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Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe

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Abstract

The *Fusarium* species predominantly found associated with *Fusarium* head blight (FHB) in wheat and other small-grain cereals all over Europe are *F. graminearum*, *F. avenaceum* and *F. culmorum*. Among the less frequently encountered species are several others which are less pathogenic or opportunistic, but also toxigenic. These include *F. poae*, *F. cerealis*, *F. equiseti*, *F. sporotrichioides*, *F. tricinctum* and, to a lesser extent, *F. acuminatum*, *F. subglutinans*, *F. solani*, *F. oxysporum*, *F. verticillioides*, *F. semitectum* and *F. proliferatum*. The species profile of FHB is due to several factors, primarily climatic conditions, particularly rain and the temperature at flowering stage, but also agronomic factors, such as soil cultivation, nitrogen fertilization, fungicides, crop rotation, and host genotype. The most frequently encountered *Fusarium* mycotoxins in FHB in Europe has proved to be deoxynivalenol and zearalenone produced by *F. graminearum* and *F. culmorum*, with the former more common in southern (warmer) and the latter in northern (colder) European areas. Nivalenol was usually found associated with deoxynivalenol and its derivatives (mono-acetyldeoxynivalenols), together with fusarenone-X, formed by *F. graminearum*, *F. cerealis*, *F. culmorum* and, in northern areas, by *F. poae*. Moreover, from central to northern European countries, moniliformin has been consistently reported, as a consequence of the widespread distribution of *F. avenaceum*, whereas the occurrence of T-2 toxin derivatives, such as T-2 toxin and HT-2 toxin, and diacetoxyscirpenol have been recorded in conjunction with sporadic epidemics of *F. sporotrichioides* and *F. poae*. Finally, beauvericin and various enniatins have recently been found in Finnish wheat colonized by *F. avenaceum* and *F. poae*.

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); ENS = Enniatins; FUS = Fusarenone-X (= 4-Acetyl-NIV); HT2 = HT-2 toxin; MAS = Monoacetoxyscirpenol; MON = Moniliformin; NEO = Neosolaniol; NIV = Nivalenol; T2 = T-2 toxin; T2ol = T-2 Tetraol; ZEN = Zearalenone; ZOH = zearalenols (α and β isomers).

Introduction

Several *Fusarium* species are widespread pathogens on small-grain cereals (soft and durum wheat, barley, oats, rye and triticale) around the world, including all European cereal-growing areas. They can cause root, stem and ear rot, resulting in severe reductions in crop yield, often estimated at between 10% and 40%. In addition, several *Fusarium* strains are capable of producing mycotoxins which can be formed in pre-harvest infected plants still standing in the fields, or in stored grain (Bottalico, 1998). Mycotoxins in wheat and barley, which constitute almost two-thirds of the world production of small-grain cereals and almost 80% of the European small-grain production, are causing great concern, because of the extent of infection and contamination of food products. However, the other less frequently-grown small grains have also been reported to contain *Fusarium* mycotoxins, though these crops appear, in general, to be less susceptible to *Fusarium* head blight (FHB) and consequently less at risk from pre-harvest toxin contamination.

The occurrence of mycotoxins in small cereal grains, particularly in wheat, is of great concern worldwide, because their presence in processed feeds and foods seems unavoidable. Consequently, they have been associated with chronic or acute mycotoxicoses in livestock and, to a lesser extent, in humans (IARC, 1993). In this respect, it appears that almost all wheat and barley crops grown in northern European areas are to some extent contaminated, at least by deoxynivalenol (DON) whereas the southern areas seem much less contaminated by *Fusarium* toxins (Bottalico, 1998; Eriksen and Alexander, 1998; Visconti, 2001). The numerous books and reviews recently published demonstrate the increasing interest in FHB and related *Fusarium* toxins in cereal grains. These deal with the several biological aspects of the causal *Fusarium* species, and with the natural occurrence, toxicology and risk assessment of toxic compounds (Chełkowski, 1989a; 1991a; IARC, 1993; Miller and Trenholm, 1994; Miller, 1995; Bottalico, 1997). In addition, several FHB problems have been discussed and published during the six European Seminars so far dedicated to *Fusarium* taxonomy, pathogenicity and mycotoxins (Chełkowski, 1989a; 1991b; Mesterházy, 1997a; Logrieco et al., 1997) as well as within joint European Fusarium projects (Nirenberg, 1995a). Moreover, it is noteworthy that specific Committees operate in several European countries on *ad hoc* programmes dealing with several aspects of FHB in small-grain cereals. Among their aims, is the improvement of the resistance to the disease and the prevention of toxin accumulation (Eriksen and Alexander, 1998; LBP, 2000; Pasquini et al., 2001).

In this paper, the most relevant aspects of the distribution of toxigenic *Fusarium* species and related mycotoxins in ear rot of small-grain cereals in European countries are reviewed, with emphasis on the occurrence of DON, nivalenol (NIV) and other trichothecene derivatives, zearalenone (ZEN), and moniliformin (MON) in infected plants standing in the field, or in freshly harvested grain. The relationship between the *Fusarium* species complex and the relative mycotoxin profile, besides underlining the toxicological risk of FHB, may be useful for predicting the mycotoxins that are most likely to be formed in infected kernels, with reference to the most conducive factors, such as predominant *Fusarium* species, geographical area, environmental conditions, agronomic factors, and host genotype. It has been reported that in organic farming systems, which employ particular safety practices, the incidence of *Fusarium* spp. appeared comparatively lower than that in conventional systems. The DON or ZEN contents in freshly harvested wheat kernels were the same, but this aspect still a critical controversial point.

