals (wheat, oats, barley, rye, triticale); and *Gibberella* stalk and ear rot, and seedling blight of maize. From the mycotoxicological point of view, the phases of disease of greatest concern are certainly scab of small cereals and ear rot of maize, for the potential accumulation of mycotoxins in grains. The etiological characteristic of both these phases, is the co-occurrence or the quick succession of several species of *Fusarium* usually referred to as a 'complex'. In fact, it is quite common to isolate up to nine different *Fusarium* species, from a single fragment of infected tissues or up to seventeen different species from freshly harvested wheat samples collected in a limited area. However only a restricted number of species have been regarded as pathogenic and generally only very few of them predominate in a particular host-agroclimatic system. But, like the strains of the pathogenic and predominant *Fusarium* species, also several strains of the other less pathogenic or opportunistic *Fusarium* species are capable of producing considerable amounts of toxins. Therefore, the toxic profile of a contaminated crop is determined not only by the predominant pathogenic species, but also by the opportunistic species included in the 'complex'.

The species predominantly found associated with head blight of wheat and other small cereals are *F. graminearum* Schwabe and its widespread teleomorph *G. zeae* (Schw.) Petch, *F. culmorum* (Wm.G. Sm.) Sacc., and *F. avenaceum* (Fr.) Sacc. (*G. avenacea* R.J. Cooke). Among the other less frequently isolated species there are *F. poae* (Peck) Wollenw., *F. crookwellense* L.W. Burgess, P.E. Nelson & T.A. Toussoun (syn. *F. cerealis* Cooke), *F. equiseti* (Corda) Sacc. (syn. *F. scirpi*) (*G. intricans* Wollenw.), *F. sporotrichioides* Sherb., and *F. tricinctum* (Corda) Sacc. Several other species may be sporadically encountered, including *F. acuminatum* Ellis & Everh. (*G. acuminata* Wollenw.), *F. subglutinans* (Wollenw. & Reink.) P.E. Nelson, T.A. Toussoun & Marasas (syn. *F. sacchari*), *F. solani* (Mart.) Sacc. (*Nectria haematococca* Berk & Broome), *F. oxysporum* Schlecht., and *F. semitectum* Berk. & Rav. (syn. *F. pallidoroseum*, *F. incarnatum*).

*Fusarium* species may be responsible for at least two kinds of maize ear rot, commonly called as 'red ear rot' mainly caused by species of the *Discolor* section, and 'pink ear rot', mainly caused by representatives of the *Liseola* section. The predominant species causing maize 'red ear rot' are *F. graminearum*, *F. culmorum*, and *F. crookwellense*. Among the other less frequently isolated species there are *F. subglutinans*, *F. avenaceum*, *F. moniliforme* J. Sheld. [syn. *F. verticillioides* (Sacc.) Nirenberg]. The species more frequently isolated from maize 'pink ear rot' and related 'random kernel rot', are essentially the widespread anamorphs of the rather rare *G. fujikuroi* (Sawada) Ito in Ito & K. Kimura, namely, *F. moniliforme*, *F. proliferatum* (T. Matsushima) Nirenberg, and *F. subglutinans*. Among the other toxigenic *Fusarium* species less frequently isolated from molded maize ears, there are: *F. equiseti*, *F. poae*, *F. sporotrichioides*, *F. acuminatum*, *F. semitectum*, *F. solani* and *F. oxysporum*.

Finally, there are many other species only sporadically isolated from cereals, but in some occasion reported as emerging problem, such as *F. anthophyllum* (A. Braun) Wollenw., *F. chlamydosporum* Wollenw. & Reink. (syn. *F. fusarioides*), *F. compactum* (Wollenw.) Gordon, *F. flocciferum* Corda, *F. heterosporum* Nees (syn. *F. reticulatum*, *F. graminum*), *F. lateritium* Nees, *F. sambucinum* Fuckel, *F. torulosum* (Berk. & Curt.) Nirenberg, and *F. venenatum* Nirenberg.

Within *F. graminearum* (*G. zeae*) were characterized two populations designated as Group 1 and Group 2, with almost the same toxigenic potentiality. The Group 1 very rarely forms perithecia in nature and mainly causes crown rot of cereals and grasses; Group 2 readily forms abundant perithecia in nature and mainly causes head blight of grain cereals and stalk and ear rot of maize. Studies on genetic diversity indicated that *F.*

*graminearum* Group 2 have greater affinity to *F. culmorum* and *F. crookwellense* than to *F. graminearum* Group 1 (Burgess *et al.*, 1997). In addition, the toxigenic strains of *F. graminearum* were classified in two chemotypes: DON and NIV producers, according to the main type B trichothecenes synthesized. Furthermore, DON-chemotype strains of *F. graminearum* were subclassified into two types: 3-AcDON and 15-AcDON producers (Miller *et al.*, 1991; Logrieco *et al.*, 1992; Szécsi and Bartok, 1995; Yoshizawa, 1997). Ecological differences in chemotype distribution may contribute to characterizing a regional grain contamination.

Toxigenic strains of *F. culmorum* can be divided into two types: DON and NIV chemotypes, according to the main type B trichothecenes produced. DON-type strains produced also AcDON (3-AcDON) (Gang *et al.*, 1995; D'Mello *et al.*, 1997).

The species *G. fujikuroi* has been subdivided into seven distinct mating populations (biological species), indicated as A to G, and covering several *Fusarium* anamorphs (Leslie, 1995). From these, the most frequently found on maize were *F. moniliforme* (A), *F. proliferatum* (D), and *F. subglutinans* (E), which were also differentiated by their toxigenic capability (Moretti *et al.*, 1997).

*F. nivale* Ces. ex Berl. & Voglino is a well known pathogen of cereals, very frequently found among the major fungi included in the species complex causing 'foot rot' and 'head blight (scab)' of small cereals. *F. nivale* is no longer considered a *Fusarium*, first it was placed in the genus *Gerlachia* [*G. nivalis* (Ces. ex Berl. & Voglino) W. Gams & E. Müller], and then transferred to *Microdochium* as *M. nivalis* (Fr.) Samuels & I.C. Hallett [teleomorph *Monographella nivalis* (Schaff.) E. Müller]. Therefore *M. nivalis* is not included in this paper dedicated to cereal fusarioses, also because it has a very low toxicity, and proved to be incapable of producing the typical *Fusarium* mycotoxins (Logrieco *et al.*, 1991).

**MYCOTOXIN PRODUCED.** *Fusarium* species occurring on cereal ears in the field are able to produce many mycotoxins, some of which of notable importance. This review focuses on the compounds most commonly found in analytical surveys of field or freshly harvested infected cereal samples. The naturally occurring *Fusarium* mycotoxins belong to three main structural groups, *i.e.* trichothecenes, zearalenones, and fumonisins. Moreover, moniliformin (MON), beauvericin (BEA), and fusaproliferin (FUP), which were also found in naturally infected cereal grains and considered as emerging toxicological problems, are briefly treated. The mycotoxin production by *Fusarium* species from cereals is illustrated in Table 1.

