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Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops

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Abstract

Recent data on the epidemiology of the common mycotoxigenic species of *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium* in infected or colonized plants, and in stored or processed plant products from the Mediterranean area are reviewed. Emphasis is placed on the toxigenicity of the causal fungal species and the natural occurrence of well known mycotoxins (aflatoxins, ochratoxins, fumonisins, trichothecenes, zearalenone, patulin, *Alternaria*-toxins and moniliformin), as well as some more recently described compounds (fusaproliferin, beauvericin) whose toxigenic potential is not yet well understood. Several *Fusarium* species reported from throughout the Mediterranean area are responsible of the formation of mycotoxins in infected plants and in plant products, including: *Fusarium graminearum*, *F. culmorum*, *F. cerealis*, *F. avenaceum*, *F. sporotrichioides* and *F. poae*, which produce deoxynivalenol, nivalenol, fusarenone, zearalenone, moniliformin, and T-2 toxin derivatives in wheat and other small grains affected by head blight or scab, and in maize affected by red ear rot. Moreover, strains of *F. verticillioides*, *F. proliferatum*, and *F. subglutinans*, that form fumonisins, beauvericin, fusaproliferin, and moniliformin, are commonly associated with maize affected by ear rot. Fumonisins, were also associated with *Fusarium* crown and root rot of asparagus and Fusarium endosepsis of figs, caused primarily by *F. proliferatum*. Toxigenic *A. alternata* strains and associated tenuazonic acid and alternariols were commonly found in black mould of tomato, black rot of olive and citrus, black point of small cereals, and black mould of several vegetables. Toxigenic strains of *A. carbonarius* and ochratoxin A were often found associated with black rot of grapes, whereas toxigenic strains of *A. flavus* and/or *P. verrucosum*, forming aflatoxins and ochratoxin A, respectively, were found in moulded plant products from small cereals, peanuts, figs, pea, oilseed rape, sunflower seeds, sesame seeds, pistachios, and almonds. Finally, toxigenic strains of *P. expansum* and patulin were frequently found in apple, pear and other fresh fruits affected by blue mould rot, as well as in derived juices and jams.

Introduction

The Mediterranean basin is a large geographical region with a temperate climate and a diversified agricultural system that includes cereals (maize, rice, wheat and other small grains), legumes (beans, peas, peanuts), several kind of vegetables (cucurbits, solanaceous, brassicas), citrus fruits (oranges, mandarin, lemons, grapefruits and limes), pome fruits (apples and pears), stone fruits (peaches, nectarines, apricots, plums and cherries), nuts (almond, hazelnuts, pistachios, walnuts) olive, grapes, soft fruits (figs, strawberries, raspberries), tubers and roots from temperate and sub-tropical plants. On these host plants, pathogenic fungi may induce plant disease and both pathogens and saprophytes can synthesise toxic secondary metabolites, which may lead to the accumulation of mycotoxins in the colonised tissues. Mycotoxin formation may begin in preharvest infected plants standing in the field and be continued or initiated in postharvest and stored products. Mycotoxins in plant products and in processed food and feed have a significant economic impact and pose a serious problem for animal and human health (IARC, 1993). Mycotoxigenic fungi

belong mainly to *Fusarium*, *Alternaria*, *Aspergillus* and *Penicillium* genera. The toxigenic *Fusarium* and *Alternaria* species are often classified as 'field fungi', because they require very high moisture content in the substrate for growth and mycotoxin synthesis (>20%). The 'storage fungi', primarily species of *Aspergillus* and *Penicillium* also grow well at lower moisture contents. Thus, *Fusarium* and *Alternaria* usually represent a high mycotoxicological risk in preharvested or freshly harvested plant that are drying, whereas toxigenic species of *Aspergillus* and *Penicillium* represent a higher risk for products in storage or being used in food and feed processing. The mycotoxicological aspects review here are related primarily to field and storage fungal diseases of important Mediterranean crops and to the mycotoxins synthesised by strains of *Fusarium* (fumonisins, trichothecenes, zearalenones, moniliformin, beauvericin and fusaproliferin), *Alternaria* (alternaric acid, alternariols, altertoxins), *Aspergillus* (aflatoxins, ochratoxins) and *Penicillium* (ochratoxins, citrinin, patulin).

FUSARIUM DISEASES

Fusarium species are distributed worldwide as important plant pathogens, as well as opportunistic colonisers of plants and agricultural commodities, or as saprophytes on debris and cellulosic plant materials. Several species cause a range of plant diseases, such as vascular wilt, root and stem rot, seedling blight, cereal ear rot, and fruit rot. *Fusarium* species are also the major cause of storage rot of fruits and vegetables and are frequently associated with cereal and legume grains, which they usually colonize before harvest. Some *Fusarium* strains can synthesise several mycotoxins, which may accumulate in infected plants before harvest or in stored or processed agricultural commodities. The occurrence of *Fusarium* mycotoxins, particularly in cereal grain is of great concern in all cereal-growing areas, and their occurrence in processed feeds and foods is often associated with mycotoxicoses in humans or domesticated animals. The most common *Fusarium* mycotoxins are trichothecenes, zearalenones, and fumonisins. In addition, moniliformin, beauvericin, and fusaproliferin may occasionally present problems.

Trichothecenes. Based on the functional group at the position in the molecule C-8, the *Fusarium*-trichothecenes may be divided into two types, A and B. Type-A trichothecenes have a functional group at C-8, other than a keto, and include: T-2 toxin (T2) and its derivatives (HT-2 toxin, T-2 triol, T-2 tetraol), produced by strains of *F. sporotrichioides*, *F. acuminatum*, and *F. poae*; diacetoxyscirpenol (DAS) and monoacetoxyscirpenol (MAS), produced by strains of *F. poae*, *F. equiseti* (syn. *F. scirpi*) (*Gibberella intricans*), *F. sambucinum* and *F. sporotrichioides*; and neosolaniol (NEO), produced by strains of *F. sporotrichioides*, *F. poae* and *F. acuminatum.* The type B-trichothecenes are characterised by the presence of a carbonyl group at C-8 and include: deoxynivalenol or vomitoxin (DON) and their mono-(3-AcDON, 15-AcDON) and di-acetylated derivatives (3,15-AcDON), produced by strains of *F. graminearum* (*Gibberella zeae*), and *F. culmorum*; nivalenol (NIV) and its monoacetylated derivative fusarenone X (FUS) and the di-acetylated derivative (4,15-AcNIV), produced by strains of *F. cerealis* (syn. *F. crookwellense*), *F. poae*, *F. graminearum* and *F. culmorum*. Trichothecenes can cause a variety of toxic effects in laboratory and farm animals including skin inflammation, digestive disorders, haemorrhagic syndrome in internal organs, blood disorders, haemolytic imbalance and depletion of the bone marrow, immunosuppression (leukopenia) and disturbance of the nervous system (IARC, 1993). Trichothecenes are responsible for several mycotoxicoses in livestock, including haemorrhagic syndrome caused by A type trichothecenes (T2, DAS and MAS), and emetic and feed refusal syndromes associated with the occurrence of B type trichothecenes (DON, NIV and FUS). T2 and DON also have been implicated in human toxicoses (ATA). However, they have not been proved to be genotoxic and IARC (1993) included the trichothecenes formed by *F. graminearum*, *F. culmorum*, *F. cerealis* and *F. sporotrichioides* amongst the compounds not yet classified for their carcinogenicity in animals or humans.

Zearalenones. Zearalenone (ZEA), produced by *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti* and *F. semitectum* (syn. *F. pallidoroseum*, *F. incarnatum*), is found associated with zearalenols (ZOH) (α- and β-zearalenol isomers). ZEA is among the most widely distributed *Fusarium* mycotoxins in agricultural commodities and is often found at relatively high concentrations, especially in maize. ZEA is both uterotrophic and estrogenic, and may cause reproductive disorders in farm animals, particularly swine. ZEA is responsible for recurring toxicoses in livestock,

characterised by hyperestrogenism in swine, infertility and poor performance in cattle and poultry, and there is a possible impact on human health. The preliminary scanty evidence of the genotoxicity of ZEA, is limited to mice and cultured mammalian and human cells, and it is not classified as human carcinogen (IARC, 1993).

Fumonisins. Fumonisins were first isolated from *F. verticillioides* (syn. *F. moniliforme*) and then also found in cultures of *F. proliferatum* and a few other *Fusarium* species with unclear ecological distributions. Amongst the characterised compounds, fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) present the greatest mycotoxicological concern. Feeds contaminated by FB_1 cause leukoencephalomalacia in horses, pulmonary oedema in swine, poor performance in poultry, and altered hepatic and immune function in cattle. Moreover, home-grown maize contaminated by FB_1 has been associated with oesophageal cancer of humans in Africa, China, and the United States. The structural similarity with sphingosines suggests a role for fumonisins as depletion agents of the complex sphingolipids from biological membranes. This that could account for their toxicity and, perhaps, their carcinogenicity. However, the evidence that cultures of *F. verticillioides* and samples of FB_1 can promote liver cancer in rats, led to the classification of fumonisins as carcinogenic to animals and possibly to humans (Group 2B) (IARC, 1993).

Moniliformin. Moniliformin (MON) has been purified from cultures of several *Fusarium* species, including: *F. proliferatum*, *F. subglutinans*, *F. avenaceum* (*G. avenacea*), and *F. tricinctum*. Diets containing material naturally contaminated with MON can cause reduced performance, haematologic disorders, and mortality in rodents, chicks, ducklings, and pigs. Its significance as a contaminant of maize and other cereal grains has not been clarified. At present, MON is regarded as cytotoxic but not genotoxic, and has yet to be associated with a natural disease outbreak in domesticated animals. MON is suspected as the cause of Keshan disease, a human heart problem that occurs in rural regions of China and South Africa (Transkei) in which maize constitutes a large portion of the diet.

Beauvericin and enniatins. Beauvericin (BEA) is a cyclic hexadepsipeptide first reported to be produced by some entomopathogenic fungi and then found in cultures of *F. semitectum*, *F. subglutinans*, and *F. proliferatum* that were isolated from maize and maize-based feed. Some of these samples were associated with animal toxicoses. In addition to its high toxicity to insects, BEA is cytotoxic to mammalian cell tissues, can cause apoptosis in both murine and human cell lines and reduce the contractility of guinea pigs smooth muscle. However, the biological activity of BEA, as found more in generally for the enniatins, seems mediated by the ability of ionophoric compounds to affect the ion transport across membranes, leading to disruption of the ionic balance of cell wall. Such activity by BEA could increase the toxicity of other *Fusarium* mycotoxins that co-occur with BEA in contaminated cereals.