Fusarium species involved and mycotoxins produced

Fusarium *head blight of small-grain cereals*

Fusarium species which are pathogenic on wheat, barley and other small-grain cereals, are responsible for two forms of disease; 'foot rot' affects roots and crowns, and includes an early stage causing seedling blight and FHB, affecting individual kernels, single ear spikelets or entire heads, leading to scab of the kernels. Infected spikelets first appear water-soaked, lose their chlorophyll and become straw-coloured. In warm, humid weather, pinkish-red mycelium and conidia develop abundantly in the infected spikelets, and the infection spreads to adjacent spikelets or through the entire head. Infected kernels become shrivelled and discoloured with a white, pink, or light brown scaly appearance as a result of the mycelial outgrowths from the pericarp. From the mycotoxicological point of view, the phase of *Fusarium* diseases of small-grain cereals which is of greatest concern is FHB because of the potential accumulation of mycotoxins in grain intended for foods and feeds.

The risks connected with the consumption of contaminated forage and straw by livestock must not be ignored. FHB causes severe damage to wheat and the other cereals, especially in areas with warm temperature and high relative humidity during the heading and flowering period. Together with favourable environmental conditions, other factors determining the severity of the disease include various agronomic factors and host genotype (LBP, 2000).

Fusarium *species involved*

The *Fusarium* species commonly isolated from small-grain cereals in Europe are illustrated in Table 1. Their ability to synthesize mycotoxins was first illustrated by Bottalico (1998). The aetiological characteristic of FHB 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 tissue, or up to seventeen different species from freshly-harvested grain samples collected in a limited area. However, only a small number of species have been regarded as pathogenic and generally, very few of them predominate in a particular host-agroclimatic system. Similar to strains of the pathogenic and predominant *Fusarium* species, several other strains of the less pathogenic or opportunistic *Fusarium* species are capable of producing considerable amounts of mycotoxins. Therefore, the mycotoxigenic profile of a contaminated crop is due not only to the predominant pathogenic *Fusarium* species, but also to the opportunistic species making up the complex. Moreover, the host genotype may play an important role in toxin accumulation. In general, less toxins are formed in the more resistant genotypes (Mesterházy et al., 1991; Mesterházy, 1997b), or in less visibly moulded ears. However, cultivars of wheat showing low FHB severity have been reported with high DON content, and wheat cultivars highly susceptible to FHB may contain lower DON levels. In general, a direct prediction of toxin contamination based on the incidence of FHB, crop yield loss, or scabby grain severity cannot be performed accurately, especially when *Microdochium nivale* or *F. avenaceum* and other *Fusarium* species which do not produce DON predominate in the FHB complex.

The species predominantly found associated with head blight of wheat and other small-grain cereals are *F. graminearum* (and its widespread teleomorph *Gibberella zeae*), *F. culmorum*, and *F. avenaceum* (*G. avenacea*). Among the other less frequently isolated species are *F. poae*, *F. cerealis* (syn. *F. crookwellense*), *F. equiseti* (syn. *F. scirpi*) (*G. intricans*), *F. sporotrichioides*, and *F. tricinctum*. Many other species may be sporadically encountered, including *F. acuminatum*, *F. subglutinans* (syn. *F. sacchari*), *F. solani*, *F. oxysporum*, *F. semitectum* (syn. *F. pallidoroseum*, *F. incarnatum*), *F. verticillioides* (syn.: *F. moniliforme*), and *F. proliferatum*.

Recent mycological, toxicological and molecular studies have clarified many peculiar characteristics of the most important FHB agents, some of which are briefly described. Two populations were previously characterized within *F. graminearum* (*G. zeae*), designated as Group 1 and Group 2. These were differentiated because of colony growth rates and colony morphology on potato dextrose agar (PDA) slants, but they had almost the same toxigenic potential. Group 1 rarely formed perithecia in nature and mainly caused crown rot of cereals and

Table 1. Mycotoxigenic species isolated from FHB of wheat in Europe

Species	Geographical incidence		Mycotoxin
	North/Centre	South	
F. graminearum	+++	+++	**DON**, **NIV**, **ZEN**, AcDON, FUS
F. avenaceum	+++	++	**MON**, BEA, ENS
F. culmorum	+++	++	**DON**, **ZEN**, ZOH,NIV
F. poae	++	+	**NIV**, **BEA**, DAS, FUS, ENS
F. equiseti	++	+	**DAS**, ZEN, ZOH
F. tricinctum	+	+	**MON**
F. cerealis	+	±	**NIV**, **FUS**, **ZEN**, ZOH
F. sporotrichioides	+	±	**T2**, **HT2**, **T2ol**, NEO
F. acuminatum	±	±	**T2**, NEO
F. subglutinans	±	—	**MON**
F. solani	±	—	—
F. oxysporum	±	—	—

AcDON = Monoacetyl-deoxynivalenols (3-AcDON, 15-AcDON); BEA = Beauvericin; DAS = Diacetoxyscirpenol; DON = Deoxynivalenol (Vomitoxin); ENS = Enniatins; FUS = Fusarenone-X (4-Acetyl-NIV); HT2 = HT-2 toxin; MON = Moniliformin; NEO = Neosolaniol; NIV = Nivalenol; T2 = T-2 toxin; T2ol = T-2 tetraol; ZEN = Zearalenone; ZOH = zearalenols (α and β isomers).

grasses, while Group 2 formed abundant perithecia in nature and mainly caused FHB in small-grain cereals (Burgess et al., 1997). Recent investigations on anamorphic and teleomorphic characteristics of the two *F. graminearum* groups, as well as on their phylogenetic relationships and on different modes of sexual reproduction, have led Aoki and O'Donnell (1999a,b) to consider *F. graminearum* Group 1 as a separate species. It was named *F. pseudograminearum* Aoki et O'Donnell. Its teleomorph was characterized through mating experiments and named *Gibberella coronicola* Aoki et O'Donnell. The existence of distinct genetic groups within several strains of *F. graminearum* collected from the USA, Northwest Europe and Nepal has also been reported. These show different pathogenic and toxigenic capabilities on wheat and maize. Moreover, from a toxicological point of view, the toxigenic strains of *F. graminearum* were classified into DON and NIV chemotypes, with NIV chemotypes being strains mainly associated with maize (Sydenham et al., 1991; Szécsi and Bartok, 1995; Yoshizawa, 1997). Furthermore, DON chemotype strains of *F. graminearum* were sub-classified into two types: DON-chemotype IA producing DON and 3-AcDON, essentially from warmer regions (mostly including the European strains), and DON-chemotype IB, producing DON and 15-AcDON from slightly cooler regions (mostly including the American strains) (Miller et al., 1991; Wang and Miller, 1994). Finally, it seems that DON and NIV chemotypes of *F. graminearum* are not distributed evenly around the climatic areas of the world, and such ecological differences in chemotype distribution may contribute to establishing specific regional grain contamination. However, some factors of variability, including a drastic change in climatic conditions at a regional as well as at a continental level can, along with close crop rotations between maize and small-grain cereals, lead to changes in the spread of *Fusarium* species.