**Table 1.** Mycotoxin production by *Fusarium* species from cereals.

<i>Fusarium</i> species	Mycotoxins <sup>1</sup>
<i>F. acuminatum</i>	<b>T2</b> , HT2, DAS, MAS, MON, NEO
<i>F. avenaceum</i>	<b>MON</b>
<i>F. chlamydosporum</i>	<b>MON</b>
<i>F. crookwellense</i>	<b>NIV, FUS, ZEN, ZOH</b>
<i>F. culmorum</i>	<b>DON, ZEN, NIV, FUS, ZOH, AcDON</b>
<i>F. equiseti</i>	<b>DAS, ZEN, ZOH, NIV, DAcNIV, MAS, FUS</b>
<i>F. graminearum</i>	<b>DON, ZEN, NIV, FUS, AcDON, DAcDON, DAcNIV</b>
<i>F. heterosporum</i>	<b>ZEN, ZOH</b>
<i>F. moniliforme</i>	<b>FB<sub>1</sub></b>
<i>F. oxysporum</i>	MON
<i>F. poae</i>	<b>DAS, MAS, NIV, FUS, T2, HT2, NEO</b>
<i>F. proliferatum</i>	<b>FB<sub>1</sub>, BEA, MON, FUP</b>
<i>F. sambucinum</i>	<b>DAS, T2, NEO, ZEN, MAS</b>
<i>F. semitectum</i>	<b>ZEN</b>
<i>F. sporotrichioides</i>	<b>T2, HT2, NEO, MAS, DAS</b>
<i>F. subglutinans</i>	<b>BEA, MON, FUP</b>
<i>F. tricinctum</i>	MON
<i>F. venenotum</i>	DAS

<sup>1</sup> Bold letters indicate the mycotoxins of great concern. Abbreviations: AcDON = Mono-acetyldeoxynivalenols (3-AcDON, 15-AcDON); AcNIV = Mono-acetylnivalenol (15-AcNIV); BEA = Beauvericin; DAcDON = Di-acetyldeoxynivalenol (3,15-AcDON); DAcNIV = Di-acetylnivalenol (4,15-AcNIV); DAS = Diacetoxyscirpenol; DON = Deoxynivalenol (Vomitoxin); FB<sub>1</sub> = Fumonisin B<sub>1</sub>; FUP = Fusaproliferin; FUS = Fusarenone-X (= 4-Acetyl-NIV); HT2 = HT-2 toxin; MAS = Monoacetoxyscirpenol; MON = Moniliformin; NEO = Neosolaniol; NIV = Nivalenol; T2 = T-2 toxin; ZEN = Zearalenone; ZOH = Zearalenols ( $\alpha$  and  $\beta$  isomers).

*Fusarium trichothecenes*. Of several trichothecene derivatives produced by *Fusarium* species, only a few have been encountered as natural contaminants of cereal products. According to some chemical features, the *Fusarium* trichothecenes can be divided in type A-trichothecenes, characterized by a functional group other than a keton at C-8, and type B-trichothecenes with only the carbonyl at C-8. The type A-trichothecenes include: a) T-2 toxin (T2) and HT-2 toxin (HT2), mainly produced by strains *F. sporotrichioides*, *F. acuminatum*, and *F. poae*; b) diacetoxyscirpenol (DAS), and monoacetoxyscirpenol (MAS), chiefly produced by strains of *F. poae*, *F. equiseti*, *F. sambucinum*, and *F. sporotrichioides*; and c) neosolaniol (NEO), mainly produced by strains of *F. sporotrichioides*, *F. poae*, and *F. acuminatum*. The type B-trichothecenes include: a) deoxynivalenol or vomitoxin (DON), and its derivatives mono- (3-AcDON and 15-AcDON) (AcDON), and di-acetylated (3,15-AcDON), produced by strains of *F. graminearum* and *F. culmorum*; and, b) nivalenol (NIV) and fusarenone X (FUS), and di-acetylated derivatives 4,15-AcNIV, produced by strains of *F. crookwellense*, *F. poae*, *F. graminearum* and *F. culmorum*.

Trichothecenes have been reported to cause a variety of toxic effects in both laboratory and farm animals, in-

cluding 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 (IARC, 1993; Rotter and Prelusky, 1996). Trichothecenes are commonly associated with several mycotoxicoses in livestock, including haemorrhagic and emetic syndromes, caused by trichothecene type A (T2, DAS, MAS) and type B (DON, NIV, FUS), respectively (IARC, 1993). Furthermore, T2 and DON were also implicated in human toxicoses. In particular, T2 and derivatives in Alimentary Toxic Aleukia (ATA) occurred in Russia in 1913 and then again in 1944 and 1952-53, in people consuming overwintered cereals (reviewed by Beardall and Miller, 1994) and more recently, in China in toxicosis associated with consumption of contaminated rice (Wang *et al.*, 1993). DON and derivatives were responsible of at least 35 toxicoses reported from 1961 in rural areas of India, China, and Japan (Ehling *et al.*, 1997). In scabby wheat (Akakabi) intoxications reported in Japan, a preeminent role seems to have been played NIV derivatives, produced by an additional strain of *Fusarium* first reported as Fn 2B, and recently recognized as a new species and named *F. kyushuiense* O'Donnell & Ueno (Ueno *et al.*, 1997). However, trichothecenes are not significant mutagenic

agents, and their very high cytotoxicity probably precludes the action of doses high enough to elicit genotoxic effects. In this regard, IARC (1993) included the trichothecenes formed by *F. graminearum*, *F. culmorum*, *F. crookwellense*, and *F. sporotrichioides* among the compounds not marked for their carcinogenicity to humans.

*Zearalenones.* Zearalenone (ZEN), is produced by *F. graminearum*, *F. culmorum*, *F. crookwellense*, *F. equiseti*, and *F. semitectum*, often associated with zearalenols (ZOH) ( $\alpha$ - and  $\beta$ -zearalenol isomers). ZEN is among the most widely distributed *Fusarium* mycotoxins in agricultural commodities, and has very often been encountered even at very high concentrations especially in maize. ZEN is a uterotrophic and estrogenic compounds that cause reproductive disorders in farm animals, especially in swine, and in laboratory animals (Ito and Ohtsubo, 1997). ZEN is responsible for recurring toxicoses in livestock, characterized by hyperestrogenism in swine, and infertility and poor performance in cattle and poultry, with a possible impact also on human health (IARC, 1993). In this regard, ZEN and its derivatives (zearalenols) have been implicated in premature pubertal changes in young children in Hungary, where estrogenic metabolites were found not only in grain-based human foodstuffs but also in the serum of young female patients showing precocious breast development (Szuets *et al.*, 1997). However, the preliminary evidence of the genotoxicity and carcinogenicity of ZEN, so far limited to mice and cultured mammalian and human cells appeared inadequate; so that the estrogenic compounds produced by *Fusarium* were classified as not carcinogenic to humans (IARC, 1993).

*Fumonisinis.* Fumonisinis were first isolated from *F. moniliforme*, and then found also in cultures of *F. proliferatum*, and in a few other less ecological important *Fusarium* species. Three series of fumonisins were described and named A, B, and C, each including four compounds, *i.e.* FA<sub>1</sub> to FA<sub>4</sub>, FB<sub>1</sub> to FB<sub>4</sub>, and FC<sub>1</sub> to FC<sub>4</sub>, respectively. The B series includes the most active fumonisins, particularly FB<sub>1</sub> which has caused the great mycotoxicological concern (Hopmans and Murphy, 1995). FB<sub>1</sub> was found to occur naturally at biologically significant levels in maize and in a variety of maize-based human foodstuffs and animal feeds worldwide (Shephard *et al.*, 1996). Feeds contaminated by FB<sub>1</sub> are known to cause leukoencephalomalacia in horses, pulmonary edema and hepatic syndrome in swine, poor

performance in poultry, and alteration in hepatic and immune function in cattle (Ross *et al.*, 1990; Osweiler, 1995). Moreover, home-grown corn contaminated by FB<sub>1</sub> has been statistically associated with high rates of esophageal cancer of humans in areas of southern Africa, China, and southeastern U.S.A. (Marasas, 1995); and similar claims have been made for northeastern Italian areas (Franceschi *et al.*, 1990). The structural similarity with sphingosines, which are components of the membrane sphingolipid complex, enables fumonisins to disrupt the biosynthesis of sphingolipids, and to provoke an increase in the concentrations of free sphinganine in blood and tissues, and of the sphinganine/sphingosine ratio in serum and urine (Merrill *et al.*, 1996). The depletion of complex sphingolipids from the biological membranes may account for the toxicity, and perhaps the carcinogenicity, of the fumonisins (Riley *et al.*, 1996). However, fumonisins appear to be non-mutagenic, but strong cancer promoters (Gelderblom *et al.*, 1996). Therefore, the evidence that cultures of *F. moniliforme* and sample of FB<sub>1</sub> are capable of promoting liver cancer in rats, together with other observations obtained from several genotoxic tests, led IARC (1993) to classify the toxins of *F. moniliforme* as carcinogenic to animals and possibly carcinogenic to humans (Group 2B).