Fusaproliferin. Fusaproliferin (FUP) is a novel sesterterpene produced by strains of *F. proliferatum* and *F. subglutinans* isolated from maize ear rot and is often found in naturally infected maize. FUP is lethal to *Artemia salina* larvae and cytotoxic to insect and human cell lines. FUP also caused high mortality in broiler chicks fed with maize cultures of *F. proliferatum* and had severe teratogenic effects in chick embryo bioassays.

Head blight of wheat and other small cereals

Causal agents and symptoms. The species predominantly found associated with head blight of wheat and other small-grain cereals in the Mediterranean region are *F. graminearum* and its widespread teleomorph *G. zeae*, *F. culmorum*, and *F. avenaceum*. Less frequently isolated species are *F. poae*, *F. cerealis*, *F. equiseti*, *F. sporotrichioides*, and *F. tricinctum.* Other species encountered sporadically include *F. acuminatum*, *F. subglutinans*, *F. solani*, *F. oxysporum*, *F. semitectum*, *F. verticillioides*, and *F. proliferatum*.

Fusarium pathogens on wheat, barley and other small-grain cereals, are responsible for two forms of disease, a 'foot rot', which affects roots and crowns, and may cause seedling blight at early stages; and a 'head blight' (FHB), which affects individual kernels, single ear spikelets or entire heads, and results in 'scab' of the kernels. Infected spikelets first appear water soaked, then lose their chlorophyll and become straw coloured. In warm, humid weather, pinkish-red mycelium, and conidia develop abundantly in infected spikelets and

the infection can spread 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 mycelial outgrowths from the pericarp. From the mycotoxicological point of view, FHB is of greatest concern, because of the potential accumulation of mycotoxins in scabby grains intended for foods and feeds. However, the risk associated with the consumption of contaminated forage and straw by domesticated animals should not be underestimated. FHB causes severe damage to wheat and the other cereals, especially in areas with warm temperatures and high relative humidity or frequent precipitation during the heading and blossoming periods. In addition to favourable environmental conditions, other factors involved in determining FHB severity, include agronomic factors (e.g. farming systems, soil management and tillage, crop rotation and preceding crop, and fungicide applied) and host genotype, e.g. varietal disease escape and varietal genetic resistance to the pathogen or the toxins it produces. The etiological characteristic of FHB is the co-occurrence, or quick succession, of several *Fusarium* species usually referred to as a 'complex'. It is not uncommon to isolate up to nine different *Fusarium* species from a single fragment of infected tissue, or to recover as many as seventeen different *Fusarium* species from freshly harvested grain samples collected in a limited geographic area. However, only a few species are regarded as pathogenic and generally only a limited number dominate in any particular host-agroclimatic system. Strains of the less-pathogenic or opportunistic *Fusarium* species can also produce considerable amounts of mycotoxins. Thus, the toxigenic profile of a contaminated crop is affected not only by the predominant pathogenic *Fusarium* species, but also by the opportunistic species making up the 'complex'. The *Fusarium* species (Table 1) most frequently isolated from 105 samples of cereals grain (wheat, barley, rice, oat, rye, and maize) collected from Mediterranean countries (Egypt, France, Greece, Lebanon, Turkey, and Yugoslavia) were *F. culmorum* and *F. graminearum* from wheat, *F. verticillioides* and *F. proliferatum* from maize, and *F. semitectum* from rice (Logrieco et al., 1990c).

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

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

[a] 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); EN = enniatins; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; FB_3 = fumonisin B_3; FUP = fusaproliferin; FUS = fusarenone-X (=4-Acetyl-NIV); FUC = fusarochromanone; HT2 = HT-2 toxin; MAS = monoacetoxyscirpenol; MON = moniliformin; NEO = neosolaniol; NIV = nivalenol; T2 = T-2 toxin; ZEA = zearalenone; ZOH = zearalenols (α and β isomers).

[b] Bold letters indicate the main mycotoxin produced.

The incidence FHB on wheat in Italy is exemplified by the data from the 2000 crop in which the incidence of the disease in the northern region averaged 4% on both soft and hard wheat, 35% and 25% on soft and hard wheat in central region and was completely absent in the southern region. The higher incidence of FHB in the central region may be attributable to warmer maritime weather (Pasquini et al., 2001). The dominant FHB species on both soft and hard wheat in Italy are *F. graminearum*, *F. culmorum*, and *F. avenaceum*. *F. graminearum* usually dominates when weather conditions near flowering are wetter and warmer than normal, while drier weather appears to be more conducive to *F. culmorum.* Similar results also have been reported from Spain and France, where the dominant species from wheat were *F. culmorum* and *F. graminearum* followed by *F. poae* (Assemat et al., 1995). Investigations on the toxigenic mycoflora associated with freshly harvested wheat grains collected from different localities in Egypt, the most common *Fusarium* species on small grains are *F. semitectum* and *F. acuminatum*, which are associated with the occurrence of ZEA, DON, NIV, and T2 (El-Kady and El-Maraghy, 1990; Atalla et al., 1999). Severe *Fusarium* head blight also occurs on barley, with significant reductions in yield and quality in several Mediterranean countries including Italy where the dominant species is *F. graminearum* (Delogu et al., 2001), and in Egypt, where the dominant species is *F. acuminatum* (Abdel-Kader et al., 1979).

Toxin formation and natural occurrence. Strains of *Fusarium* species commonly isolated from cereals in the Mediterranean area synthesise different mycotoxins, some at very high concentrations (Table 2). For example, Bakan et al. (2001b) analysed 60 strains of *F. culmorum* isolated from wheat grain collected from different wheat-growing areas in France. They found that 60/60 strains produced ZEA (up to 1700 mg kg^{-1}); 24/60 produced DON (up to 51.9 mg kg^{-1}); 35/60 produced NIV (up to 12 mg kg^{-1}); 12/60 produced FUS (up to 8.4 mg kg^{-1}); 5/60 produced 15-AcDON (up to 28 mg kg^{-1}); and 13/60 produced 3-AcDON (up to 21 mg kg^{-1}).

The mycotoxins most frequently encountered in field surveys of FHB of wheat across of the Mediterranean countries are DON and its derivatives, produced by

Table 2. Profile of mycotoxin production by strains of *Fusarium* from cereal grains in Mediterranean countries[a]

Fusarium species	Strain (ITEM)	Origin	Mycotoxins (mg kg^{-1})					
			ZEA	ZOH	DON	15-AcDON	3-AcDON	MON
F. culmorum	349	Italy	30	ND	20	ND	100	ND
	328	Italy	100	20	5	ND	140	ND
	345	France	270	ND	50	ND	31	ND
	682	Portugal	20	0.2	15	ND	9.5	ND
	627	Yugoslavia	40	0.8	1.9	ND	5.3	ND
	628	Yugoslavia	350	1.4	12	ND	37	ND
F. graminearum	623	Portugal	6	ND	7.7	21.6	ND	ND
	625	Yugoslavia	50	ND	38	90	ND	ND
	626	Yugoslavia	5	ND	14	32	ND	ND
	655	Yugoslavia	22	ND	14	38	ND	ND
	656	Yugoslavia	60	ND	22	71	ND	ND
	657	Yugoslavia	40	ND	21	51	ND	ND
F. cerealis[b]	619	Yugoslavia	60	4.1	ND	ND	ND	ND
F. equiseti	365	Italy	7.4	1.6	ND	ND	ND	ND
	367	Italy	ND	ND	ND	ND	ND	ND
F. avenaceum	314	Italy	ND	ND	ND	ND	ND	2000
	321	Italy	ND	ND	ND	ND	ND	67
	683	Portugal	ND	ND	ND	ND	ND	1200
	620	Yugoslavia	ND	ND	ND	ND	ND	1330
	621	Yugoslavia	ND	ND	ND	ND	ND	40

[a]Strains were grown on autoclaved maize kernels at 25 °C for 4 weeks. ND = not detected.
[b]This strain produced also fusarenon-X (29.2 mg kg^{-1}) and nivalenol (<1 mg kg^{-1}).

F. graminearum and *F. culmorum*. Freshly harvested grain in Italy contained up to 1000 ng g^{-1} of DON in samples of durum and up to 330 ng g^{-1} of DON in soft wheat (Pascale et al., 2001). Similar data were obtained from French wheat, where DON was present at levels up to 650 ng g^{-1} in 90% of freshly harvested grain samples (Bakan et al., 2001a).

NIV and FUS, which usually co-occur with DON, are also common in ears of small-grain cereals affected by FHB in Mediterranean localities and are usually attributed to the activity of *F. graminearum* and *F. culmorum* NIV-chemotypes, and to *F. poae* and *F. cerealis*. In particular, NIV, at levels up to 232 ng g^{-1}, was reported in samples of freshly harvested French wheat (Bakan et al., 2001a).

ZEA, which is produced mainly by *F. graminearum* and *F. culmorum* and commonly co-occurs with DON and its derivatives, is among one of the most frequently encountered mycotoxins in grain from FHB-diseased small-grain cereals throughout the Mediterranean countries. In particular, ZEA (up to 16 ng g^{-1}) was found, together with DON, in surveys of freshly harvested French wheat (Bakan et al., 2001a).

Several strains of *F. avenaceum* isolated from infected samples produced MON, BEA, and other hexadepsipeptides (enniatins B, B_1 and A_1) *in vitro* at high levels (up to 3703 mg kg^{-1}). Recently, these toxins also were found as natural contaminants in wheat kernels that were dominated by *F. avenaceum* (Logrieco et al., 2002).

Ear rot of maize

Fusarium species are responsible for at least two types of maize ear rot, roughly a 'red ear rot' or 'red fusariosis', caused mainly by species of the *Discolor* section, and 'pink-ear rot' or 'pink fusariosis' caused mainly by representatives of the *Liseola* section (Bottalico, 1998). The dominant species causing maize 'red ear rot' are *F. graminearum*, *F. culmorum*, *F. cerealis*, and *F. avenaceum*. The species frequently isolated from maize 'pink-ear rot' are essentially the widespread anamorphs of *F. verticillioides*, *F. proliferatum*, and *F. subglutinans*. Other toxigenic *Fusarium* species less frequently isolated from both types of maize ear rot include *F. equiseti*, *F. poae*, *F. sporotrichioides*, *F. acuminatum*, *F. semitectum*, *F. solani*, and *F. oxysporum*. Finally, there are some species isolated sporadically only from maize, although they may occasionally be reported as a regional problem, such as *F. anthophilum*, *F. chlamydosporum* (syn. *F. fusarioides*), *F. compactum*, *F. heterosporum* (syn.: *F. reticulatum*, *F. graminum*), *F. lateritium*, *F. sambucinum*, *F. torulosum*, and *F. venenatum*.