Toxigenic strains of *F. culmorum* have been split into two types in relation to the main type B trichothecenes produced, that is DON and NIV chemotypes. Strains of DON-types also produced AcDON (3-AcDON) (Miller et al., 1991; Gang et al., 1998; D'Mello et al., 1997), while strains of NIV-type were also able to produce FUS. In field trials, DON and NIV chemotypes exhibited different aggressiveness toward winter rye (Gang et al., 1998).

Within the section *Sporotrichiella*, a *Fusarium* species described as 'powdery *F. poae*' and responsible for the occurrence of T-2 toxin and its deacetylated derivative HT-2 toxin in Norwegian cereals, has recently been reported. Powdery *F. poae*, which was routinely identified as *F. poae* appears morphologically similar to *F. poae*, but with a mycotoxin profile similar to that of *F. sporotrichioides*. Preliminary results of molecular sequencing indicated that all strains of powdery *F. poae* are closer to *F. sporotrichioides* than those of *F. poae* and *F. kyushuense*. Unfortunately, both *F. poae* and powdery *F. poae* genotypes seemed to have the same geographic and host distribution. This stresses the need for a method which can be used for their distinction.

Fusarium acuminatum sensu lato has recently been split into two subspecies, on the basis of morphological and ecological characteristics, namely *F. acuminatum* subsp. *acuminatum*, and *F. acuminatum* subsp. *armeniacum* (Burgess et al., 1997). Isolates of *F. acuminatum* subsp. *armeniacum* were able to produce larger amounts of T2 and NEO than those produced by *F. acuminatum* spp. *acuminatum* (Logrieco et al., 1992). In addition, some preliminary phylogenetic evidence, apart from the peculiar T2 synthesis capability, suggested a closer genetic affinity between *F. acuminatum* subsp. *armeniacum* and *F. sporotrichioides* than with *F. acuminatum* subsp. *acuminatum*.

Three species were differentiated within *F. sambucinum* on the basis of morphological and toxicological characteristics, namely *F. sambucinum sensu stricto* a producer of DAS, NEO, T2 and ENB; *F. torulosum* a producer of ENB; and *F. venenatum* mainly a DAS producer (Nirenberg, 1995b; Altomare et al., 1995).

F. avenaceum (Fr.) Sacc. is not generally accepted as a homogeneous species. On the basis of the host range and of morphological and ecological characteristics observed in limited Australian environments, this species was recently split into three subspecies, that is *F. avenaceum* subsp. *avenaceum* Sangalang et al.; *F. avenaceum* subsp. *aywerte* Sangalang and Burgess; and *F. avenaceum* subsp. *nurragi* Sommerell and Burgess (Burgess et al., 1994). The restricted niche and the host specialization of the latter two subspecies led to the assumption that only the former could be worldwide in distribution, and all the past references in the literature which related to *F. avenaceum* (Fr.) Sacc. should probably refer to *F. avenaceum* subsp. *avenaceum*. In addition, a number of north European strains of *F. avenaceum* from wheat and barley were not clearly distinguished by cladistic analysis, and even though clustered into several sub-groups, showed no pathogenic specialization on wheat and barley (Yli-Mattila et al., 1997).

Furthermore, notwithstanding the different hosts and ecological conditions that distinguish most European areas, there is only scant evidence of the occurrence of molecular diversity within the population of *F. avenaceum* occurring on small-grain cereals. This situation certainly requires future investigation, especially in relation to strains of *F. tricinctum* which appear to belong to the same geno-species, and which show the same mycotoxin profile, notably in relation to the production of MON (Schütt et al., 1998).

Fusarium nivale 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-grain cereals. *F. nivale* is no longer considered to be a *Fusarium* species. It was first placed in the genus *Gerlachia* (*G. nivalis*), then transferred to *Microdochium* as *M. nivale* (teleomorph *Monographella nivalis*) (Gams, 1989). *M. nivale* has a very low, if any, mycotoxin producing ability (Logrieco et al., 1991), and proved incapable of producing the typical *Fusarium* trichothecenes and zearalenone *in vitro* (Chełkowski et al., 1991; Nakajima and Naito, 1995). No typical *Fusarium* toxins were found in naturally *M. nivale*-infected ears of wheat and rye, even with a blight incidence of up to 46% and 65% respectively (Chełkowski et al., 1991).

Fusarium species and mycotoxins associated with fusarium head blight in Europe

Fusarium species occurring on cereal ears in the field can produce many mycotoxins, some of which are of notable importance. This section focuses on the compounds most commonly found in analytical surveys of field or freshly harvested infected kernel samples. The naturally occurring *Fusarium* mycotoxins belong to three main structural groups, that is trichothecenes, ZEN and MON. In addition, beauvericin (BEA) has also been found in naturally infected small-grain cereal and is considered as an emerging toxicological problem. The principal toxicological features of these mycotoxins are briefly outlined by Bottalico (1998).