*Moniliformin.* Moniliformin (MON) has been purified from cultures of several *Fusarium* species, including: *F. proliferatum*, *F. subglutinans*, *F. avenaceum*, and *F. tricinctum* (Chelkowski *et al.*, 1990). Although diets containing culture material naturally contaminated with MON, or amended with purified MON, can cause reduced performance, hematologic disorders, myocardial hypertrophy, and mortality in rodents, chicks, ducklings, and pigs (Ledoux *et al.*, 1995; Harvey *et al.*, 1997), its significance as a contaminant of maize and other cereal grains has not yet been clarified. In particular, it was considered in the past to explain the toxicity of *F. moniliforme* cultures, which was definitely attributed to FB<sub>1</sub>; but at present MON is regarded as cytotoxic but not genotoxic, and has never been associated with field outbreaks in livestock. However, MON was suspected to cause the Keshan disease, a myocardic human impairment occurring in rural areas of China and South-Africa (Transkei) where there is high maize consumption.

*Beauvericin.* Beauvericin (BEA) 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*, some of which were isolated from maize and maize-based feed for swine, and involved in animal toxicoses (Plattner and Nelson, 1994; Moretti *et al.*, 1995). Besides its high toxicity to insects (Gupta *et al.*, 1991), BEA was found to be cytotoxic to mammalian cell tissues, and was reported to cause apoptosis on both murine and human cell lines (Macchia *et al.*, 1995). In addition, BEA showed toxic effects on the contractility of guinea pigs smooth muscle (Krska *et al.*, 1997). However, the biological activity of BEA, like that of the enniatins, seems due to the ability of such ionophoric compounds to affect ion transport across membranes, leading to disruption in the ionic balances of cell wall. Such an activity of BEA could strengthen that of other *Fusarium* mycotoxins commonly associated with this toxin in contaminated cereals.

*Fusaproliferin*. Fusaproliferin (FUP) is a novel sesquiterpene first purified from a culture of a *F. proliferatum* strain from maize ear rot in northern Italy (Ritieni *et al.*, 1995), and then found 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 *Artemia salina* larvae, and cytotoxic to SF-9 insect cells and IARC/CL 171 human B lymphocytes (Logrieco *et al.*, 1996). Moreover, high mortality in broiler chicks fed with the maize culture of *F. proliferatum* was reported by Ramakrishnan and Wu (1994), and severe teratogenic effects were observed in chick embryo bioassays by Ritieni *et al.* (1997b).

## **FUSARIUM TOXINS IN CEREALS, IN EUROPE**

**FUSARIUM TOXINS IN CEREAL GRAINS.** *Fusarium* mycotoxins are worldwide occurring in cereal grains and frequently reported even at high concentrations, in fresh or stored grains, as well as in cereal based food and feed (Shotwell, 1991; Yoshizawa, 1991). An updated outline of the occurrence of *Fusarium* mycotoxins in European countries (EC), compiled mainly utilizing the reviews of Smith *et al.* (1994), and Eriksen and Alexander (1998), with the addition of many other original surveys, is summarized in Table 2. As can be inferred from this table, the occurrence of *Fusarium* mycotoxins in EC countries is quite similar to that generally found in the rest of world, with a higher risk in EC countries for FB<sub>1</sub>, DON, and T2, and a relatively lower risk for NIV and MON.

In an evaluation of several reports on the occurrence of *Fusarium* toxins in small cereal grains in Europe published up to 1995, with emphasis on Nordic countries (Denmark, Finland, Germany, Norway, Sweden, UK), Pettersson (1995) indicated DON as the most frequently encountered compound, followed by NIV, HT2, and T2. Other *Fusarium* toxins less frequently found and at lower concentrations were AcDON (3-AcDON), FUS, NEO, ZEN, ZOH. In particular, DON was found (sample incidence-mean of toxin in  $\mu\text{g kg}^{-1}$ ): wheat (57% - 173), barley (53% - 189), oats (72% - 408), and rye (57% - 85); and, in comparison, NIV was found in: wheat (17% - 22), barley (14% - 28), oats (18% - 47), and rye (5% - 5). DON concentrations were normally less than 1 mg kg<sup>-1</sup>, but the highest levels recorded were up to 28.538 mg kg<sup>-1</sup> in wheat in 1987 in Bavaria (Germany); up to 62.05 mg kg<sup>-1</sup> in barley in 1988 in Norway; up to 5.6 mg kg<sup>-1</sup> in oats in 1989 in Norway; and up to 0.59 mg kg<sup>-1</sup> in rye in 1985 in Denmark. Moreover, in an evaluation of the risk assessment limited to Nordic areas, the *Fusarium* toxin daily intake was calculated to be 23.93  $\mu\text{g}$  for DON (0.40-0.80  $\mu\text{g kg}^{-1} \text{bw}^{-1}$ ) and 4.48  $\mu\text{g}$  for NIV (0.07-0.14  $\mu\text{g kg}^{-1} \text{bw}^{-1}$ ) (Eriksen and Alexander, 1998; and references therein stated). A similar risk of exposure to DON can be assumed for southern European countries like Italy, characterized by a lower contamination of domestic grains, but with large imports from suspect geographical areas.

However, the extent of *Fusarium* mycotoxin contamination in cereal crops and processed grains, appears in general consistently lower in areas of north America, Europe, and Japan (DON mean = 0.1 mg kg<sup>-1</sup>) if compared with data reported for areas of south America, Africa and southern China (DON mean = 2.0 mg kg<sup>-1</sup>). But, a more recent assessment indicated an increase in DON contamination (mean = 0.15-1.6 mg kg<sup>-1</sup>), especially in particular years and locations in wheat from Canada and from central-northern Europe (IARC, 1993; Mueller and Reiman, 1997).

The lower EC risk seen in Table 2 for NIV and MON in comparison with the rest of world, is mainly due to the increase in reports of NIV from Japan (Yoshizawa, 1997), and MON from southern African countries. In surveys performed by MAFF-UK (1993), MON was found in low concentrations in samples of retail whole maize (up to 0.25 mg kg<sup>-1</sup>) and maize products (meal, flour) (up to 0.25 mg kg<sup>-1</sup>) from UK, USA, France, and Italy. But, higher MON contaminations were found in similar surveys in samples from Gambia (up to 3.160 mg kg<sup>-1</sup>) and Transkei (South-Africa) (up to 2.730 mg kg<sup>-1</sup>).

**Table 2.** Occurrence of *Fusarium* mycotoxins in cereal grains, worldwide (W) and in European countries (EC)<sup>1</sup>.