Fusarium *red ear rot of maize*

Causal agents and symptoms. The dominant species causing maize 'red ear rot' are *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. avenaceum*, and *F. subglutinans*, which represent 90–95% of all isolated *Fusarium* strains from plants with this disease. Others species isolated include: *F. sporotrichioides*, *F. poae*, *F. equiseti*, and *F. acuminatum*, and to a lesser extent *F. verticillioides* and *F. proliferatum*. *F. graminearum* is becoming more widely distributed in continental parts of Europe (e.g. northern Italy, France, and Yugoslavia) and is often associated with many other *Fusarium* species. In maize 'red ear rot', symptoms usually appear at the tip of the ear, with a reddish mould eventually covering the ear extensively. The blue–black perithecia of the teleomorph, *G. zeae*, are commonly observed on infected husks and ear shanks. The severity of the disease and the occurrence and prevalence of the causal species varies from region to region and year to year, depending on the climatic parameters (temperature, relative humidity, rain, and location), and agronomic practices (farming systems, tillage, crop rotation, fertilization, planting area, irrigation, and measures taken for disease and pest control). In this context, the host genotype (e.g. constituents, maturity class, *Fusarium*-susceptibility) can be very important. The *Fusarium* profile, and consequently mycotoxin accumulation, can change drastically if there is insect damage. In particular, on maize ears damaged by the European corn-borer, which is an important vector of *Fusarium* species of the *Liseola* section, e.g. *F. verticillioides* and *F. subglutinans* are isolated more frequently than those of the *Discolor* Section, e.g. *F. graminearum*, *F. culmorum*, and *F. cerealis* (Lew et al., 1991). Control of the European corn borer can thus reduce of the levels of FB_1 and MON in diseased ears, but has little effect on the occurrence of ZEA, DON or NIV, mycotoxins which are produced by *Fusarium* species in the *Discolor* section.

Toxin formation and natural occurrence. In a study of ZEA production by 70 *Fusarium* strains from grain and vegetative parts of cereals, collected from several hosts all over Italy, all the 15 assayed strains

of *F. graminearum* and 15/16 strains of *F. culmorum* produced ZEA (up to 1500 and 6300 mg kg^{-1}), whereas ZEA was not produced by representatives of *F. avenaceum* (19 strains), *F. equiseti* (2), *F. sporotrichioides* (3), *F. tricinctum* (5), *F. solani* (1), *F. oxysporum* (1), *Microdochium nivale* (5) or an unidentified *Fusarium* spp. (3) (Bottalico and Logrieco, 2001). T2 was produced by the *F. sporotrichioides* strains in this study (Bottalico and Logrieco, 2001). Additional data on the potential toxigenic capability of *Fusarium* strains from cereals collected from some Mediterranean localities are illustrated in Table 2 (Logrieco et al., 1990c). The occurrence of toxigenic *Fusarium* strains, including *F. semitectum* and *F. acuminatum*, capable of producing ZEA and DAS was reported in freshly harvested maize kernels from several locations in Egypt (Abdel-Mallek et al., 1994).

Epidemics of maize red ear fusariosis, induced by *F. graminearum* and *F. culmorum*, usually result in contamination of the maize ears by DON, NIV, ZEA, and rarely T2 derivatives. The levels of toxin present are not always proportional to diseases severity. High concentrations of DON (up to 2.8 mg kg^{-1}), NIV (up to 1.3 mg kg^{-1}) and ZEA (up to 1.75 mg kg^{-1}) were found in freshly harvested infected maize ear samples in France (Bakan et al., 2001a). In addition, 107/156 samples of dried maize collected at 12 sites in France were contaminated by ZEA (up to 1.45 mg kg^{-1}) (Bakan, 2001). Occurrence of severe maize red-ear rot, caused primarily by *F. graminearum* and associated DON contamination (up to 67 mg kg^{-1}), has also been reported in Italy in years with cool rainy autumns, and especially on late maturing hybrids (FAO maturity class 500–700) (Bottalico and Logrieco, 2001). Furthermore, ZEA often is associated with DON. ZEA was found in Italy (up to 42 mg kg^{-1}) (Bottalico and Logrieco, 2001), Yugoslavia (up to 10 mg kg^{-1}) (Bočarov-Stančić et al., 1997), and in Egyptian commercial cereals (maize, rice, and wheat), although at relatively low incidence (24 out of 135 samples) and concentrations (5–50 ng g^{-1}) (Abd Alla, 1997).

NIV and FUS have often been reported from Mediterranean maize-growing areas associated with red-ear rot. The formation of these compounds may be due to the NIV chemotype of *F. graminearum*, when all three toxins occur together. NIV and FUS can be found with ZEA when the plant is infected with *F. cerealis*. In particular FUS (up to 0.9 mg kg^{-1}) and NIV (up to 0.7 mg kg^{-1}) were associated with ZEA (1300 mg kg^{-1}) in samples of *Fusarium*-infected maize ears collected in Italy and Austria (Bottalico ad Logrieco, 2001).

In infected maize ears, the main *Fusarium* species capable of producing MON are *F. subglutinans*, *F. proliferatum*, and *F. avenaceum*. While *F. subglutinans* is more widespread in northern Mediterranean localities, *F. proliferatum* is increasingly reported from southern locations. In infected maize ears, *F. subglutinans* appears to be a better producer of MON (up to 400 mg kg^{-1}) than *F. avenaceum* (up to 38 mg kg^{-1}), whereas the toxigenicity of *F. proliferatum* (up to 200) is similar to that of *F. subglutinans* (Logrieco et al., 1995). MON is potentially one of the more common mycotoxins in maize ear rot in Mediterranean basin, because it can be produced in both southern and northern areas. Its production is not limited to the predominant ear-rot agents *F. proliferatum*, *F. subglutinans*, and *F. avenaceum*, but is also produced by other less widespread or opportunistic species, including *F. acuminatum*, *F. tricinctum*, *F. chlamydosporum*, and *F. oxysporum* (Schütt et al., 1998).

Fusarium *pink-ear rot of maize*

Causal agents and symptoms. The species frequently isolated from maize 'pink-ear rot' is usually *G. fujikuroi* and its anamorphs *F. verticillioides*, *F. proliferatum*, and *F. subglutinans*. In maize 'pink-ear rot', the kernels may be infected through the silk but the pathogen may also be introduced into the ear from the tip by other types of external infections or be present as a systemic endophyte. Thus, ear colonisation is a complex process. In association with a more generalised colonisation originating from airborne inoculum colonising the ears from the tip, a 'random kernel rot' phase of the disease also may occur, in which randomly scattered kernels, either individuals or in groups, usually tan to brown, develop pink mycelium under wet conditions. In such cases, the mycotoxin (fumonisins) accumulation in infected kernels may begin early in the maize ear and continue as the kernels reach physiological maturity (Warfield and Gilchrist, 1999).

Maize 'pink-ear rot' is commonly observed in several Mediterranean areas, including Italy (Bottalico et al., 1995), France (Le Bars and Le Bars, 1995), Spain (Rapior et al., 1993), and Egypt (Fadl-Allah, 1997). The dominant species, *F. verticillioides*, is usually associated with *F. proliferatum* and, to a lesser extent, with

F. subglutinans. In particular, *F. proliferatum* usually occurs together with *F. verticillioides* in more southern areas, e.g. Italy (Logrieco et al., 1995), France (Melcion et al., 1998), and Egypt (Fadl-Allah et al., 1997), but is displaced by *F. subglutinans* in northern areas, e.g. Croatia (Jurjević et al., 1997). Logrieco et al. (1995) examined 42 samples of preharvest maize ear rot collected in 1992–1993 from different maize fields throughout Italy and found the following incidence of *Fusarium* species. *F. proliferatum* (34%) and *F. moniliforme* (54%) were the dominant species, followed by *F. equiseti* (8%) and *F. graminearum* (2%), and to a much lesser extent by *F. chlamydosporum*, *F. culmorum*, *F. oxysporum*, *F. semitectum*, *F. solani*, *F. sporotrichioides*, and *F. subglutinans*. This was the first report of a high incidence of toxigenic *F. proliferatum* strains as causal agents of maize ear rot, and strongly suggests a more significant role for *F. proliferatum* in maize ear rot and in associated fusariotoxicoses, than has previously been thought, especially since *F. proliferatum* is relatively easy to confuse with other closely related species in the *Liseola* section.

Toxin formation and natural occurrence. *Gibberella fujikuroi* has been divided into eight distinct mating populations (biological species), designated as A–H, including several *Fusarium* anamorphs that differ in their host preference and toxigenic capability. Of these, the three most commonly recovered from maize are *F. verticillioides* (mating population A), *F. proliferatum* (MP D), and *F. subglutinans* (MP E). Most strains of *F. verticillioides* from Italy produced FB_1 (up to 3800 mg kg^{-1}) and traces of BEA. Strains of *F. proliferatum* produced FB_1 (up to 2500 mg kg^{-1}), MON (5300 mg kg^{-1}), BEA (200 mg kg^{-1}) and FUP (300 mg kg^{-1}). Strains of *F. subglutinans* produced high amounts of MON (3300 mg kg^{-1}), BEA (180 mg kg^{-1}) and FUP (1500 mg kg^{-1}) (Moretti et al., 1997; Bottalico and Logrieco, 2001).

A survey of fumonisin production by 28 strains of *F. verticillioides* and 1 strain of *F. proliferatum* from Italy, Spain, and France showed that all of the assayed strains produced FB_1 (up to 4100 mg kg^{-1}) and FB_2, with the highest average production by strains from maize (average 1300 mg kg^{-1}), followed by those from wheat (average 770 mg kg^{-1}), barley (average 320 mg kg^{-1}), and sorghum (10–15 mg kg^{-1}); whereas only traces of fumonisins were produced by 11 strains of *F. subglutinans* (Visconti and Doko, 1994). *Fusarium* species from Spanish maize-based animal feeds (Sanchis et al., 2001) were dominated by *F. verticillioides*, which produced FB_1 (up to 4200 mg kg^{-1}) and FB_2 (up to 1100 mg kg^{-1}), followed by *F. proliferatum* which also produced FB_1 (up to 3900 mg kg^{-1}) and FB_2 (up to 1700 mg kg^{-1}); whereas none of the very few strains of *F. subglutinans*, *F. graminearum*, *F. avenaceum*, and *F. semitectum* produced any fumonisins. Mycotoxin production by 26 strains from Italian preharvest maize ear rot samples was as follows: all the 26 assayed strains (100%) produced FB_1 (up to 2250 mg kg^{-1}); 22 strains (85%) also produced BEA (up to 200 mg kg^{-1}); and 12 strains (46%) produced together FB_1 and BEA and also MON (up to 5300 mg kg^{-1}) (Logrieco et al., 1995).