Fusarium *species associated with FHB in Europe*

A profile of the *Fusarium* complex species associated with FHB in wheat in Europe is presented in Table 1. Regarding the three predominant species, it appears that *F. graminearum* is the most common in moist-warm continental climates, such as central and south-eastern Europe. In contrast, *F. culmorum* and *F. avenaceum* are more often found in maritime and cooler European areas. However, the influence of several factors favourable to the disease can lead to a change in *Fusarium* profile from region to region, as well as from year to year, as exemplified by the following regional situations.

The data on the incidence of FHB on wheat in Italy in the year 2000 (Pasquini et al., 2001), showed an incidence of the disease in the northern regions (Lombardia, and Emilia-Romagna) (average of 4% on both soft and hard wheat), which was lower than that in central regions (Umbria, Toscana, Lazio and Abruzzo) (average of 35% and 25% on soft and hard wheat, respectively). The disease was absent in the southern regions (Molise, Puglia and Sicily). The higher FHB incidence in central regions can be explained by the more pronounced influence of maritime warmer weather. In addition, the data obtained in the year 2000 showed a slightly higher incidence of FHB on soft wheats than on hard wheat. Other data indicate that hard cultivars were more susceptible than soft cultivars (Balmas et al.1999). FHB is also commonly observed on barley cultivated from central (Lazio) to northern (Emilia-Romagna and Lombardia) Italian regions (Delogu et al., 2001).

The species predominantly found associated with FHB of both soft and hard wheat in Italy are *F. graminearum* (mostly Group 2), *F. culmorum* and *F. avenaceum*. A prevalence of *F. graminearum* and *F. culmorum* was observed when weather conditions around the time of flowering were wetter and warmer than normal, while drier weather appears more conducive to *F. culmorum* and less to *F. graminearum.* In particular, *F. graminearum* (32%), *F. avenaceum* (31%), *F. culmorum* (25%) and, to a lesser extent, *F. cerealis*, *F. poae* and *F. tricinctum* were mostly isolated from FHB of soft wheat (Balmas and Corazza, 1994; Casulli et al., 1995). During three years of observations (1994–1996) of FHB in hard wheat throughout Italy, Pancaldi and Torricelli (1999) found *F. culmorum* and *F. graminearum*, associated with *F. avenaceum*, predominant in all three years, and also *F. verticillioides*, *F. tricinctum* and *F. cerealis* in 1994. In 1996, *F. poae* was isolated with high frequency. Balmas et al. (1999) confirmed the predominance of *F. graminearum* and *F. culmorum* from blighted heads of durum and bread wheat and stressed the prevalence of the former in northern Italy and of the latter in central and southern Italy. Moreover, among the other occurring species involved in FHB of hard wheat were reported

F. avenaceum, *F. acuminatum*, *F. cerealis*, *F. chlamydosporum*, *F. semitectum*, *F. compactum*, *F. equiseti, F. poae*, *F. solani*, *F. oxysporum*, *F. proliferatum*; *F. subglutinans* and *F. sporotrichioides* (Balmas et al., 1999; Pasquini et al., 2001).

A similar *Fusarium* profile observed in Italy was reported for FHB in 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 a partial displacing of *F. graminearum* by *F. avenaceum* and *F. culmorum*, was reported for southern Germany (Bavaria), north-eastern regions of Austria, Croatia, Swiss midlands, and Czech and Slovak Republics. In regions from central to northern Germany an emerging prevalence of *F. poae* and *F. sporotrichioides* was noted, in addition to the three predominant species, together with the occasional appearance of *F. tricinctum*, *F. equiseti*, *F. cerealis*, *F. acuminatum* and *F. solani* (Mauler-Machnik and Suty, 1997; Müller and Reiman, 1997; Schütze et al., 1997). In FHB of durum wheat in Austria, *F. graminearum* (26.8%) was dominant, with *F. avenaceum* (11.1%) *F. poae* (2.6%), *F. culmorum* (1.9%), *F. equiseti* (1.8%), *F. cerealis* (<1%), *F. oxysporum* (<1%) *F. subglutinans* (<1%) and *F. solani* (<1%) (Adler et al., 1995). In the eastern part of Croatia, the following results were found: *F. graminearum* (51.08%), *F. verticillioides* (20.38%), *F. avenaceum* (12.61%), *F. subglutinans* (9.05%), *F. poae* (1.91%) and *F. solani* (1.53%); whereas the species isolated from barley included *F. graminearum* (71.37%), *F. verticillioides* (13.65%), *F. avenaceum* (6.61%), *F. subglutinans* (6.61%), and *F. poae* (1.76%). In the Swiss midlands, the *Fusarium* species most represented in FHB in winter wheat were *F. graminearum* (up to 42% of diseased ears) and *F. avenaceum* (Schachermayr and Fried, 2000). A profile of the *Fusarium* species complex similar to that found in central Europe was reported for the Czech and Slovak Republics where *F. verticillioides* and *F. proliferatum* were isolated from wheat and barley (Srobárová and Pavlová, 1997; Srobárová, 1997). In the Czech Republic the spectrum of species on spring barley in 1997–98 included: *F. culmorum* (70%), *F. poae* (20%), *F. avenaceum* (2%), and to a much lesser extent, *F. stilboides*, *F. aquaeductuum*, *F. merismoides* and *F. gigas*. An increase in FHB severity in spring barley in recent years has been reported, and this change appears to be related to a drastic shift in environmental conditions as well as to agro-technical factors and host genotype. In particular, a different *Fusarium* spp. complex was observed on barley depending on the previous crop: *F. tricinctum* and *F. poae* were dominant after sugar beet, *F. culmorum* and *F. oxysporum* after maize, and *F. culmorum*, *F. poae* and *F. tricinctum* after small-grain cereals.