Toxin <sup>2</sup>	Positive/assayed samples				Range ( $\mu\text{g kg}^{-1}$ ) <sup>a</sup>	
	No.		%		W	EC
	W <sup>a</sup>	EC <sup>b</sup>	W	EC		
DON	3491/7369	3771/6288	47	60	1-67000	4-67000
DAS	126/2103	49/1535	6	3	1-31500	20-31500
FB <sub>1</sub>	365/497	154/179	73	86	1-117520	50-4670
MON	102/288	37/148	35	25	50-11570	50-750
NIV	867/2181	745/4608	40	16	2-37900	3-7800
T2	350/4656	519/4383	8	12	1-38890	1-14000
ZEN	2277/18018	845/5745 <sup>c</sup>	13	15	2-275800	1-175000

<sup>1</sup> Data from (a) Smith *et al.* (1994), and (b) Eriksen and Alexander (1998). EC<sup>b</sup>, and W<sup>a</sup> data are based on different surveyed periods.

<sup>2</sup> Abbreviations: DON = Deoxynivalenol (Vomitoxin); DAS = Diacetoxyscirpenol; FB<sub>1</sub> = Fumonisin B<sub>1</sub>; MON = Moniliformin; NIV = Nivalenol; T2 = T-2 toxin; ZEN = Zearalenone.

#### **FUSARIUM SPECIES AND MYCOTOXINS IN HEAD BLIGHT OF SMALL GRAINS**

Occurrence of *Fusarium* species in small cereal head blight. *Fusarium* species pathogens on wheat, barley and other small grains, are responsible for two forms of disease, the 'foot rot' affecting roots and crown, and in an early stage causing also seedling blight; and 'head blight' (scab), affecting from individual grains or single ear spikelets to entire heads. As mentioned before, the head blight is the most important cereal fusariosis phase for the potential accumulation of toxins in scabby grains intended for foods and feeds, but the risk connected with the use of contaminated forage and straw by livestock must not be neglected.

In general, according to the temperature, the *Fusarium* species causing head blight of wheat and other small cereals in Europe can be broadly arranged from warm to cold areas as follows: *F. graminearum*, *F. avenaceum*, and *F. culmorum*. Among the less isolated there are *F. poae* and *F. tricinctum*, followed by *F. equiseti*, *F. crookwellense*; *F. sporotrichioides*, *F. acuminatum*, *F. subglutinans*, *F. solani* and *F. oxysporum*. The profile of head blight *Fusarium* complex, proceeding from southern to northern areas, can be exemplified by some situations reported for some characteristic climatic areas. These situations indicated that *F. graminearum*, particularly Group 2, and its widespread teleomorph *G. zeae*, were the most common in moist-warm continental climates, such as cen-

tral and southeastern Europe; whereas, *F. culmorum* and *F. avenaceum* were more often found in maritime and cooler European areas (Table 3).

In central to northern Italy *F. graminearum* (mainly Group 2) (32%), *F. avenaceum* (31%), *F. culmorum* (25%) and, to a lesser extent, *F. crookwellense*, *F. poae* and *F. tricinctum* were isolated from head blight of wheat (Balmas and Corazza, 1994; Casulli *et al.*, 1995). The same *Fusarium* profile was reported for head blight of wheat in other similar southern European localities, including Portugal, Spain, and France (Maurin and Chenet, 1993; Assemat *et al.*, 1995).

A slightly shifted spectrum of the predominant species, characterized by an increasing incidence of *F. avenaceum* and *F. culmorum* partially displacing *F. graminearum*, was reported for northeastern France, Austria, Switzerland, and southern Germany (Bavaria). In addition, in the latest surveys, an emerging prevalence of *F. poae* and *F. sporotrichioides* was also noted especially in central to northern Germany, together with the occasional appearance of other species, including *F. tricinctum*, *F. equiseti*, *F. crookwellense*, *F. acuminatum* and *F. solani* (Adler *et al.*, 1995; Mauler-Machnik and Suty, 1997; Mueller and Reiman, 1997; Schütze *et al.*, 1997). A *Fusarium* species complex comparable with that found in central Europe was also reported in Czech and Slovak Republics (Srobárová and Pavlová, 1997), and Croatia (Jurkovic and Cosic, 1997).

**Table 3.** Toxigenic *Fusarium* species isolated from head blight of wheat in Europe.

Species	Occurrence		Mycotoxin <sup>1</sup>
	North/Center	South	
<i>F. graminearum</i>	+++	+++	DON, AcDON, NIV, FUS, ZEN
<i>F. avenaceum</i>	+++	++	MON
<i>F. culmorum</i>	+++	++	DON, NIV, ZEN, ZOH
<i>F. poae</i>	++	±	DAS, NIV, FUS
<i>F. equiseti</i>	+	±	DAS, ZEN, ZOH
<i>F. crookwellense</i>	+	±	NIV, FUS, ZEN, ZOH
<i>F. tricinctum</i>	+	±	MON
<i>F. sporotrichioides</i>	+	-	T2, HT2, NEO
<i>F. acuminatum</i>	±	±	T2, NEO
<i>F. subglutinans</i>	±	-	MON
<i>F. solani</i>	±	-	-
<i>F. oxysporum</i>	±	-	-

<sup>1</sup> Abbreviations: AcDON = Mono-acetyldeoxynivalenol (3-AcDON, 15-AcDON); DAS = Diacetoxyscirpenol; DON = Deoxynivalenol or vomitoxin; FUS = Fusarenone X; HT2 = HT-2 Toxin; MON = Moniliformin; NEO = Neosolaniol; NIV = Nivalenol; T2 = T-2 toxin; ZEN = Zearalenone; ZOH = Zearalenols ( $\alpha$  and  $\beta$  isomers).

In cooler maritime areas of the northwestern European countries, including some northwestern areas of France, The Netherlands, Belgium, England, and Scotland, the most commonly *Fusarium* species involved in ear blight of small grain cereals were *F. culmorum*, *F. graminearum*, *F. avenaceum*, and *F. poae*. However, the latest surveys report an increasing prevalence of *F. culmorum* and a greater importance of *F. poae* and *F. avenaceum* on all small grain cereals, especially in years less conducive to *F. graminearum* infections (Daamen *et al.*, 1991; Parry *et al.*, 1995; Polley and Turner, 1995; De Nijs *et al.*, 1996).

In northeastern Europe such as in Poland, the latest wheat surveys covering various climatic areas, indicate the predominance of *F. poae* (64%), followed by *F. tricinctum* (15%), *F. avenaceum* (8%), *F. culmorum* (6%), and *F. graminearum* (4%) (Golinski *et al.*, 1997). However, previous surveys indicated an annual variation in relation to different environmental conditions and locations, with a wider occurrence of *F. graminearum* especially on oats in southern areas, and a greater relevance of *F. poae*, *F. culmorum* and *F. avenaceum* on wheat and barley in central to northern locations (Chelkowski, 1989b). Moreover, an emerging relevance of *F. crook-*

*wellense* and *F. sporotrichioides*, together with the sporadic presence of *F. equiseti* and *F. oxysporum* were also reported (Perkowski *et al.*, 1997a). In northwestern Russia (Leningrad region), the *Fusarium* profile seemed to have shifted to Nordic species, and the most frequently isolated species were *F. avenaceum*, *F. sporotrichioides*, *F. culmorum*, and *F. poae*; while *F. graminearum* was almost absent (Schipilova and Gagkaeva, 1997).

In southeastern Europe such as in Hungary, the *Fusarium* species most frequently found in winter wheat samples in the early 1990s, were *F. equiseti* (16%), *F. poae* (16%), *F. sporotrichioides* (14%), *F. graminearum* (13%), and *F. semitectum* (10%), and to a lesser extent, *F. culmorum* (6%), *F. chlamydosporum* (6%), *F. avenaceum* (6%), *F. acuminatum* (5%), and *F. oxysporum* (2%). However, in earlier 1970s and 1980s surveys, quite different *Fusarium* profiles were recorded, with an alternate predominance of *F. graminearum* and *F. culmorum*, combined with a frequent or rare relevance of *F. poae* and *F. oxysporum*, respectively (Tóth, 1997). Instead, in near areas of Bulgaria, Romania, and southern European part of Russia (Bielorussia, Krasnodar, Ukraine), a higher and less variable predominance of *F. graminearum* was reported (Ablova, 1997; Klechkovskaya, 1997).