High levels of FB_1 are frequently reported in freshly harvested maize ears in Spain (Rapior et al., 1993), Italy (up to 250 mg kg^{-1}) (Pietri et al., 1995; Visconti et al., 1996), France (up to 3.3 mg kg^{-1}) (Bakan et al., 2001a), the Adriatic Zadarska region of Croatia (up to 5753 mg kg^{-1}) (Jurjević et al., 1999) and Egypt (up to 780 ng g^{-1} in maize and maize-based products) (El-Sayed et al., 2001). In contrast, fumonisin levels are often lower in the central and northeastern Mediterranean areas (Bottalico, 1998). In the central Mediterranean areas, due to the co-occurrence of both the principal fungal producers of FB_1, i.e. *F. verticillioides* and *F. proliferatum*, the probability of finding fumonisin toxins in maize is higher than that in northern Mediterranean regions. Thus, Marín et al. (1995) found *F. proliferatum* strains that could produce fumonisins under grain conditions drier than normally suitable for *F. verticillioides*. These results suggest that the species recovered might vary with the weather conditions and appear in succession in the ripening maize ears. Given the widespread formation of fumonisins in preharvest *Fusarium*-infected maize ears, the occurrence of fumonisins in commercial grains and in maize-based foodstuffs is to be expected. In dried maize collected at 12 locations of France in 1997, FB_1 occurred in 107/156 samples (up to 4 mg kg^{-1}) (Bakan, 2001). In Spanish maize-based foods for human consumption purchased from different retail markets during two distinct surveys (Sanchis et al., 2001), 8/50 and 17/36 samples were contaminated with up to 200 ng g^{-1} or 940 ng g^{-1} fumonisins, respectively. Furthermore, analyses of moistened or moulded maize samples involved in disease out breaks in farm animals in Spain, from 1994 to 1999, revealed the presence of fumonisins in 18/48 samples (up to 1800 ng g^{-1}), associated with little if any presence of DON and ZEA (Jiménez and Mateo, 2001). Fumonisin

B_1 was also found (up to 86 ng mg^{-1}) in 14/96 sample of Spanish beer (Torres et al., 1998).

In maize pink-ear rot, associated with large amounts of FB_1 produced by *F. verticillioides* and *F. proliferatum*, the co-occurrence of MON, BEA or FUP produced by *F. proliferatum* and/or *F. subglutinans* has also been reported. MON is expected throughout the Mediterranean maize growing areas, because MON can be produced not only by the predominant ear rot agents, e.g., *F. proliferatum*, *F. subglutinans*, and *F. avenaceum*, but also by other less widespread or opportunistic species, e.g. *F. acuminatum*, *F. tricinctum*, *F. chlamydosporum*, and *F. oxysporum* (Schütt et al., 1998). In Italy, high levels of MON (up to 8200 mg kg^{-1}) were found in preharvest selected maize ears heavily infested with *F. proliferatum* in association with *F. verticillioides* (Bottalico and Logrieco, 2001).

The co-occurrence of BEA, with FB_1 and/or MON in maize ears infected by *F. proliferatum* was first reported in preharvest *F. proliferatum*-infected samples in Sardinia, and then in samples from other Italian localities (up to 520 mg kg^{-1}) (Bottalico and Logrieco, 2001). It is probably safe to assume that BEA is more widespread in Mediterranean areas than has been reported, because this toxin is also produced by several other *Fusarium* species found on maize ears, e.g. *F. subglutinans*, *F. avenaceum*, *F. acuminatum*, *F. equiseti*, *F. poae*, and *F. oxysporum.*

An important emerging toxicological problem appears to be associated with FUP. This fusariotoxin is commonly produced, often together with BEA, by strains of *F. proliferatum* and *F. subglutinans.* Since the first recovery of FUP from infected maize ears in Italy (up to 500 mg kg^{-1}), this toxin has been increasingly found in maize pink-ear rot associated with *F. proliferatum* (Bottalico and Logrieco, 2001).

Stalk rot of maize

Causal agents and symptoms. Several *Fusarium* species are commonly isolated from stalk rot of maize in Mediterranean regions, but the most common are *F. verticillioides, F. graminearum*, and *F. culmorum*. *F. verticillioides* is usually the most prominent and may spread within plants through systemic infections originating from seedborne inoculum. It also penetrates roots and stalks directly and may enter plants through wounds. The most important pathway for *F. verticillioides* to infect maize seed is through infections of the silks (Munkwold et al., 1997). *F. verticillioides* is commonly isolated from maize plants even in the absence of visible symptoms. This behaviour resembles that of some true endophytes and in this symptomless state this fungus may contribute to mycotoxin accumulation in both diseased and 'healthy' tissues and kernels (Bacon and Hinton, 1996). However, when plants are stressed by biotic or abiotic factors, they can be massively colonised by endophytic strains of *F. verticillioides* and develop typical stalk rot symptoms. *F. verticillioides* and *F. graminearum* are usually isolated from visibly infected plants. *F. culmorum* is common in cooler areas (Bottalico et al., 1989). Many other species can be isolated from maize, including *F. equiseti*, *F. semitectum*, *F. oxysporum*, and *F. acuminatum* in southern areas and *F. subglutinans*, *F. avenaceum*, *F cerealis*, and *F. poae* in central to northern areas (Bottalico, 1998). With respect to pathogenicity, maize seedling assays confirmed the aggressiveness of *F. verticillioides* and the strong pathogenicity of *F. graminearum* and *F. culmorum*, while indicating moderate to weak activity for *F. subglutinans*, *F. crookwellense*, *F. avenaceum*, *F. equiseti*, *F. oxysporum*, and *F. poae* (Pronczuk et al., 1991).

Toxin formation and natural occurrence. Samples of rotted stalk of maize collected in Italy were contaminated by ZEA (up to 7.4 mg kg^{-1}), DON (up to 0.67 mg kg^{-1}) and ZON (up to 0.086 mg kg^{-1}) which were produced by strains of *F. culmorum* and *F. graminearum* (ZEA, ZON, DON) and *F. equiseti* (ZEA and ZON) (Bottalico et al., 1985).

Fusarium *crown and root rot of Asparagus*

Symptoms and causal agents. The main symptom of *Fusarium* crown and root rot of Asparagus (*A. officinalis*) is the premature yellowing of the fronds in midsummer, consisting of chlorosis and wilting of individual stalks, progressive discoloration of plants and destruction of roots and crown. The *Fusarium* species most frequently reported as the casual agents of the disease are *F. oxysporum* f. sp. *asparagi* and *F. proliferatum*. The relative importance of these species depends on geographic location (Elmer, 1995). In Italy, diseased asparagus plants were colonized by *F. proliferatum* (72%) and *F. oxysporum* (28%) as the dominant species, with less frequently recovered species (23%), including *F. equiseti*, *F. solani*,

F. acuminatum subsp. *acuminatum*, *F. compactum*, *F. scirpi*, and *F. graminearum*. *F. proliferatum* was isolated more frequently from crowns than roots and *F. oxysporum* was isolated more frequently from roots than crowns (Logrieco et al., 1998).

Toxin formation and natural occurrence. On autoclaved maize kernels, nine of the *F. proliferatum* strains produced high levels of FB_1 (up to 2500 mg kg^{-1}), FB_2 (950 mg kg^{-1}), BEA (90 mg kg^{-1}) and FUP (300 mg kg^{-1}). In addition, FB_1 (up to 0.5 mg kg^{-1}), and FB_2 (0.1 mg kg^{-1}) were detected in samples of crowns and stems of asparagus plants, respectively (Logrieco et al., 1998). The natural occurrence of fumonisins in asparagus tissues colonized by *F. proliferatum* indicates a possible risk for human health (Nigh et al., 1999; Seefelder et al., 2002).

Fusarium *fig endosepsis*

Causal agents and symptoms. Fig endosepsis, also called pink rot or soft rot, is a serious disease of fruits of the Common Fig (*Ficus carica*) associated with several *Fusarium* species, particularly *F. verticillioides*, *F. solani* and *F. dimerum*, but also *F. proliferatum*, *F. subglutinans*, *F. lactis*, and *F. ramigenum* (Moretti et al., 2000).

Toxin formation and natural occurrence. All strains of *Fusarium* species isolated from rotted figs in Italy produced fusaric acid on autoclaved maize kernels at very low levels, with the exception of one strain of *F. subglutinans* which produced high levels of this toxin. In addition, strains of *F. subglutinans* and *F. proliferatum* produced BEA and FUP. Fumonisins were detected in cultures of all strains of *F. proliferatum*. Low levels of fumonisins were found in some samples of rotted fig fruits (Moretti et al., 2000).

Fusarium *on other crops*

Samples of dry pea (*Pisum sativum*) seeds were contaminated with several strains of *Fusarium* and contained T2 toxin (Saber et al., 1998), presumably produced by strains of *F. acuminatum*. Toxigenic *Fusarium* strains (presumably *F. semitectum* and *F. acuminatum*), which can produce ZEA and DAS, were recorded in sunflower (*Helianthus annuus)* seeds from several locations in Egypt (Abdel-Mallek et al., 1994). Also in Egypt, toxigenic strains of *F. verticillioides*, producing FB_1 (up to 73 ng ml^{-1}), were reported in the predominant fungal flora associated with decayed sugarcane (*Saccharum officinarum*) (Ziedan and Hegazy, 2002). *Fusarium* toxins have also been found at very low levels in beans, peanuts, chilli, curry, ginger, garlic, coriander, black tea, and medicinal plants. Although these food categories are usually consumed in small amounts and do not appear to represent a real risk, they could contribute to an intake of *Fusarium* toxins.

In general, exposure of plants to *Fusarium* toxins and their relative toxicological risk have not yet been well established. For example, toxigenic *Fusarium* species are important pathogens and root colonisers of various fruits (e.g. banana, mango, and pineapple) and vegetables (e.g. red clover and potato), but so far, there are no reports of the natural occurrence of *Fusarium* mycotoxins in products obtained from these plants.

ALTERNARIA DISEASES

The genus *Alternaria* is ubiquitous and abundant in the atmosphere as well as in soil, seeds, and agricultural commodities. It includes plant pathogenic and saprophytic species that may affect crops in the field or can cause harvest and postharvest decay of plant products. Of the many species belonging to *Alternaria*, the most common is *A. alternata* (*A. tenuis*), which is widespread across plants, seasons and geographic regions, and contains host-specific pathogenic strains, as well as opportunistic and saprophytic forms causing spoilage of freshly harvested crops. Species of *Alternaria* produce many secondary metabolites, mostly phytotoxins, which play an important role in the pathogenesis of plants. Some species, in particular *A. alternata*, can produce mycotoxins in infected plants and/or in agricultural commodities. The major *Alternaria* mycotoxins belong to three structural classes: the tetramic acid derivative, tenuazonic acid; the dibenzopyrone derivatives, alternariol, alternariol mono-methyl ether and altenuene; and the perylene derivatives, the altertoxins (Bottalico and Logrieco, 1998).