In cooler maritime areas of the north-western European countries, including north-western areas of France, the Netherlands, Belgium, England, and Scotland, the most common *Fusarium* species involved in FHB in small-grain cereals were *F. culmorum*, *F. graminearum*, *F. avenaceum* and *F. poae*. However, the latest available surveys report an increasing prevalence of *F. culmorum* and a greater importance of *F. poae* and *F. avenaceum*, especially in years less conducive to *F. graminearum* infection (Daamen et al., 1991; Parry et al., 1995; De Nijis et al., 1996). In the United Kingdom, which was characterised by very high FHB in wheat, surveys carried out during 1998 indicated the south west and Wales as the most affected regions, with a prevalence of *F. culmorum* and *F. graminearum* as *Fusarium* causal agents (Polley and Turner, 1995). In the Netherlands, the number of freshly harvested cereal samples infected with *Fusarium* differed considerably between 1991 (34%) and 1993 (83%), possibly due to the humid and dry weather conditions which occurred at flowering. The most predominant species in 1991 were *F. culmorum* and *F. avenaceum* isolated only in northern areas, while in 1993 the most predominant species were *F. poae*, *F. culmorum* and *F. cerealis*, which were isolated throughout the country (De Nijs et al., 1996).

In north-eastern Europe, for example in Poland, the wheat surveys carried out during the late 1990s and covering various climatic areas, indicated the predominance of *F. poae* (64%), followed by *F. tricinctum* (15%), *F. avenaceum* (8%), *F. culmorum* (6%), and *F. graminearum* (4%), together with an emerging occurrence of *F. sporotrichioides*, and a sporadic presence of *F. equiseti* and *F. oxysporum* (Perkowski et al., 1997a; Goliński et al., 1997). However, previous surveys carried out during the 1980s indicated an annual variation in relation to the location and the environmental conditions, showing a wider occurrence of *F. graminearum*, *F. avenaceum* and *F. cerealis* especially on wheat and oats in southern areas, and a greater relevance of *F. poae*, *F. culmorum* and *F. avenaceum* on wheat, triticale and barley in central to northern locations (Perkowski et al., 1990; 1997a). In the seasons between 1985 and 1989, the most frequently isolated species was *F. avenaceum*, both from wheat and triticale, (Lew et al., 1993). Similar results were obtained for freshly harvested kernels of spring barley in the Lublin region, where in three seasons (1986–88) the most frequently isolated *Fusarium* species was *F. avenaceum*, followed by *F. culmorum*, and to a much lesser extent *F. graminearum* (Lacikowa and Kiecana, 1991). Finally, *F. avenaceum* proved to be the dominant *Fusarium* species on triticale. In fact, in 1986 the most important species infecting heads of winter triticale were *F. avenaceum* (39%), *F. culmorum* (20%), *F. graminearum* (14%), and others (6%), including *F. cerealis*, *F. poae* and *F. equisieti*. The following year, 1987, was characterized by a long snowy winter, very conducive to *M. nivale* (64%), and in these conditions the spread of *Fusarium* species was suppressed, with a lower presence of *F. avenaceum* (24%), *F. culmorum* (6%), and *F. graminearum* (5%) (Perkowski et al., 1988). But, in the next period from 1987 to 1989, the percentage of FHB triticale heads infected by *F. avenaceum* was again higher with incidences of 23%, 83%, and 40% (Lew et al., 1993).

In Russia, FHB of small-grain cereals is very widespread and the loss of the wheat crop has reached 25–50%. In north-western regions of Russia, the most frequently isolated species were *F. culmorum*, *F. avenaceum*, *F. tricinctum*, *F. poae*, and *F. sporotrichioides*, while *F. graminearum* was almost absent (Ablova, 1997; Schipilova and Gagkaeva, 1997). In central areas (Moscow region), *F. culmorum*, *F. avenaceum*, *F. sporotrichioides* and *F. poae*, were the dominant species, followed by *F. equiseti*, *F. subglutinans*, *F. tricinctum*, *F. graminearum*, *F. sambucinum*, and *F. acuminatum*. In the warm and humid conditions of the south-eastern European part of Russia (Bielorussia, Krasnodar, Ukraine) and in the Far East, a higher and less variable predominance of *F. graminearum* was reported, together with *F. avenaceum*, *F. poae*, *F. sambucinum* and *F. verticillioides*.

In south-eastern Europe, for example 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%) (Tóth, 1997). 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 occurrence of *F. poae*, *F. semitectum* and *F. oxysporum* (Tóth, 1991). In contrast, in the neighbouring northeast areas of Romania and Bulgaria, the most widespread species was *F. culmorum*.

The northern European situation can be exemplified by the following Norwegian surveys, also confirmed by Finnish observations. The *Fusarium* species most frequently encountered during the latest Norwegian surveys of cereal samples collected from the central to the southern part of the country included, in decreasing order, *F. avenaceum*, *F. tricinctum*, *F. poae*, *F. graminearum*, *F. culmorum*, *F. equiseti*, *F. torulosum*, and *F. sporotrichioides* (Langseth et al., 1997). However, a prevalence of *F. tricinctum*, *F. poae* and *F. culmorum* was observed on spring wheat when weather conditions around the time of flowering were drier and warmer than normal. Wetter and colder weather was conducive to *F. avenaceum* and *F. graminearum* and less to *F. culmorum* (Elen et al., 1997). Other less frequently represented species were *F. acuminatum*, *F. cerealis*, *F. flocciferum*, *F. oxysporum*, *F. pallidoroseum*, *F. proliferatum*, *F. sambucinum*, *F. sporotrichioides*, *F. chlamydosporum* and *F. verticillioides* (Langseth et al., 1997; Kosiak et al., 1997). The Danish and Finnish field situations appear similar, as confirmed from the profile of *Fusarium* species reported by Thrane (2000). In particular, the profile of detected *Fusarium* spp. included, in decreasing order, *F. avenaceum* and *F. tricinctum* as the most frequently represented, followed by *F. poae* and *F. culmorum*, and then to a lesser extent, by *F. graminearum*, *F. equiseti*, and *F. sporotrichioides*. However, in Denmark, *F. culmorum* is believed to be the major cause of FHB of barley.