The northern European situation can be exemplified by the Norwegian surveys (Elen *et al.*, 1997; Langseth *et al.*, 1997). The *Fusarium* species most frequently encountered during the latest surveys of cereal samples (wheat, barley, oats) collected from central to southern part of the country included, in decreasing order, *F. avenaceum*, *F. poae*, *F. tricinctum*, *F. culmorum*, *F. graminearum*, *F. equiseti*, *F. torulosum*, and *F. sporotrichioides* (Langseth *et al.*, 1997). In addition, a prevalence of *F. culmorum* on *F. avenaceum* and *F. graminearum* was observed on spring wheat when weather conditions around the time of flowering were drier and warmer than normal, while wetter and colder weather was more conducive to *F. graminearum* and *F. avenaceum*, and less to *F. culmorum* (Elen *et al.*, 1997). Similar observations on barley and oats indicated the higher susceptibility of these cereals under wetter and colder weather conditions than under drier and warmer ones, primarily to *F. culmorum* and then to *F. avenaceum* and *F. graminearum* (Elen *et al.*, 1997). The resulting *Fusarium* profiles were found in relation to the differences in the occurrence of trichothecenes in Norwegian barley, oats and wheat grain (Langseth and Elen, 1996). Furthermore, an increasing prevalence of *F. poae* and associated NIV derivatives, was even more frequently recorded especially in latest surveys of oats and barley, also in neighbouring Nordic countries, including Finland (Yli-Mattila *et al.*, 1997), Denmark, and Sweden (Pettersson and Olvång, 1995). Other less represented or only sporadically occurring species, were *F. acuminatum*, *F. crookwellense*, *F. flocciferum*, *F. oxysporum*, *F. pallidoroseum*, *F. proliferatum*, *F. sambucinum*, *F. sporotrichioides*, *F. chlamydosporum* and *F. moniliforme* (Kosiak *et al.*, 1997; Langseth *et al.*, 1997).

It seems of interest to note that the occurrence of *F. moniliforme* in infected wheat heads in Bulgaria, Czechoslovakia (Parry *et al.*, 1995), Croatia (Jurkovic and Cosic, 1997), Hungary (Tóth, 1997), and even in Norway (Kosiak *et al.*, 1997); as well as of toxigenic strains *F. proliferatum* and *F. moniliforme* in freshly harvested wheat and oats kernels from southern European countries (Italy, France, Portugal, Greece, and Turkey) (Bottalico *et al.*, 1989a), is indicative of the spreading trend of these fumonisin-producing species. As a consequence, it seems that the potential occurrence of FB<sub>1</sub>, till now limited to maize, must be extended also to wheat and other small grains.

*Occurrence of Fusarium mycotoxins in cereal head blight.* The surveys carried out directly in the field or on freshly harvested grains, confirmed the general findings mainly obtained from post-harvest samples. The field survey clearly indicated that the mycotoxins most fre-

quently encountered in head blight of wheat, barley and triticale in all European countries are DON, 3AcDON, and ZEN produced by *F. graminearum* and *F. culmorum* occurring in succession from southern to northern areas (Table 3).

Selected *F. graminearum* and *F. culmorum* infected ears of wheat and triticale collected in Poland, were found to be contaminated by DON (up to 30.4 mg kg<sup>-1</sup>), and 3AcDON (up to 30 mg kg<sup>-1</sup>). In addition, a large survey of grain field samples (200 samples) carried out in 1994-95 crop years in central Poland showed the occurrence of DON, 3-AcDON, and 15-AcDON, in wheat, barley, and oats, with the highest DON level in barley (average of 0.12 mg kg<sup>-1</sup>) (Golinski *et al.*, 1997; Perkowski *et al.*, 1997a). Similar data were obtained for durum wheat in Austria, where in preharvest ears primarily infected by *F. graminearum* and with a lower presence of *F. culmorum*, high amounts of DON (up to 8.2 mg kg<sup>-1</sup>), and lower levels of ZEN (0.33 mg kg<sup>-1</sup>) (Adler *et al.*, 1995) were found. But, a higher occurrence of ZEN, associated with DON was found in neighbouring Slovakia (Srobárová and Pavlová, 1997). In Germany, the severity of wheat head blight and DON content in scabby grains were correlated with the preceding crops, and higher DON concentrations were found in wheat following maize for silage (up to 0.3 mg kg<sup>-1</sup>) and maize for grains (up to 0.5 mg kg<sup>-1</sup>) (Obst *et al.*, 1997).

In Norway, the concentrations of DON in a large number of *Fusarium* infected ear samples of wheat, barley and oats, collected in 1995 under weather conditions that were very conducive to *F. graminearum* attacks, were 2.36, 0.36 and 0.69 mg kg<sup>-1</sup>, respectively (Elen *et al.*, 1997). The occurrence of DON was also reported in Finland (up to 1.18 mg kg<sup>-1</sup>) (Hietaniemi and Kumpulainen, 1991), and in The Netherlands (up to 0.5 mg kg<sup>-1</sup>) together with ZEN (up to 0.2 mg kg<sup>-1</sup>) (De Nijis *et al.*, 1996). The occurrence of mycotoxins in UK small cereals, was generally related to the severity of head blight *Fusarium* infections, and highest DON concentrations were found in grains infected with *F. graminearum* and *F. culmorum* (Turner and Jennings, 1997).

In southern localities of Russia (Krasnodar), *Fusarium* wheat head blight is widespread (losses up to 25-50%), and high amounts of DON (up to 36.25 mg kg<sup>-1</sup>) are frequently found in freshly harvested scabby grains (Leonov *et al.*, 1990; Tutelyan, 1996). Severe infestations of wheat ears by *F. graminearum* are also reported in Bulgaria (scab incidence of 37.2%), with an average of DON content of 0.176 mg kg<sup>-1</sup> (Vrabcheva *et al.*, 1996).

Associated with DON frequently NIV and FUS were found, produced mainly from southern to north-

ern European localities by *F. graminearum* NIV-chemotype and *F. culmorum* NIV-chemotype. In addition, NIV and FUS can be produced by *F. poae*, especially in Sweden and other Nordic countries where this fungus represents the major source of NIV contamination, and also by *F. crookwellense* in grains from central to northeastern Europe (Eriksen and Alexander, 1998). Samples of *Fusarium* infected wheat collected in central Poland in 1987 were found to be contaminated by NIV (up to 0.01 mg kg<sup>-1</sup>) and dideoxy-NIV (up to 0.15 mg kg<sup>-1</sup>), together with DON (up to 40 mg kg<sup>-1</sup>) 3-AcDON (up to 0.10 mg kg<sup>-1</sup>), 15-AcDON (up to 2.0 mg kg<sup>-1</sup>), and ZEN (up to 2.0 mg kg<sup>-1</sup>) (Perkowski *et al.*, 1990). These findings were confirmed during the latest large surveys for scabby field grains particularly infected by *F. poae*, and the highest NIV (average 0.056 mg kg<sup>-1</sup>) and FUS (average 0.052 mg kg<sup>-1</sup>) levels were found in oats (Golinski *et al.*, 1997; Perkowski *et al.*, 1997a). In UK grains, the NIV concentrations were generally related to the severity of scab, and highest levels of NIV were associated with *F. poae* and *F. culmorum*; while higher DON concentrations were found in grains infected by *F. graminearum* and *F. culmorum* (Turner and Jennings, 1997).