Tenuazonic acid. Tenuazonic acid (TA) is a well-known mycotoxin and phytotoxin, produced primarily by *A. alternata* (*A. tenuis*), but also by other phytopathogenic *Alternaria* species, including

A. capsici-annui, *A. citri*, *A. kikuchiana*, *A. japonica*, *A. longipes*, *A. porri*, *A. radicina*, *A. tenuissima*, and *A. tomato*. Tenuazonic acid is also produced by other species of fungi including, *Pyricularia oryzae* and *Phoma sorghina*. TA is toxic to a wide spectrum of viruses, bacteria, fungi and plants. Tenuazonic acid is toxic to mice, chick embryos and chickens. In particular, increasing TA in chicken feed from sublethal to lethal levels progressively reduced feed efficiency, suppressed weight gain and increased internal haemorrhaging. Sorghum grain colonised by *P. sorghina* that contained TA were associated with the human haematological disorder known as Onyalay (Bottalico and Logrieco, 1998).

Alternariols. Alternariol (AOH) and alternariol monomethyl ether (AME), are produced primarily by *A. alternata*, but also by other *Alternaria* species, including *A. brassicae*, *A. capsici-annui*, *A. citri*, *A. cucumerina*, *A. dauci*, *A. kikuchiana*, *A. tenuissima*, *A. tomato*, and *A. solani.* AME is also produced by *A. longipes* and *A. porri.* Toxic activity of the alternariols is greater towards bacteria than forwards fungi. AOH and AME are usually found in combination and have some teratogenic and fetotoxic effects in pregnant mice (Bottalico and Logrieco, 1998).

Altertoxins. Altertoxins I, II and III (ATX-I, ATX-II, and ATX-III) are produced primarily by *A. alternata*, but also by other *Alternaria* species, including *A. mali*, *A. radicina*, *A. tenuissima*, and *A. tomato*. The great interest in these toxins is related to their mutagenic activity, in particular that of ATX-III, whose mutagenicity is approximately ten times lower than that of aflatoxin B_1 (Bottalico and Logrieco, 1998).

Black mould of tomato

Causal agents and symptoms. *Alternaria* tomato fruit rot occurs on green and ripe fruit affected by physiological alterations, e.g. nutritional deficiency, skin sunburn, etc. The disease is favoured by warm rainy weather or dew formation on the fruit surface and is more severe if infection occurs when the fruit is ripe rather than green. The disease may cause substantial losses, especially of tomatoes for canning. Fruit rot lesions are circular to irregular in shape, slightly sunken and dark green to almost black from the abundant sporulation of the fungus. *Alternaria* rot sometimes develops on the fruit beneath the sepals and can colonise the internal tissues without an obvious external infection. In southern Italy, *A. alternata* is the dominant fungal species although *A. tenuissima* has also been recovered.

Toxin formation and natural occurrence. Sixteen of seventeen strains of *A. alternata* from black mould tomatoes collected in Italy, produced *in vitro* high amounts of TA (up to 4200 mg kg^{-1}), AOH (600 mg kg^{-1}), AME (100 mg kg^{-1}), ALT (30 mg kg^{-1}), and ATX-I (13 mg kg^{-1}), while three strains of *A. tenuissima* produced only very low levels of TA. In naturally infected tomato fruits collected in southern Italy, *Alternaria*-mycotoxins were present as TA (up to 7200 ng g^{-1}), mainly associated with AME (up to 270 ng g^{-1}) and AOH (up to 1300 ng g^{-1}) (Bottalico and Logrieco, 1998).

Black rot of olive

Causal agents and symptoms. Olives are often affected by *Alternaria* species, particularly if the fruits remain on the soil for a long time after ripening. Surface physical damage of the olive fruit due to unfavourable conditions, e.g. low temperature and insects, etc., is a major precondition for fungal penetration into the fruit pulp and for subsequent mycelial proliferation.

Toxin formation and natural occurrence. A study of mycotoxin production by strains of *A. alternata* isolated from olives showed that the toxigenic potential of *A. alternata* is much higher on rice than that it is on olives. In particular, the production of TA, AME and AOH on rice was 1000, 150, and 60 times higher than on olives. Moreover, ALT and ATX-I, which were produced on rice at average levels of 5.9 and 5 mg kg^{-1}, respectively, were not detected in olive cultures. The amount of TA produced on rice by these isolates of *A. alternata* is very high, up to 9800 mg kg^{-1} with an average over 6000 mg kg^{-1} (Visconti et al., 1986).

In moulded or damaged olive samples collected in Apulia (Italy), 4/13 samples were contaminated with two to four *Alternaria* mycotoxins, i.e. AOH, AME, ALT, ATX I or TA. The highest levels of contamination were found in a badly damaged sample containing 2900, 2300, 1400, and 260 ng g^{-1} of AME, AOH, ALT and TA, respectively. No mycotoxins were detected in olive oil for human consumption (six samples) or olive husks (three samples) collected from oil mills after the first pressing (Visconti et al., 1986).

Black and grey rot of citrus fruits, with reference to mandarin

Causal agents and symptoms. Mandarin fruits with *Alternaria* black rot have been observed in several locations in southern Italy at harvest time. In the first stage of the disease, the fruit have no visible symptoms, but later the surface turns dark, beginning at the peduncle. In advanced stages of the disease, the fruit usually falls to the ground. Two kinds of *Alternaria* heart rot are distinguished, based on the colour of the diseased tissues (grey and black). The causal agents produce grey and black colonies when cultured on potato-sucrose-agar under light. The black colour is associated with sporulation, whereas the grey colour is associated with felty grey mycelium with conidiophores growing on the aerial hyphae. Both strains colonise mandarin, but the black strain also colonises oranges and lemons (Logrieco et al., 1990a).

Toxin production and natural occurrence. Grey strains of *A. alternata* from mandarin fruits in southern Italy produced TA (up to 85 mg kg^{-1}), AOH (0.92 mg kg^{-1}), AME (0.17 mg kg^{-1}), and ATX-I (0.2 mg kg^{-1}). A black strain produced TA (up to 93 mg kg^{-1}), AOH (8.7 mg kg^{-1}), and AME (6.3 mg kg^{-1}), but did not produce ATX-I or ALT. Toxin formation occurred more readily on a rice substrate than in whole mandarin fruits. On autoclaved rice the grey strain of *A. alternata* produced TA (up to 6800 mg kg^{-1}), AOH (12 mg kg^{-1}), AME (7.5 mg kg^{-1}), ATX-I (83 mg kg^{-1}), and ALT (25 mg kg^{-1}), while the black strain produced TA (up to 170 mg kg^{-1}), AOH (20 mg kg^{-1}), AME (20 mg kg^{-1}), ATX-I (7.5 mg kg^{-1}), and ALT (50 mg kg^{-1}) (Logrieco et al., 1990). Oranges and lemons can also be contaminated with *Alternaria* and some toxins were detected when artificially infected with *A. citri*. Investigations carried out on the natural occurrence of mycotoxins in infected fruits showed that samples of the two kinds of mandarin heart rot contained different mycotoxin profiles. In black rot samples, TA, AME, and AOH (up to 87, 1.4, and 5.2 mg kg^{-1}) were found, whereas TA (up to 174 mg kg^{-1}) was the only detectable mycotoxin in grey rot samples. None of the natural *Alternaria* rot samples contained ALT or ATX-I (Logrieco et al., 1990a).

Black point of small-grain cereals

Causal agents and symptoms. Cereal grains are frequently infected by species of *Alternaria*, in particular *A. alternata*, which can cause a disease called 'black point', which consists of a discoloration of the germ and the seed due to mycelial and conidial masses. This

Table 3. Incidence of *Alternaria* species in cereal grains from Mediterranean countries

Cereal	Origin	Samples examined No.	Mean % of infected kernels[a]	Mean % of *Alternaria* infected grain			
				Total	*A. alternata*	*A. triticina*	Other[b]
Barley	Greece	1	100	86	53	33	0
	Lebanon	2	100	59	33	26	5
	Turkey	5	70	0	0	0	0
	Yugoslavia	2	100	17	15	2	0
Maize	France	1	95	0	0	0	0
Oat	Greece	1	100	49	45	4	0
Rice	Egypt	10	92	<1	0	<1	0
Rye	Greece	1	100	10	5	5	0
	Greece	1	70	0	0	0	0
Wheat	France	6	93	45	31	14	0
	Greece	5	93	58	42	9	0
	Italy	13	97	50	42	9	3
	Lebanon	5	72	0	0	0	0
	Turkey	10	98	48	37	11	2
	Yugoslavia	23	100	7	5	2	0

[a]Percentages are based on 50 kernels per sample.
[b]*A. tenuissima* and *Alternaria* anamorph of *Pleospora infectoria*.

disease is frequent and serious when persistent rainfall, heavy dews or irrigation occur during kernel development, although a high incidence has also been observed in relatively dry weather.

In a survey carried out on grain samples (wheat, barley, rice, oat, rye, and maize) from Egypt, France, Greece, Italy, Lebanon, Portugal, Turkey, and Yugoslavia, the highest percentage of *Alternaria* infection was found in kernels of barley (up to 86%), wheat and oats, whereas rice and maize were for the most part uninfected. Two predominant species of *Alternaria* were isolated, *A. triticina* and *A. alternata*. Occasionally, *A. tenuissima* and the *Alternaria* state of *Pleospora infectoria* were also recorded (Table 3) (Logrieco et al., 1990b).

Toxin production and natural occurrence. Investigations of mycotoxin production on autoclaved rice by strains of *A. alternata* from wheat, collected in Italy, Yugoslavia, Greece, Lebanon, Egypt, and Turkey, showed that 14/14 produced TA (up to 6000 mg kg^{-1}); 13/14 produced AOH (120 mg kg^{-1}), and AME (59 mg kg^{-1}); 13/14 produced ALT (37 mg kg^{-1}); 14/14 produced ATX-I (32 mg kg^{-1}), and 13/14 produced ATX-II (100 mg kg^{-1}) (Logrieco et al., 1990b). Additional investigations carried out with some of these isolates grown on wheat and rice showed that TA production was greater on wheat (up to 8700 mg kg^{-1}) than on rice, while the opposite was observed for the benzopyrenes and perylene derivatives (Table 4) (Bottalico and Logrieco, 1992). A high incidence of toxigenic strains was found among 176 isolates of *A. alternata* from barley collected in Spain, i.e., 88.6% produced TA, 15.3% produced AOH and 9% produced AME (Sanchis et al., 1993).