Fusarium *mycotoxins associated with FHB in Europe*

Deoxynivalenol. Field surveys indicate that the mycotoxins most frequently encountered in FHB of wheat throughout the European countries are DON and its derivatives, produced by *F. graminearum* and *F. culmorum*.

A survey carried out by Pascale et al. (2000, 2001) in Italy, on freshly harvested cereal grains in 1998–2000, showed the occurrence of DON in samples of soft wheat from Emilia-Romagna, both in traditionally grown (incidence 5/36, DON average in contaminated samples 0.146 mg kg^{-1}, range 0.055–0.33 mg kg^{-1}), and biologically-grown cultivars (incidence 18/35, DON average 0.070 mg kg^{-1}, range 0.050–0.105 mg kg^{-1}). Levels of DON higher than that in soft wheat were also found in samples of hard wheat collected from the same region in 1998 (incidence 24/26, DON average 0.202 mg kg^{-1}, range 0.060–1.000 mg kg^{-1}).

Deoxynivalenol was also found in samples of *T. dicoccum* grown biologically (incidence 11/13, DON average 0.237 mg kg^{-1}, range 0.125–0.350 mg kg^{-1}), and in samples of *T. spelta* grown biologically (incidence 7/7, DON average 0.119 mg kg^{-1}, range 0.070–0.220 mg kg^{-1}). Moreover, DON was found in untreated samples of triticale (*T. secalotricum*) (incidence 8/10, DON average 0.116 mg kg^{-1}, range 0.050–0.200 mg kg^{-1}), and barley (incidence 2/20, DON average 0.805 mg kg^{-1}, range 0.070–1.540 mg kg^{-1}). A survey carried out in 1999 showed decreasing contamination from northern Italian regions (Lombardia, Emilia Romagna), to central regions (Toscana, Umbria), with a very low DON incidence in southern regions (Puglia, Basilicata, Sicilia). In that year, contamination was, in general, higher, than that of 1998, and the difference in susceptibility of hard wheat to soft wheat was more marked. The data obtained from surveys carried out in 2000, confirmed both the decreasing DON contamination from northern to southern Italian regions, and the greater susceptibility of hard wheat than soft wheat. In particular, the data obtained in Lombardy showed a degree of contamination for hard wheat (incidence 2/2, DON average 3.385 mg kg^{-1}, range 1.610–6.465 mg kg^{-1}) significantly higher than that of soft wheat (incidence 2/2, DON average 1.371 mg kg^{-1}, range 0.380–3.010 mg kg^{-1}).

In Poland, samples of wheat ears naturally infected with *F. graminearum* and *F. culmorum* were contaminated with DON (100%) and 3AcDON (80%) at very high concentrations (up to 30.4 and 29.54 mg kg^{-1}, respectively), and the levels of DON and 3AcDON in the chaff were 5 and 50 times higher than those in the kernels (Visconti et al., 1986). In addition, analysis of cereal grain field samples (200 samples) carried out in 1994–95 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^{-1}) (Chełkowski, 1989b; Perkowski et al., 1990, 1997b; Goliński et al., 1997). A similar analysis of freshly harvested small-grain cereal from western Poland, showed the occurrence of DON at an average concentration of 0.6 and 1.530 mg kg^{-1} in 1996 and 1997. The DON content in kernels was well correlated to scab symptoms, because the species involved in FHB mostly included the mycotoxin-producing species *F. graminearum* (up to 23%) and *F. culmorum* (up to 25%). Finally, samples of triticale kernels from ears infected by *F. graminearum* and *F. culmorum* were all found contaminated by DON (1.6–16.4 mg kg^{-1}) and 3-AcDON (7–1.4 mg kg^{-1}), with higher concentrations in chaff (9.9–33.2 and 5.2–16 mg kg^{-1}, respectively) (Perkowski et al., 1988).

In Austria, 48 freshly harvested kernel samples of durum wheat predominantly infected by *F. graminearum* and with a lower presence of *F. culmorum*, contained high amounts of DON (up to 8.2 mg kg^{-1}), together with low levels of ZEN (up to 0.330 mg kg^{-1}) (Adler et al., 1995). DON was associated with FHB in neighbouring Slovakia and in the Swiss midlands, together with a high occurrence of ZEN (Srobárová and Pavlová, 1997) in ears of winter wheat heavily contaminated with *F. graminearum*.

In Bavaria, Germany, DON was found in 87% of wheat samples at very high levels (mean 3.96 mg kg^{-1}, max 43.8 mg kg^{-1}), together with ZEN, NIV and T2 (Lepschy-von Gleissenthal et al., 1989). In addition, DON levels between 0.27 and 0.36 mg kg^{-1} were found in field samples of barley, rye and oats (Lepschy-von Gleissenthal et al., 1989). In the same country, Obst et al. (1997) observed that the severity of wheat FHB and DON content in scabby grains were correlated with the nature of the preceding crops. Higher DON concentrations were found in wheat following maize for silage (up to 0.3 mg kg^{-1}) and maize for grain (up to 0.5 mg kg^{-1}). Furthermore, it was observed that the occurrence of DON in harvested wheat and rye was higher in conventional farming systems than in organic systems. In the south-western areas of Germany (Baden-Wuerttemberg), DON (up to 4.76 mg kg^{-1}, mean 0.4 mg kg^{-1}) was found in 89% of several freshly harvested barley seed infected by *Fusarium*, together with low levels of 3-AcDON and ZEN in 48 and 68% of the samples, respectively (Müller and Schwadorf, 1993a). In the same south-western area of Germany, a very high contamination of DON (incidence 96%, mean 1.632 mg kg^{-1}, max 20.538 mg kg^{-1}) associated with 3-AcDON (incidence 59%, mean 0.007 mg kg^{-1}, max 0.018 mg kg^{-1}), ZEN, α-ZOH, NIV, T2, and HT2, was found in 84 farm samples of wheat for feed use collected in a year characterised by heavy rainfall in the summer months (Müller and Schwadorf, 1993b).