Severe infections of *F. avenaceum*, *F. tricinctum*, and to a lesser extent, *F. subglutinans* in central to northeastern European countries were usually responsible for MON occurrence in scabby grains. Significant levels of MON were found in head blight of small cereals in Poland (up to 0.495 mg kg<sup>-1</sup>) (Golinski *et al.*, 1997) and in Austria (up to 0.88 mg kg<sup>-1</sup>) (Lew *et al.*, 1993; Adler *et al.*, 1995).

Finally, epidemics of *F. sporotrichioides* and *F. poae* in cold European localities may lead to the occurrence of T-2 derivatives (T2, HT2), and DAS and MAS, respectively. To this regard, a large Polish survey of scabby field grains showed the occurrence of T2 and HT2 in wheat, barley and oats, with the highest concentrations in oats (average 0.302 mg kg<sup>-1</sup>), predominantly infected by *F. sporotrichioides*. In addition, in barley heads infected by *F. sporotrichioides* collected in south-eastern areas of Poland, T2 (up to 2.4 mg kg<sup>-1</sup>), HT2 (up to 0.37 mg kg<sup>-1</sup>) and T2-tetraol (up to 0.21 mg kg<sup>-1</sup>) were found (Perkowski *et al.*, 1997a, b).

#### **FUSARIUM SPECIES AND MYCOTOXINS IN EAR ROT OF MAIZE**

*Occurrence of Fusarium species in maize ear rot.* The relative incidence of *Fusarium* species and related mycotoxins found in maize ear rot diseases in Europe, are tentatively separated into 'red fusariosis' and 'pink fusariosis' (and related 'random kernel rot'), and sum-

marized in Table. 4.

The distribution and the prevalence of the different *Fusarium* species and of the two kinds of ear rot disease are largely governed by environmental conditions, primarily temperature, as well as by many other factors including agrotechnical practices (Arino and Bullermann, 1994). In general, red fusariosis is particularly severe in years and locations with heavy rainfall and cool temperatures during the summer and early fall (Bocarov-Stancic *et al.*, 1997; Ellend *et al.*, 1997) whereas pink fusariosis prevails in drier and warmer climates and southern areas (Bottalico *et al.*, 1995). However, a critical change in the profile of maize ear rot *Fusarium* complex is even more frequently recorded in several maize growing areas of the world, probably due to the introduction of new genotypes or as consequence of tillage practices more conducive to disease, also in relation to the spreading of maize to new areas. In effect, in surveys performed since 1980's, *F. graminearum* together with *F. moniliforme* prevailed as the causal agents of ear rot in most maize growing areas of the world; whereas in the last ten years the most frequently reported dominant species was *F. moniliforme* associated with *F. subglutinans*, and followed only to a lesser extent by *F. graminearum*. In this context, the emerging trend of *F. proliferatum* not only on maize but also on wheat is even more frequently recorded (Bottalico, 1997).

In maize 'red ear rot' the pathogen usually starts from the tip of the ear and develops a reddish mold covering the ear extensively. On the infected husks and ear shanks the brownish perithecia of the teleomorph *G. zeae* are commonly observed. *F. graminearum* is increasingly distributed from central to northern European areas, and is usually associated with many additional *Fusarium* species, whose occurrence and prevalence changes from region to region, and year to year, depending mainly on the temperature and rain. Associated with *F. graminearum* there are *F. subglutinans* and *F. avenaceum* which prevail from central to northern European areas, respectively (Chelkowski, 1989c; Krska *et al.*, 1996; Bocarov-Stancic *et al.*, 1997; Levic *et al.*, 1997; Palaversic *et al.*, 1997a). In central European localities, together with *F. graminearum*, *F. subglutinans* and *F. avenaceum*, which represent almost 90-95% of all isolated *Fusarium* strains, the other species isolated every year, but to a lesser extent, include: *F. moniliforme*, *F. proliferatum*, *F. crookwellense*, and *F. culmorum*. In addition, among species found sporadically there are *F. sporotrichioides*, *F. poae*, *F. equiseti*, and *F. acuminatum*. Besides, during the last ten year surveys, the most prevalent ear rot *Fusarium* species recorded in the main maize growing areas of Austria, Slovenia and Poland, was *F. subglutinans* (Krska *et al.*, 1996; Milevoj, 1997).

**Table 4.** Toxigenic *Fusarium* species and associated mycotoxins isolated from ear rot of maize in Europe.

Species	Occurrence		Mycotoxin <sup>1</sup>
	North/Center	South	
‘Red ear rot’ or ‘Red fusariosis’			
<i>F. graminearum</i>	+++	+	DON, AcDON, NIV, FUS, ZEN
<i>F. subglutinans</i>	++	±	MON, BEA, FUP
<i>F. avenaceum</i>	++	±	MON
<i>F. moniliforme</i>	+	++	FB <sub>1</sub>
<i>F. crookwellense</i>	+	±	NIV, FUS, ZEN, ZOH
<i>F. culmorum</i>	+	-	DON, NIV, ZEN, ZOH
<i>F. proliferatum</i>	±	++	FB <sub>1</sub> , MON, BEA, FUP
<i>F. sporotrichioides</i>	+	-	T2, HT2, NOS
<i>F. poae</i>	+	-	DAS, NIV
<i>F. equiseti</i>	+	±	DAS, ZEN, ZOH
<i>F. acuminatum</i>	±	±	T2, NEO
‘Pink ear rot’ or ‘Pink fusariosis’ (and ‘Random kernel rot’)			
<i>F. moniliforme</i>	+	+++	FB <sub>1</sub>
<i>F. proliferatum</i>	±	+++	FB <sub>1</sub> , MON, BEA, FUP
<i>F. subglutinans</i>	+++	+	MON, BEA, FUP
<i>F. graminearum</i>	+	±	DON, AcDON, NIV, FUS, ZEN
<i>F. culmorum</i>	+	±	DON, NIV, ZEN, ZOH
<i>F. equiseti</i>	+	±	DAS, ZEN, ZOH
<i>F. solani</i>	±	+	-
<i>F. semitectum</i>	±	+	ZEN, ZOH
<i>F. crookwellense</i>	±	±	NIV, FUS, ZEN, ZOH
<i>F. sporotrichioides</i>	±	-	-
<i>F. oxysporum</i>	-	+	-

<sup>1</sup> Abbreviations: AcDON = Mono-acetyldeoxynivalenols (3-AcDON, 15-AcDON); BEA = Beauvericin; DAS = Diacetoxyscirpenol; DON = Deoxynivalenol or Vomitoxin; FB<sub>1</sub> = Fumonisin B<sub>1</sub>; FUP = Fusaproliferin; FUS = Fusarenone X; HT2 = HT-2 toxin; MON = Moniliformin; NEO = Neosolaniol; NIV = Nivalenol; T2 = T-2 toxin; ZEN = Zearalenone; ZOH = Zearalenols ( $\alpha$  and  $\beta$  isomers).