Alternaria *blackmould of other crops*

Sunflower. The sunflower head rot disease, caused by *A. alternata*, usually starts from the external basal part of the head and then, favoured by humid atmosphere, the mycelium colonises the seeds through the internal tissues. The superficial lesions become covered with dark spores. Sunflower seeds from infected heads collected in south Italy were contaminated with AOH and AME, up to 1.8 and 0.13 mg kg^{-1}, respectively (Bottalico and Logrieco, 2001).

*Oilseed rape (*Brassica napus*).* All of the 11 isolates of *A. alternata* from samples of siliquae black spot or from oilseed rape (nine from seeds and four from siliquae) collected all over Italy, when cultured on autoclaved rice produced TA (up to 12000 mg kg^{-1}), AOH (200 mg kg^{-1}), AME (200 mg kg^{-1}), ATX-I (250 mg kg^{-1}), and ATX-II (70 mg kg^{-1}) (Visconti et al., 1992). However, *Alternaria* mycotoxins, i.e. TA, AOH, AME, ANE, ATX-I, ATX-II, and ATX-III were not found in 16 samples of oilseed rape collected in oil processing plants, although the samples were contaminated with strains of *A. alternata* capable of producing high amounts of these mycotoxins on rice (Visconti et al., 1992). Strains of *A. alternata* were predominant

Table 4. Profile of mycotoxin production by strains of *A. alternata* from cereal grains in Mediterranean countries[a]

Alternaria strain	Origin	Mycotoxins (mg kg^{-1})[b]					
		TA	AME	AOH	ALT	ATX-I	ATX-II
ITEM-752	Greece	2500	19	62	3	4	6
ITEM-753	Greece	3500	52	120	37	7	5
ITEM-754	Greece	2800	7	48	10	3	2
ITEM-755	Greece	1400	5	5	1	2	3
ITEM-756	Yugoslavia	6000	21	69	14	5	ND
ITEM-757	Lebanon	3100	ND	ND	3	2	5
ITEM-758	Lebanon	4700	20	47	2	4	3
ITEM-761	Lebanon	1900	26	17	ND	26	100
ITEM-759	Egypt	2200	16	56	3	2	16
ITEM-760	Turkey	3200	13	9	2	5	13
ITEM-762	Italy	3200	32	80	4	32	3
ITEM-763	Italy	2800	59	100	7	15	11

[a]Strains grown on autoclaved rice kernels at 25 °C for 4 weeks. ND = not detected.
[b]Abbreviations: TA = tenuazonic acid; AME = alternariol monomlethyl ether; AOH = alternariol; ALT = altenuene; ATX-I = altertoxin I; ATX-II = altertoxin II.

among the mycoflora isolated from 20 oilseed rape samples collected in Catalonia (Spain), but none of the examined samples was contaminated with *Alternaria* mycotoxins (Vinas et al., 1994).

*Pepper (*Capsicum annuum*).* Black rot infection of *A. alternata* occurs on pepper fruits when they are over ripened or injured by sunlight. The colonised spots on the fruit surface reveal a dense, velvety-olive green or black spore mass. Moreover, the mycelium develops in the internal tissues invading either the pericarp or the placenta. Furthermore, the fungus can continue the colonisation during low-temperature refrigerated transport and storage. Samples of peppers with black spot, collected in southern Italy, were contaminated with low concentrations of TA (up to 54 ng g^{-1}) AME (49 ng g^{-1}), and AOH (640 ng g^{-1}) (Bottalico and Logrieco, 2001).

*Melon (*Cucumis melo*).* The black mould disease of melon is caused by *A. alternata*, but strains of *A. cucumerina* have also been found. Fruit which has suffered sun injury is especially prone to infection. Mycelium, especially in a humid atmosphere, can colonise large areas and develop in the internal tissues, while the superficial lesions become covered with dark spores. Samples of melon showing superficial black spot of *A. alternata*, collected in southern Italy, were contaminated with traces of TA (8 ng g^{-1}) and AME (5.1 ng g^{-1}) (Bottalico and Logrieco, 2001).

ASPERGILLUS DISEASES

Aspergillus is among the most ubiquitous fungal genera, mostly including saprophytic species which colonise plant debris or deteriorated agricultural commodities, but also a few strains which are able to colonise living plants. Besides their economic significance as spoilage agents of plant products, particularly grains and legumes, some species are capable of producing mycotoxins which are able to elicit tremendous effects on human and animal health. The mycotoxins of great interest produced by *Aspergillus* include aflatoxins and ochratoxins.

Aflatoxins. Aflatoxins are produced by some strains of *A. flavus*, which form essentially aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2), and by most strains of *A. parasiticus*, which also form aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). Of these four main aflatoxins, AFB_1 and AFG_1, occur most frequently and in the largest amounts in plant products. Aflatoxin-producing strains of *A. flavus* and *A. parasiticus* are distributed worldwide in soil and air and are classifiable as storage fungi and field fungi, since some strains are phytopathogenic. These can infect plants in the field and then colonise harvested or stored plant products. Aflatoxins can thus accumulate in many important agricultural commodities. Plant products which become particularly contaminated are those from tropical and sub-tropical areas and include maize and other cereal grains, groundnuts and other legumes, cottonseed and other oilseeds, cassava and other roots and tubers, most nut crops, most fresh and dried fruits, spices and herbs, and most vegetables. Aflatoxins may also contaminate many processed foods and feeds. Aflatoxins exhibit many adverse biological effects on living organisms, such as a broad range of toxic activities, which are related to their reaction with nucleic acid and cell nucleoproteins, and the ultimate effect of these reactions on protein synthesis and cellular integrity. Aflatoxins are primarily potent hepatotoxins, causing aflatoxicoses in man and animals. They occur in farm animals, both as chronic diseases characterised by an impairment of resistance and immune responsiveness, which result in a reduction in growth rate and feed efficiency; and as acute poisoning characterised by severe clinical disease, liver tumours, and death. Because of their mutagenic, teratogenic, and carcinogenic potency, aflatoxins are classified within Group 1, as compound carcinogenic to humans (IARC, 1993).

Ochratoxins. Ochratoxins are produced by a few strains of *A. ochraceus* and allied species belonging to section *Circumdati* (particularly *A. alliaceus*), by almost all strains of *A. carbonarius* and other strains of the closely related *A. niger*, and by some *Penicillium* species, primarily *P. verrucosum*. These three groups of species show different behaviours in respect to ecological niches, the plant products affected and their geographical occurrence. *A. ochraceus* and its allied species grow at warm temperature and down to a_w 0.80, and can sporadically affect cereals, other plant products and stored food, as well as coffee and cocoa beans during sun-drying. *P. verrucosum* grows well below 30 °C and down to a_w 0.80, therefore it is usually found on cereals in cool temperate regions of northern Europe and Canada, though occasionally in Mediterranean sea localities. *A. carbonarius* grows at moderate to high

temperatures and is associated with maturing fruits, especially grapes.

Of the nine described ochratoxins, only ochratoxin A (OTA) plays a role as an environmental toxin. Field cases of ochratoxicosis in farm animals, mainly pigs and poultry, have been reported in many areas of the world. Its primary manifestation is a chronic nephropathy, characterised by kidney lesions and secondarily by carcinogenicity to the upper urinary system. However, the doses at which the renal tumours were observed in rodents were higher than those that caused nephrotoxicity. OTA has also been found in human blood samples, most notably in people living in the Balkans where a fatal human kidney disease (Balkan Endemic Nephropathy) occurs. This syndrome is usually associated with an increased incidence of tumours of the upper urinary tract. However, the clinical and epidemiological data available were deemed inadequate for calculating the carcinogenicity potency of OTA in humans and led to the inclusion of this toxins in Group 2A (IARC, 1993).

Black rot of grapes

Causal agents and symptoms. The disease is particularly severe in the warmer grape-producing southern localities of Spain, France, Italy, Greece, Egypt, and Morocco. The fungus survives on plant debris in the soil and the conidia are disseminated in vineyards by warm (25–30 °C) air currents. The fungus causes infection via injuries, essentially in mature berries, and then under warm conditions it spreads throughout the bunch. Infection and colonisation of the bunch may also occur through injuries caused by careless handling. The disease is caused by species of *Aspergillus* section *Nigri*, and in particular by *A. carbonarius*/*A. niger*. Because of its black spores, this fungus is highly resistant to sunlight, and survives sun-drying. It is the source of OTA in grapes, dried vine fruits, and wine, especially red wine. OTA is formed prior to alcoholic fermentation (Zimmerli and Dick, 1996) and it is more commonly detected in red wines (54%), than in rosé (40%) and white wines (25%), with concentrations higher in red wines. (Otteneder and Majerus, 2000). In addition, contamination of wine from northern areas is lower than that of wine from southern or warmer areas (for red wine 12% and 95%, respectively) (Otteneder and Majerus, 2000).

Of the 692 strains of *Aspergillus* isolated in Italy from black moulded grapes, 87% belonged to section *Nigri*, represented by *A. carbonarius* (26.6%) and the closely related species *A. niger* (28.8%), and by *A. japonicus* (44.6%) (Bottalico et al., 2002). Investigations carried out by Sage et al. (2002), showed that 6/11 grape samples collected from southern France were contaminated by potentially ochratoxigenic strains and all 14 strains of *A. carbonarius* tested were able to produce OTA (up to 87 mg kg^{-1}).

Toxin formation and natural occurrence. In Italy, 56 samples of red (38), rosé (8), white (9) and dessert (1) wine were contaminated with high levels of OTA ranging from <10 to 7600 ng l^{-1} with red wines more contaminated than rosé and white wines (Visconti et al., 1999). Additional data reported by Castellari et al. (2000), indicated the presence of OTA in samples of red wine from Italy (7/7, up to 500 ng l^{-1}) and Spain (1/1, 74 ng l^{-1}), and in samples of white wine from Italy (1/1, 12 ng l^{-1}) and France (1/1, 11 ng l^{-1}). In Italy, OTA was found in 14/96 red wines (up to 3200 ng l^{-1}) and in 6 out of 15 special dessert white wines (up to 3800 ng l^{-1}), with the distribution of OTA higher in wines produced in southern regions (Pietri et al., 2001).

In Spain, OTA was found in 267 samples of wines of different types and 18 samples of grape products. Contamination was evident in 92%, 91%, and 65% of red, rosé, and white wines (mean OTA concentration of 54, 31, and 20 ng l^{-1} (Burdaspal and Legarda, 2000). In addition, OTA was detected in 74 % of 47 samples of sherry wines (mean 40 ng l^{-1}), in 83% of 12 samples of sparkling wines (mean 12 ng l^{-1}), and in 94% of 16 samples of dessert wines (mean 1000 ng l^{-1}). In a preliminary study by Filali et al. (2001) all of the 30 Moroccan samples of wine were contaminated with OTA (up to 3200 ng l^{-1}).