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*, were 2.36, 0.36 and 0.69 mg kg^{-1}, respectively (Elen et al., 1997). Moreover, analyses of 5000 freshly harvested wheat, barley and oats samples surveyed during the period 1988–1996, showed a mean year concentration of DON from 0.035 mg kg^{-1} in 1996 to 1072 mg kg^{-1} in 1988, with a maximum DON content from a single sample of 62.050 mg kg^{-1}. The percentages of oats, barley and wheat containing more than 1 mg kg^{-1} of DON were 12.5, 6.6 and 3.1 respectively. Oats generally contained greater amount of DON in the northern areas in Norway (Trondelag region) than in southern areas, whereas barley and wheat were more affected in southern areas (Langseth and Elen, 1996; 1997).

In other Northern countries, the occurrence of DON was reported in Finland (up to 1.18 mg kg^{-1}) (Hietaniemi and Kumpulainen, 1991), and in the Netherlands (up to 0.5 mg kg^{-1}) together with ZEN (De Nijis et al., 1996). The occurrence of mycotoxins in UK small-grain cereals was generally related to the severity of FHB infection, and highest DON concentrations were found in grains infected with *F. culmorum* and *F. graminearum* (Turner and Jennings, 1997). However, the amount of DON produced was lower than that expected from the severity of ear blight, especially when the predominance of *M. nivale* supressed the infections by toxigenic *Fusarium* species, as reported for winter wheat ear blight surveyed in 1998 in southwest England and Wales.

In southern localities of Russia (Krasnodarski krai), FHB in wheat is widespread (losses up to 25–50%), and high amounts of DON and its derivatives were frequently found in freshly harvested scabby grains. In particular, a very high occurrence of DON (incidence of 100%, range 7.25–36.25 mg kg^{-1} was found in 15 selected kernel samples from several fields of wheat affected by FHB predominantly caused by *F. graminearum*, associated with consistent levels of 4,7-dideoxy-NIV (0.16–1.25 mg kg^{-1}) (Leonov et al., 1990).

Severe infestations of wheat ears by *F. graminearum* are also reported in Bulgaria (scab incidence of 37.2%), with an average DON content of 1.8 mg kg^{-1} in 67% of 140 samples collected after harvest throughout the country (DON average in positive samples 0.180 mg kg^{-1}). Together with DON, 3-AcDON and 15-AcDON were found in 2.1% and 0.7% of the samples, respectively (both up to 0.100 mg kg^{-1}) (Vrabcheva et al., 1996; Vrabcheva and Vrabchev, 1997).

In the Czech Republic, DON and 15-AcDON were found in 71% and 12% of the samples. In one sample, the DON concentration was higher than the limit stipulated in Czech food safety regulations (2 mg kg^{-1}). In addition, levels of DON, sometimes higher than the hygiene limit, were found in spring barley grown after maize, and predominantly contaminated by *F. culmorum*.

Although mould growth was reported in Finnish rye in the field, only small amounts of DON were detected in rye kernels. In autumn 1998, in spite of the wet summer, DON was observed in 70% of rye samples, in the concentration range 0.005–0.050 mg kg^{-1}.

Nivalenol and fusarenone-X (FUS). The occurrence of NIV and FUS, mainly co-occurring with DON, was frequently found in ears of cereals affected by FHB from southern to northern European localities, where they were attributed to the presence of *F. graminearum* NIV-chemotypes and to *F. culmorum* NIV-chemotypes. In addition, NIV and FUS formation has also been attributed to the activity of *F. poae*, especially in Sweden and other northern countries and to *F. cerealis*, particularly in grains from central to northeast Europe (Bottalico et al., 1990; Eriksen and Alexander, 1998).

In Polish samples collected in 1987, NIV (up to 0.01 mg kg^{-1}) and 4,7-dideoxy-NIV (=7-deoxy-DON) (up to 0.15 mg kg^{-1}), were found together with DON, 3-AcDON, 15-AcDON, and ZEN (Perkowski et al., 1990). These findings, which for the first time reported the occurrence of 15-AcDON and 3-AcDON in European wheat, were confirmed during the latest surveys of scabby field grains, particularly those infected with *F. poae*, which showed highest NIV (0.056 mg kg^{-1}) and FUS (0.052 mg kg^{-1}) levels in oats (Goliński et al., 1997; Perkowski et al., 1997a). Moreover, samples of freshly harvested oat kernels collected at different locations in Poland, were contaminated by NIV (0.05 mg kg^{-1} (Chełkowski et al., 2000).

In Germany, in 1987, NIV (0.04–0.29 mg kg^{-1}) was found in 30% of wheat samples (Lepschy-von Gleissenthal et al., 1989). In the southwest area of Germany (Baden-Wuerttemberg), samples of wheat for feed use collected in a year characterised by heavy rainfall in the summer months, showed traces of NIV (incidence 26%, mean 0.009 mg kg^{-1}, max 0.032 mg kg^{-1}) associated with DON, 3-AcDON, ZEN, αZOL, T2, and HT2 (Müller and Schwadorf, 1993b).

A survey of 15 selected field kernel samples of wheat from Krasnodarski Krai in the USSR, predominantly colonized by *F. graminearum*, showed high levels of 4,7-dideoxy-NIV (0.16–1.25 mg kg^{-1}) associated with DON (Leonov et al., 1990).

In UK grains, 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).

In the Czech Republic, during a screening of 50 wheat samples collected at harvest in 1999, NIV and FUS were found in 69% and 2% of the samples. Concentrations of NIV lower than the legislated limit (2 mg kg^{-1}), were found in spring barley, particularly when cultivated after small-grain cereals predominantly infected by *F. poae*, *F. culmorum* and *F. tricinctum*.

Zearalenone. Zearalenone, mainly produced by *F. graminearum* and *F. culmorum* and commonly associated with DON and its derivatives, is among the mycotoxins most frequently encountered in FHB in small-grain cereals throughout the European countries.