In maize ‘pink ear rot’, more commonly observed in southern to central Europe, the more frequently isolated species together with *F. moniliforme* are *F. subglutinans* and *F. proliferatum*. In central European areas, *F. proliferatum* is displaced by *F. subglutinans*, and this species predominates as the maize pink ear rot agent from central to northern areas. In effect, *F. proliferatum* was very commonly reported together with *F. moniliforme* in Italy (Logrieco *et al.*, 1995), but in Austria (Krska *et al.*,

1997); Croatia (Jurjevic *et al.*, 1997), Slovak Republic (Piecková and Jesenská, 1997; Srobárová, 1997), and even more so in Poland (Kostechí *et al.*, 1995) the occurrence of *F. proliferatum* was scantily recorded; while in corresponding locations *F. subglutinans* was often isolated in an amount even higher than that of *F. moniliforme*. Associated with *F. moniliforme*, *F. subglutinans*, and *F. proliferatum* many other species were commonly isolated from maize pink ear rot *Fusarium* complex. The

relative species incidence reported by Levic *et al.* (1997) during three year (1994-96) surveys of freshly harvested maize ears in Yugoslavia, included: *F. moniliforme* (63%), *F. subglutinans* (51%), *F. graminearum* (12%), *F. proliferatum* (10%), *F. oxysporum* (6%), and *F. solani* (2%). Other less frequently isolated species included *F. equiseti*, *F. sporotrichioides*, *F. chlamydosporum*, *F. crookwellense*, and *F. semitectum*.

Investigations on how seedborne kernel infection by *F. moniliforme* occurs, proved that this fungus was able to reach kernels by several pathways. In particular, Munkwold *et al.* (1997) reported that the most important pathway for *F. moniliforme* to reach the kernels is through silk infections at silking stage. Therefore, silk infections of *F. moniliforme* could be responsible for the occurrence of 'random kernel rot', appearing as randomly scattered either individual or groups of kernels, usually tan to brown, developing pink mycelium under wet conditions. So that, in association with a more generalized 'ear pink rot' originated from airborne inoculum colonizing the ears from the tip, also a 'random kernel rot' phase of this disease was frequently observed, especially in dry summers. *F. moniliforme* is distributed all over Europe, but the 'random kernel rot' is in general much more severe in southern warm areas, associated with hot, dry seasons.

In southern Italy where *F. moniliforme* largely predominates and environmental conditions are often conducive for high intensity of disease, the 'random kernel rot' is quite prevalent. Together with *F. moniliforme*, a very high incidence of *F. proliferatum* was found. Among the other occasionally isolated species from maize ear rot in Italy there were *F. equiseti*, *F. graminearum*, *F. chlamydosporum*, *F. culmorum*, *F. compactum*, *F. oxysporum*, *F. semitectum*, *F. solani*, *F. sporotrichioides*, and *F. subglutinans* (Logrieco *et al.*, 1995; Riti *et al.*, 1997a). The higher occurrence of *F. proliferatum* in Italy, focused 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 also reported for adjacent European countries (Levic *et al.*, 1997; Srobárová, 1997).

*Occurrence of Fusarium mycotoxins in maize ear rot.* Epidemics of red ear fusariosis, induced by *F. graminearum* and *F. culmorum*, are usually responsible for the occurrence in maize ears of ZEN, DON, and 3AcDON, (Chelkowski, 1989c; Bocarov-Stancic *et al.*, 1997; Palaversic *et al.*, 1997a). A survey of 85 freshly harvested maize ear samples, collected in eastern part of Austria in 1996, mostly contaminated by *F. grami-*

*nearum*, contained DON (up to 2.44 mg kg<sup>-1</sup> in 95% of samples), 15-AcDON (up to 1.11 mg kg<sup>-1</sup> in 54% of samples), 3-AcDON (up to 0.11 mg kg<sup>-1</sup> in 3.5% of samples), and ZEN (up to 0.75 mg kg<sup>-1</sup> in 70% samples) (Ellend *et al.*, 1997). A similar situation, but with higher amounts of DON (up to 334 mg kg<sup>-1</sup>), 15-AcDON (up to 165 mg kg<sup>-1</sup>), 3-AcDON (up to 2.6 mg kg<sup>-1</sup>), and ZEN (up to 17 mg kg<sup>-1</sup>), was found in selected samples of maize ears infected by *F. graminearum*, collected in 1990-91 in Poland (Grabarkiewicz-Szczesna *et al.*, 1996). DON, was occasionally found (up to 67 mg kg<sup>-1</sup>) in selected *F. graminearum* infected maize ears also in northern Italy (Bottalico *et al.*, 1989b).

Associated with DON and ZEN were also usually recorded NIV and FUS produced by DON- and NIV-chemotypes of *F. graminearum*. However, the occurrence of NIV and FUS in red fusariosis of maize in European areas appeared to be related more to the spreading of *F. crookwellense* than of *F. graminearum*. In effect most *F. crookwellense* strains collected from Finland, Germany, Yugoslavia, Italy, and Poland proved to be capable to synthesize NIV and FUS (Bottalico *et al.*, 1990). In addition, in the latest Polish surveys of infected maize samples, together with DON (up to 2.1 mg kg<sup>-1</sup>), 15-AcDON (up to 5.4 mg kg<sup>-1</sup>) and ZEN (up to 99.6 mg kg<sup>-1</sup>), related to the occurrence of *F. graminearum*, just as high concentrations of NIV (up to 42.2 mg kg<sup>-1</sup>) and FUS (up to 0.8 mg kg<sup>-1</sup>) were also found, explainable only by considering the co-occurrence of *F. crookwellense* (Grabarkiewicz-Szczesna *et al.*, 1996).

Epidemics of *F. sporotrichioides* usually led to the accumulation of T-2 derivatives in infected ears. Polish surveys of maize ears in 1984-85 showed *F. sporotrichioides* the predominant fungus in approximately 2% of the samples; and, in corresponding hand-selected heavily damaged kernels up to 1,715 mg kg<sup>-1</sup> of total type-A trichothecenes (T2, HT2, NOS, T-2 triol, T-2 tetraol) were found (Chelkowski *et al.*, 1989c).

Finally, sporadic epidemics of *F. poae* were responsible not only for the accumulation in infected ears of DAS and MAS, but also of NIV and FUS. In fact, in Austrian maize samples together with DON and ZEN likely produced by *F. graminearum* and *F. culmorum*, were found NIV and FUS probably formed also by *F. crookwellense* and *F. poae*, and DAS presumably accumulated by *F. poae* (Bottalico *et al.*, 1989b). Moreover, in Poland where *F. poae* is a widespread agent of maize ear rot, NIV and FUS, together with DAS and MAS, were significantly produced by almost all the strains (13/14), collected from different locations, and NIV was found in naturally infected maize ears either in grains (up to 32.5 mg kg<sup>-1</sup>) and in cobs (up to 13.5 mg kg<sup>-1</sup>) associated with FUS (up to 2.4 mg kg<sup>-1</sup>) (Chelkowski *et al.*, 1994a).

There is increasing evidence of the occurrence of FB<sub>1</sub> in maize and maize based food and feed all over Europe (Pestka *et al.*, 1994; Sanchis *et al.*, 1994; Doko *et al.*, 1995; Patel *et al.*, 1997). Investigations carried out in Italy have revealed that *F. moniliforme* was the most frequently isolated fungus from infected maize plants and from commercial maize kernels associated with FB<sub>1</sub> at levels of up to 5.31 mg kg<sup>-1</sup> (Pietri *et al.*, 1995). However, the occurrence of FB<sub>1</sub> seems relevant in southwestern European areas, *i.e.* Portugal, France (Dragoni *et al.*, 1996), and Italy (Doko and Visconti, 1993), in association with a more severe 'random kernel rot' of maize (Bottalico, 1997); while FB<sub>1</sub> levels are significantly lower in central to northeastern European areas, including

(up to 250 mg kg<sup>-1</sup>), BEA (up to 40 mg kg<sup>-1</sup>) and MON (up to 200 mg kg<sup>-1</sup>) (Logrieco *et al.*, 1995).