Immunoaffinity HPLC on 31 samples of red wine originating from Mediterranean sea countries and 15 samples of vinegar, revealed that 72% of the wine samples and 100% of the vinegar samples were contaminated with OTA (up to 3.4 and 0.25 ng ml^{-1}) (Markaki et al., 2001). In southern France, 8/11 musts from grape samples were contaminated with OTA (up to 460 ng l^{-1}), and there was a strong correlation between the presence of *A. carbonarius* on grapes and OTA in musts (Sage et al., 2002). The average levels of OTA in locally produced red and white wines in Cyprus in 1999 year were 0.12 and 0.2 µg kg^{-1} (Ioannou-Kakouri et al., 2001). Sanchis et al. (2001) also found OTA in samples of beer from Europe. Thirty-seven out of 38 Spanish samples were contaminated with OTA

(up to 75 ng l^{-1}) and it was present in all 41 samples from other EU countries (up to 121 ng l^{-1}).

Aspergillus *moulding of* cereals

Causal agents and symptoms. Among the many fungal species causing moulding of cereals in Mediterranean areas there are, in particular, some aflatoxigenic strains of *A. flavus* and *A. parasiticus*, and some ochratoxigenic strains of *A. ochraceus* and *P. verrucosum*. These toxigenic strains, essentially with an opportunistic or saprophytic behaviour, may colonise cereals before harvest under wet favourable climatic conditions, but particularly after harvest and during storage and processing when the water content of the product is favourable (>15–17%).

Aflatoxigenic fungi belonging to the *A. flavus* group, namely *A. flavus* and *A. parasiticus*, were reported in Spain to occur widely in cereal grain even at high contamination levels, i.e. maize (100%), wheat (100%), sorghum (94%), barley (74%), and rice (34%), but no aflatoxins were found in 165 samples collected from markets (Jiménez and Mateo, 2001). However, analysis of moistened or moulded maize samples associated with problems in farm animals in Spain, during 1994–1999, revealed aflatoxins in 13 of 38 samples (up to 3300 ng g^{-1}), and OTA in 3 of 18 samples (up to 150 ng g^{-1}). Other species of *Aspergillus*, occurring in commercial cereal samples from Spanish markets, with proven toxigenicity, were *A. niger* (OTA), *A. glaucus*, (OTA), *A. ochraceus* (OTA), *A. candidus* (OTA) and *A. fumigatus* (OTA), and in lower proportions *A. chevalieri* (sterygmatocystin (STG)), *A. clavatus* (PAT), *A. terreus* (patulin (PAT)), *A. versicolor* (STG) and *A. amstelodami* (STG) (Jiménez and Mateo, 2001).

Investigations carried out on the toxigenic mycoflora associated with freshly harvested wheat grains collected from different localities in Egypt led to the isolation, together with other fungal genera, of some representatives of *Aspergillus* associated with the occurrence of aflatoxins and ochratoxins (El-Kady and El-Maraghy, 1990). Additional surveys carried out by Atalla et al. (1999) on the mycoflora associated with diseased wheat grains collected from different regions, led to the isolation of some representatives of *Aspergillus* associated with the presence of AFB_1, AFB_2, AFG_1, AFG_2, OTA, OTB, and STG. It can be presumed that wheat grains may be colonised to some extent, from harvest to storage, by *A. flavus, A. parasiticus, A. ochraceus*, and *A. versicolor*. Furthermore, Abdel-Mallek et al. (1994) reported the occurrence of toxigenic *Aspergillus* strains in maize kernels. These were capable of producing aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) (presumably from *A. flavus* and *A. parasiticus*), STG (presumably from *A. versicolor*), and OTA (presumably from *A. ochraceus*).

Toxin formation and natural occurrence of aflatoxins and ochratoxins. Aflatoxins (traces to 7 ng g^{-1}) were reported in 36 of 58 wheat samples collected from experimental field plots in northern Italy (Lombardy). This was probably due to bad climatic conditions at harvest time and/or in appropriate postharvest drying procedures (Perenzin et al., 2001). Such data confirmed the widespread occurrence of aflatoxigenic strains of *Aspergillus* in Italy and stressed the importance of the postharvest care of grains. In Croatia, OTA was present in 76% of 94 wheat samples (up to 160 ng g^{-1}) and in 33% of 51 maize samples (up to 40 ng g^{-1}) (Puntaric et al., 2001). In Cyprus, in 1997–1999, the average of positive samples (>0.2 ng mg^{-1} OTA) of locally grown cereals (maize and barley) and cereal products (wheatflour and biscuits) was 23%. The range of toxin levels (0.3–1 ng mg^{-1}) was lower than the maximum tolerable limits (5 ng mg^{-1}) adopted by EU countries for wheat (Ioannou-Kakouri et al., 2001). Analysis of 100 maize-based Spanish product revealed that only one feed sample was contaminated with AFB_1 (0.15 mg kg^{-1}) and AFB_2 (0.08 mg kg^{-1}) (Sanchis et al., 1995).

Aspergillus *on other crops*

The aflatoxigenic capability of 91 strains of *Aspergillus* belonging to 11 species groups of Raper and Fennell's nomenclature and 22 strains of *Penicillium*, isolated from plant products collected in Italy, confirmed the production of aflatoxins (AFB_1 and AFB_2) by strains of *A. flavus* (11 out of 33) and *A. parasiticus* (two out of two), capable of producing AFB_1, AFB_2, AFG_1, and AFG_2. Aflatoxins were not produced by representatives of the other *Aspergillus* groups, including *A. glaucus* (17 strains), *A. cervinus* (1), *A. fumigatus* (1), *A. ochraceus* (6), *A. niger* (12), *A. candidus* (2), *A. flavus* (33), *A. wentii* (9), *A. cremeus* (1), *A. flaviceps* (1), *A. terreus* (1), and unclassified *Aspergillus* spp. (7); or by 22 strains of *Penicillium*.

The ochratoxigenic capability of 195 strains of *Aspergillus* isolated from Spanish animal mixed feeds and raw plant products (maize, soybeans and peas) was determined. They included 1 strain of *A. alliaceus*, 12 of *A. candidus*, 45 of *A. flavus*, 15 of *A. fumigatus*, 12 of *A. ochraceus*, 2 of *A. tamarii* Kita, 10 of *A. terreus*, 10 of *A. versicolor*, 23 of *Eurotium amstelodami*, 31 of *E. chevalieri*, 15 of *E. rubrum*, and 19 of *A. niger*. OTA was found in cultures of 2 strains of *A. ochraceus*, 1 of *A. versicolor*, 1 of *A. fumigatus*, the only assayed strain of *A. alliaceus* and 2 of *A. niger* (Sanchis et al., 2001).

Peanuts (Arachys hypogea)
Determinable levels of aflatoxins were found in 14 of 41 samples of raw shelled peanuts imported into the United States from Egypt in 1981, with three samples exceeding the maximum tolerable concentration of 26 ng g^{-1} (Jelinek et al., 1989). Surveys carried out in Cyprus on 1580 unsorted samples of field peanuts indicated, for 1997 an incidence of 6.7% and a level of total aflatoxins, (up to 780 μ g kg^{-1}) confirming that peanuts are very susceptible to aflatoxin contamination (Ioannou-Kakouri et al., 2001). Aflatoxigenic strains belonging to the *A. flavus* group, namely *A. flavus and A. parasiticus* occur widely in Spanish peanuts Jiménez and Mateo (2001). AFB_1 (10 ng kg^{-1}) was found in one sample. In samples of Spanish peanuts, other species of *Aspergillus,* with proven toxigenicity were *A. niger, A. glaucus* and *A. ochraceus* (OTA), and in lower proportion *A. candidus, A. fumigatus*, and *A. chevalieri* (STG), *A. clavatus* (PAT), *A. terreus* (PAT) *A. versicolor*, and *A. amstelodami* (STG) (Jiménez and Mateo, 2001).

Almond (Prunus amygdalus)*, Hazelnut* (Corylus avellana)*, and Pistachio* (Pistacia vera)
The results of a preliminary survey on the occurrence of aflatoxigenic *Aspergillus* strains and aflatoxins in freshly harvested and processed (peeled, sliced, diced, and grounds) almonds, showed that a negligible aflatoxin risk, if any, was associated with Italian processed products (principally ground almond), whereas traces of aflatoxins were usually associated with whole almonds from Morocco (Bottalico and Logrieco, 2001). *A. flavus* and *A. parasiticus*, were reported by Jiménez and Mateo (2001) to occur in Spanish nut samples, i.e. pistachios (84%), almonds (76%), and hazelnuts (76%). AFB_1 (95 ng kg^{-1}) and AFB_2 (15 ng kg^{-1}) were found in one sample of almonds. Other species of *Aspergillus* with proven toxigenicity, occurring in nuts samples, were *A. niger* (OTA), and in lower proportions *A. glaucus* and *A. ochraceus* (OTA), *A. candidus, A. fumigatus, A. chevalieri, A. versicolor*, and *A. amstelodami* (STG), *A. clavatus* and *A. terreus* (PAT) (Jiménez and Mateo, 2001). Aflatoxins were also found in 4 of 11 samples of pistachios from Sicily (up to 45.07 ng g^{-1} of AFB_2 and AFG_2), in three out of seven samples from Greece (up to 87 ng g^{-1} of AFB_1, AFB_2, AFG_1, and AFG_2) and in three out of six samples from Turkey (up to 102 ng g^{-1} of AFB_1, AFB_2, AFG_1, and AFG_2) (Barbagallo and Russo, 1999).

Common Fig Tree (Ficus carica)
Dried fig samples (110) imported from Turkey were surveyed by Gelosa (1990). Sixty-one percent were contaminated with strains of *Aspergillus* and *Penicillium* and contained aflatoxins (14% up to 340 ng g^{-1}). In Cyprus, some samples of local figs from the 1998 crop were contaminated with aflatoxin, but the levels (up to 5 μg kg^{-1}) were within the maximum permitted levels (10 μg kg^{-1} total aflatoxins, of which AFB_1 should be not more than 5 μg kg^{-1}) (Ioannou-Kakouri et al., 2001).

Pea
Samples of dry pea seeds were contaminated by several strains of *Aspergillus* and aflatoxins (Saber et al., 1998).

Oilseed rape
Three of 40 strains of *A. flavus* isolated from 20 oilseed rape samples collected from Catalonia (Spain) were aflatoxigenic. One sample of oilseed rape was contaminated with AFB_1 (0.25 ng g^{-1}) (Vinas et al., 1994).