Austrian pre-harvest hard wheat ears, predominantly infected by *F. graminearum* and with a lower presence of *F. culmorum*, contained low levels of ZEN (0.33 mg kg^{-1}) which were associated with high amounts of DON (up to 8.2 mg kg^{-1}) (Adler et al., 1995). In Slovakia, a high occurrence of ZEN, together with DON, was found associated with FHB in wheat mainly infected by *F. graminearum* (Srobárová and Pavlová, 1997).

In Bavaria, Germany, ZEN (up to 1.56 mg kg^{-1}) was found in 58% of wheat samples, mainly associated with DON (Lepschy-von Gleissenthal et al., 1989). It was also observed that the amount of ZEN in harvested wheat was higher in conventional farming systems (up to 0.74 mg kg^{-1}) than in organic systems (up to 0.47 mg kg^{-1}). A survey of wheat collected on farms from the southwest area of Germany, in a year characterised by heavy rain in the summer months, showed high contamination of ZEN (incidence 80%, mean 0.178 mg kg^{-1}, max 8.036 mg kg^{-1}), co-occurring with α-ZOH (incidence 5%, mean 0.023 mg kg^{-1}, max 0.071 mg kg^{-1}), DON, 3-AcDON, NIV, T2, and HT2 (Müller and Schwadorf, 1993b).

In the Netherlands, ZEN (up to 0.2 mg kg^{-1}) was found in freshly harvested cereal samples, together with DON (De Nijis et al., 1996), and in Bulgaria ZEN was found in 69% of 140 wheat samples for human consumption in concentrations up to 0.120 mg kg^{-1} (mean in positive sample of 0.017 mg kg^{-1}) (Vrabcheva et al., 1996).

T-2 toxin derivatives. Epidemics of *F. sporotrichioides* and *F. poae* in cold European localities may lead to the occurrence of T-2 derivatives (T2, HT2, T2ol), and DAS and MAS, respectively. In Poland scabby field grains contained T2 and HT2 in wheat, barley and oats, with the highest concentrations in oats (average 0.302 mg kg^{-1}, predominantly infected by *F. sporotrichioides* (Perkowski et al., 1997a). In addition, T2 (up to 2.4 mg kg^{-1}), HT2 (up to 0.37 mg kg^{-1}) and T2-tetraol (up to 0.21 mg kg^{-1}) were found in barley heads infected by *F. sporotrichiodes* collected in southeast areas of Poland (Perkowski et al., 1997b). Moreover, samples of freshly harvested oats kernels collected at different locations in Poland were contaminated by DAS (0.02 mg kg^{-1}), T2 (0.06 mg kg^{-1}) and HT2 (0.02 mg kg^{-1}) (Chełkowski et al., 2000).

In Bavaria, Germany, cereal samples containing low levels of T2 (0.005–0.60 mg kg^{-1}) were found in 38% of wheat samples (Lepschy-von Gleissenthal et al., 1989). Wheat collected from farms in Baden-Wuerttemberg showed the occurrence of T2 (incidence 26%, mean 0.82 mg kg^{-1}, max 0.249 mg kg^{-1}) associated with HT2 (incidence 7%, mean 0.010 mg kg^{-1}, max 0.020 mg kg^{-1}), DON, 3-AcDON, ZEN, α-ZOH and NIV (Müller and Schwadorf, 1993b).

In the Czech Republic, HT2, T2ol and DAS were found in 28%, 10% and 6% of the wheat samples analysed in 1999. In addition, T2 (0.055 mg kg^{-1}) was found in 1 of 140 samples of freshly harvested wheat from Bulgaria (Vrabcheva et al., 1996).

Moniliformin. Severe infections of *F. avenaceum*, *F. tricinctum*, and to a lesser extent *F. subglutinans*, from central to north-east European countries, were usually responsible for MON occurring in scabby grains (Kostechi et al., 1995). In particular, in Poland, significant levels of MON were found in scabby kernels obtained in 1988 from ears of wheat and triticale (7.2–25.2 and 2.4–5.1 mg kg^{-1}) highly infected by *F. avenaceum* (Lew et al., 1993). MON was also reported in freshly harvested durum wheat in Austria (up to 0.88 mg kg^{-1}) (Adler et al., 1995). In all these surveys, the MON content in kernels was well correlated with the presence of *F. avenaceum* (up to 38%) (Kostechi et al., 1995; Chełkowski et al., 2000).

Beauvericin. Beauvericin was detected as a natural contaminant of wheat kernels collected in Poland (Kostecki et al., 1997) and co-occurred with ENS in Finland (up to 3.5 mg kg^{-1}). In addition, most Finnish strains of *F. avenaceum* and *F. poae* isolated from contaminated wheat samples produced BEA and other esadepsipeptides in high amounts on autoclaved maize (up to 3,703 mg kg^{-1}) (Logrieco et al., 2002).

Fumonisins. It is interesting to note that the occurrence of *F. verticillioides* and *F. proliferatum* in infected wheat ears in Norway (Kosiak et al., 1997), Bulgaria, the Czech and Slovak Republics (Parry et al., 1995; Srobárová and Pavlová, 1997; Srobárová, 1997) Croatia and Hungary (Tóth, 1997), South Russia and Italy (Pasquini et al., 2001, Pancaldi and Alberti, 2001) as well as of toxigenic strains of (*F. verticillioides* and *F. proliferatum*) in freshly harvested wheat and oats kernels from southern European countries (Italy, France, Portugal, Greece, and Turkey) (Bottalico et al., 1989; Infantino et al., 2001), is indicative of the spread of these fumonisin-producing species. As a consequence, it seems that the occurrence of FB_1, until now limited to maize, can be expected in infected wheat and other small grains. The occurrence of strains of *F. proliferatum* able to produce fumonisins B_1, B_2 and B_3 in rye grains (Fadl-Allah et al., 1997) confirms the above indication of the spread of this phytopathogenic species, and of the potential occurrence of its carcinogenic toxins in small grains.

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