Therefore, due to the co-occurrence of both the principal fungal source of FB<sub>1</sub>, the probability of finding such carcinogenic toxins in maize is higher in southern than in central to northern European localities. Marín *et al.* (1996) found *F. proliferatum* capable of growing and synthesizing fumonisins under drier grain conditions than are suitable for *F. moniliforme*. This competitive behaviour suggested that there may be a different prevalence of these species in relation with the weather conditions, as well as their possible succession on ripening maize ear rot.

In central to northern European localities, *F. proli-*

**Table 5.** Toxigenic *Fusarium* species and associated mycotoxins isolated from stalk rot of maize, in Europe.

Species	Occurrence		Mycotoxin <sup>1</sup>
	North/Center	South	
<i>F. culmorum</i>	+++	+	DON, ZEN, ZOH
<i>F. graminearum</i>	++	++	DON, AcDON, ZEN
<i>F. moniliforme</i>	+	+++	–
<i>F. subglutinans</i>	++	+	–
<i>F. crookwellense</i>	+	±	–
<i>F. equiseti</i>	±	+	ZEN, ZOH
<i>F. avenaceum</i>	+	±	–
<i>F. poae</i>	+	–	NIV
<i>F. oxysporum</i>	±	±	–
<i>F. chlamyosporum</i>	–	±	–
<i>F. acuminatum</i>	–	±	–
<i>F. semitectum</i>	–	±	–

<sup>1</sup> Abbreviations: AcDON = Mono-Acetyldeoxynivalenols (15-AcDON, 3-AcDON); DON = Deoxynivalenol or Vomitoxin; NIV = Nivalenol; ZEN = Zearalenone; ZOH = Zearalenols ( $\alpha$  and  $\beta$  isomers).

Austria (Krska *et al.*, 1997), Switzerland (Pittet *et al.*, 1992), Croatia (Palaversic *et al.*, 1997a), Slovak Republic (Piecková and Jesenská, 1997), Germany (Usleber *et al.*, 1994), Czech Republic (Ostrý and Ruprich, 1997), Romania, and Poland (Chelkowski *et al.*, 1994b). In southern European areas, together with *F. moniliforme* is frequently found also *F. proliferatum*, which represents an additional FB<sub>1</sub> source. In particular, Logrieco *et al.* (1995) reported that almost all strains of *F. proliferatum* collected all over Italy produced FB<sub>1</sub> (up to 2,250 mg kg<sup>-1</sup>). In addition, selected maize ears mainly infected by *F. proliferatum* were found to be contaminated by FB<sub>1</sub>

*feratum* is largely displaced by *F. subglutinans* which mainly substitutes also *F. moniliforme*. Consequently, in maize from central to northern Europe, together with negligible amount of FB<sub>1</sub> it is very common to find high amount of MON produced by *F. subglutinans*. In fact, MON was very commonly found in Polish and Austrian samples of maize ears infected by *F. subglutinans* (Kostechi *et al.*, 1995, 1997; Krska *et al.*, 1996), and very high amounts of MON (up to 530 mg kg<sup>-1</sup>) were found in all infected maize ears in Poland during years with severe epidemics of *F. subglutinans* (incidence up to 95-100%) (Lew *et al.*, 1996). The high occurrence of MON

in maize ear rot in central to northern European areas (Austria, Poland) seemed related not only to the spread of *F. subglutinans*, but also to the frequent occurrence of *F. avenaceum* (Lew *et al.*, 1991; Sharman *et al.*, 1991; Krska *et al.*, 1997) and, to a lesser extent, also of *F. proliferatum*. Therefore, MON appears to be one of the more common mycotoxins occurring in ear rot of maize in Europe, because it can be produced from south to north by *F. proliferatum*, *F. subglutinans* and *F. avenaceum*.

In maize ear rot caused by *F. moniliforme*, *F. subglutinans* and *F. proliferatum*, together with the formation of either FB<sub>1</sub> by *F. moniliforme* and *F. proliferatum*, and/or MON by *F. subglutinans* and *F. proliferatum*, the occurrence of BEA in Poland (up to 15.8 mg kg<sup>-1</sup>) (Logrieco *et al.*, 1993; Kostechi *et al.*, 1995), Austria (Krska *et al.*, 1996), and Italy (Moretti *et al.*, 1994; Bottalico *et al.*, 1995; Ritieni *et al.*, 1997b), and of the novel fusariotoxin FUP in Italy (Ritieni *et al.*, 1997b) were also reported. Both, BEA and FUP are commonly produced by strains of *F. subglutinans* and *F. proliferatum* (Moretti *et al.*, 1995, 1997; Logrieco *et al.*, 1996). In addition, BEA (up to 250 mg kg<sup>-1</sup>) and FUP (up to 500 mg kg<sup>-1</sup>) were found in fifteen out of thirty-nine selected samples of preharvest maize ears collected all over Italy during 1993-94, and predominantly infected by *F. proliferatum* (Ritieni *et al.*, 1997b).

**FUSARIUM SPECIES AND MYCOTOXINS IN STALK ROT OF MAIZE.** Several *Fusarium* species are usually isolated from stalk rot of maize in Europe, but the most commonly found are *F. graminearum*, *F. culmorum*, and *F. moniliforme*. While *F. graminearum* predominates in southern to central European areas, and *F. culmorum* seems rather prevailing in more northern areas, (Krüger, 1989; Lew *et al.*, 1997; Palaversic *et al.*, 1997b), *F. moniliforme* is mainly spreading in southern areas (Bottalico *et al.*, 1989b). Many species were isolated with the main colonizers including, *F. equiseti*, *F. semitectum*, *F. oxysporum*, and *F. acuminatum* in southern areas (Bottalico, 1997), and *F. subglutinans*, *F. avenaceum*, *F. crookwellense*, and *F. poae* in central to northeastern areas (Pronczuk *et al.*, 1991; Lew *et al.*, 1997; Milevoj, 1997) (Table 5). However, the assays performed by Pronczuk *et al.* (1997) on maize seedlings confirmed the strong pathogenicity of *F. graminearum* and *F. culmorum*, and indicated a moderate to weak activity for *F. subglutinans*, *F. crookwellense*, *F. avenaceum*, *F. equiseti*, *F. oxysporum*, and *F. poae*, in decreasing order. A particular role in maize stalk rot is played by *F. moniliforme*. This fungus may spread through plants by systemic infection originating from seedborne inoculum, or may penetrate roots and stalks directly, or may enter plants through wounds. *F. moni-*

*forme* is commonly isolated from maize plants even in the absence of visibly symptoms, as happens with some true endophytes. However, when plants are stressed by biotic or abiotic factors, they can be massively invaded and subjected to the typical stalk rot symptoms. In warm temperate and subtropical maize growing areas, *F. moniliforme* is recognized as the primary agent of stalk and ear rot of this cereal.

Samples of stalk rot of maize collected in Italy were found to be contaminated by ZEN (up to 7.4 mg kg<sup>-1</sup>), DON (up to 0.668 mg kg<sup>-1</sup>), and ZOH (up to 0.086 mg kg<sup>-1</sup>), produced by strains of *F. culmorum* (ZEN, ZOH, DON) and *F. equiseti* (ZEN and ZOH) (Bottalico, 1997). The occurrence of ZEN, DON, 3-AcDON and 15-AcDON was also reported in Austrian maize stalks predominantly infected by *F. graminearum*, whereas in leaves severely infected by *F. poae* NIV was found (Lew *et al.*, 1997). In additional surveys, Lew *et al.* (1997) found DON (up to 4.82 mg kg<sup>-1</sup>), 15-AcDON (up to 2.39), 3-AcDON (up to 1.66), ZEN (up to 2.96) and NIV (up to 1.39 mg kg<sup>-1</sup>) also in samples of silo maize consisting of the maize whole plants without ears.

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