Sunflower seeds
Aspergillus strains, capable of producing the four aflatoxins and STG, and strains of *A. ochraceus*, capable of producing OTA, were recorded in sunflower seeds collected from several Egyptian localities (Abdel-Mallek et al., 1994). High levels of aflatoxins (up to 230 ng g^{-1}) were found in 7% of 135 samples of sunflower seeds in Tunisia (Jelinek et al., 1989). *A. flavus* and *A. parasiticus* were reported in samples of sunflower seeds from Spain, together with other toxigenic *Aspergillus* species, including *A. niger,* but in lower proportions also *A. glaucus, A. ochraceus, A. candidus*, *A. fumigatus, A. chevalieri, A. versicolor*, and *A. amstelodami* (STG), and *A. clavatus* and *A. terreus* (PAT) (Jiménez and Mateo, 2001).

Fruit juices of apple (Pyrus malus) *and guava* (Psidium guajava)
Investigations carried out by Abdel-Sater et al. (2001), on the natural contamination of Egyptian canned fruit juices and beverages, showed that the most prevalent fungi were members of *Aspergillus* and *Penicillium.* In addition, TLC analyses revealed that all five pear beverages examined contained AFB_1 and AFG_1 (both up to 30 ng ml^{-1}), and two of five guava juices contained AFB_1 (up to 12 ng ml^{-1}).

Dried raisins
Aspergillus flavus, A. niger, A. fumigatus, and *Eurotium chevalieri* were among the most frequently encountered fungi in 100 samples of dried raisins collected from retail markets throughout Egypt. Selected strains produced AFB_1, AFB_2, AFG_1, AFG_2, OTA, fumagillin, and STG. In addition, TLC analyses revealed the presence of AFB_1 (up to 300 ng g^{-1}) in two samples, and OTA (250 ng g^{-1}) in a third sample (Youssef et al., 2000).

Sesame (Sesamum indicum) *seeds and tahini (sesame butter)*
Tahini, an ingredient of many sweet foods widely consumed in Turkey and the Middle East, is produced by milling dehulled and roasted sesame seeds. Aflatoxigenic strains of *A. flavus* and *A. parasiticus* were reported to grow on sesame seed and the first surveys carried out in Turkey have shown the potential risk of contamination for commercial sesame seeds and tahini (Nilüfer and Boyacioglu, 2002).

Sugarcane
Aflatoxigenic strains of *A. flavus* and ochratoxigenic strains of *A. niger* were the predominant fungal flora associated with decayed sugarcane in Egypt (Ziedan and Hegazy, 2002).

PENICILLIUM DISEASES

Penicillium is certainly the most complex genus in terms of numbers of species and their range of habitats. They are of great economic importance primarily as a cause of food and feed spoilage. However, a few species are among the most common and destructive agents of postharvest diseases, affecting most kinds of fruits and vegetables. Several species also cause decay of grains and legumes during harvest, storage or transit, especially at temperatures slightly below normal and at moisture contents slightly above normal. Moreover, certain species decay hay and others colonise processed foods and feeds, including meats, cheeses, and spices. In addition to the losses caused by the rotting of fruits and vegetables, as well as by the deterioration of seeds, grains and processed foods, and feeds, the fungus may produce several mycotoxins that can accumulate in infected products. Notable is the occurrence of OTA in small-grain cereals colonised by *P. verrucosum*, which contaminates cereal-based foods and feeds, as well as malt and beer.

Patulin. PAT is among the most widespread of fungal metabolites, produced by many species of *Penicillium*, as well as by species of *Aspergillus* and *Byssochlamys*. Of great interest is the production of PAT by strains of *P. expansum* colonising apple and other fruits and forming toxins in rotted fruits, which is then transmitted to juices and jams. However, PAT is also formed in cereal grains and cereal-based foods and feeds by certain strains of *P. expansum, P. griseofulvum*, and *P. roquefiortii*.

Citrinin. Citrinin (CIT) is produced by at least by 14 species of *Penicillium* and three species of *Aspergillus*. Of particular importance is the formation of CIT by strains of *P. citrinum* causing yellowing of rice, and by strains of *P. verrucosum* colonising cereal grains and legumes. Citrinin is commonly associated with OTA in barley and other cereal grains naturally colonized by *P. verrucosum*. CIT is a nephrotoxin associated with OTA in certain mycotoxicoses of pigs and chickens and suspected also to be involved in human diseases such as yellowed-rice toxicosis and Balcanic endemic nephropathy.

Penicillic acid. Penicillic acid (PAC) is produced by many species of *Penicillium*, and in particular by strains of *P. aurantiogriseum* which cause 'blue-eye' of maize. Penicillic acid was found in samples of maize and other cereal grains, as well as in samples of dried bean. Penicillic acid is cytotoxic to plant and animal cell lines and is moderately genotoxic to microorganisms.

Penicillium *moulding of cereals*

Causal agents and symptoms. Favourable weather conditions, such as high rainfall and mild temperature

before harvest, can be conducive to colonisation of cereal ears by saprophytic or opportunistic fungi, including toxigenic *Penicillium* strains. The activity of such toxigenic strains may continue during harvest and storage under favourable conditions. Jurjević et al. (1999) reported a high occurrence (up to 93.6%) of species of *Penicillium* in freshly harvested maize samples collected from 14 regions of Croatia, whilst Jiménez and Mateo (2001) reported a wide occurrence of toxigenic *Penicillium* species, particularly *P. griseofulvum* (PAT), *P. variabile, P. islandicum, P. purpurogenum*, *P. chrysogenum* (PAC), and *P. capsulatum* in samples of cereal grains collected all over Spain markets

Toxin formation and natural occurrence. Samples (two out of four) of freshly harvested maize from Croatia, collected in 1996, which were characterised by high amount of rainfall and mild temperature at harvest, were found colonised by *Penicillium* species (up to 82%), and contaminated by OTA (up to 224 ng g^{-1}) (Jurjević et al., 1999). Sample of cereals from Cyprus were contaminated (23%) with low concentrations of OTA (up to 0.9 ng g^{-1}), with the highest contamination in barley, followed by wheat, while the contamination was quite negligible for maize (Ioannou-Kakouri et al., 2001). Investigations carried out on the toxigenic mycoflora associated with freshly harvested wheat grains collected from different localities in Egypt led to the isolation, together with other fungal genera, of some representatives of *Penicillium* associated with the occurrence of CIT (El-Kady and El-Maraghy, 1990). In addition, the occurrence of toxigenic *Penicillium* strains capable of producing CIT and rubratoxins was also recorded in maize kernels (Abdel-Mallek et al., 1994). Moistened or moulded wheat samples associated with problems in farm animal in Spain, revealed PAT in three of five samples (up to 38 ng g^{-1}) (Jiménez and Mateo, 2001).

Blue mould rot of apple and pear

Causal agents and symptoms. The disease is caused by strains of *P. expansum,* a pathogen which is generally considered to be a wound parasite. Mould growth normally occurs only where the surface tissue of fruit has been damaged. Infection commonly follows insect or storm damage during preharvest, rough gathering at harvest or strong washing and sorting procedures after harvest. During storage, infection can occur even at 0 °C, but decay proceeds slowly during cold storage, and usually only develops rapidly when fruits are returned to warm temperatures. The species includes strains with different pathogenicities, as determined by lesion diameter on fruits, and with different capacities to produce PAT (from 2 to 100 mg kg^{-1}).

Toxin formation and natural occurrence. Under laboratory conditions, PAT can be produced by a variety of different moulds on apples, grape juice and grains, but in natural conditions PAT is essentially known as a metabolite of *P. expansum* contaminating apples and apple juice. Patulin is stable in apple juice, grape juice, and dried maize, but not in oranges, orange juice, flour, bread, cheese, or wet maize. However, occasionally PAT has been detected in other naturally brown rot fruits like bananas, pineapples, grapes, peaches, apricots, and tomatoes. Fruit juices converted to cider or wine do not contain PAT because it is destroyed by alcoholic fermentation. High levels of PAT (up to 250 mg kg^{-1}) have been reported in 52% of the 104 Spanish apple samples (Jelinek et al., 1989), but the level of contamination in the juices is usually lower, probably due to the disposal of apples affected by rot. To this regard, the occurrence of PAT was reported in 43 out of 100 samples of Spanish apple juices in concentrations of 10–170 ng ml^{-1} (Prieta, 1994). Investigation carried out on Italian products, showed PAT in all five samples of apple juices examined (up to 60 ng ml^{-1}), in five out of six pear juices (up to 25 ng ml^{-1}), in two out of six peach juices (up to 3 ng ml^{-1}), in one out of three apricot juices (12 ng ml^{-1}) and in more than 50% of 20 samples of jam (up to 75 ng g^{-1}) (Valletrisco et al., 1983).

A very high incidence (100%) of PAT even though at low levels (5–75 ng ml^{-1}) was found in 44 samples of Turkish apple juices (Karadenizm and Eksi, 1997). But a similar survey carried by Gökmen and Acar (1998) showed higher PAT contamination of 215 apple juices from Turkey with 100% incidence and toxin range of 7–376 ng ml^{-1}. In France, PAT was found in 100% (up to 610 ng g^{-1}) and 69% (up to 300 ng g^{-1}) of 27 samples of concentrated apple juices, and 13 samples of apple cider (Jelinek et al., 1989). Laidou et al. (2001) reported the formation of PAT in pear inoculated with *P. expansum* and its diffusion in the apparently sound flesh, in concentration surpassing the accepted maximum Greek limits (50 ng ml^{-1}). Investigation carried out by Martins et al. (2002) on the presence of

mycotoxins in 351 samples of seven different varieties of apples with small rotten areas, collected throughout Portugal, revealed the occurrence of PAT (up to 80.5 mg kg^{-1}) and CIT (up to 5 mg kg^{-1}) found either together (20%) or alone (69% and 4% samples).

Penicillium *on other crops*

None of the strains of *Penicillium* isolated from 20 oilseed rape samples collected all over Catalonia (Spain) were mycotoxigenic (Vinas et al., 1994). However, strains of *Penicillium* species, including strains of *P. citrinum*, capable of producing, CIT were recorded in sunflower seeds collected from several Egyptian localities (Abdel-Mallek et al., 1994). In addition, Jiménez and Mateo (2001) reported the wide occurrence of *Penicillium* species, particularly *P. griseofulvum*, *P. variabile* and *P. chrysogenum* in 35 samples of sunflower seeds collected from Spain markets. *P. chrysogenum* and *P. oxalicum* were reported by Youssef et al. (2000) to be among the most frequently encountered fungal species in 100 dried raisins fruits collected from retail markets in Egypt. Selected strains were able to produce PAC and CIT. Jiménez and Mateo (2001) reported the wide occurrence of *Penicillium* species, particularly *P. griseofulvum, P. variabile*, and *P. chrysogenum* in 38 samples of peanuts collected from Spanish markets. Toxigenic strains of *Penicillium* spp. producing PAC (up to 48 ng ml^{-1}) were reported to be among the predominant fungal flora associated with decayed sugarcane in Egypt (Ziedan and Hegazy, 2002).

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