Phytopathologia Mediterranea (2012) 51, 1, 131–147

Review

Diversity of black Aspergilli and mycotoxin risks in grape, wine and dried vine fruits

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Summary. Mycotoxin risk in the grape product chain is primarily due to ochratoxin A (OTA) occurrence in wine and dried vine fruits. *Aspergillus carbonarius* and the *A. niger* group are the main agents of *Aspergillus* bunch rot of grape, and they, especially *A. carbonarius*, are responsible for OTA contamination worldwide. Fumonisin B_2 (FB_2) represents an additional potential mycotoxin risk in the grape-wine product chain and *A. niger*/*A. awamori* were recently reported as the FB_2 producers in grapes. A deeper understanding of the species diversity of black Aspergilli, together with specific knowledge of their ecology and epidemiology, can help to predict their occurrence. From this perspective several studies have been done regarding prevention and control of black Aspergilli and reduction of mycotoxin risk at all stages, from vineyard management to wine-making procedures. In this review a comprehensive overview of all these aspects is presented.

Key words: *Aspergillus* section *Nigri*, ochratoxin A, fumonisins, mycotoxin risk management.

The grape chain

Importance of grape and grape products worldwide

Grapes and grape-derived products have a significant worldwide importance. In particular, according to data from the Food and Agriculture Organization (FAO), global grape production has a monetary value of $55 million (USD); most grapes are used for wine-making (71%), about 27% are consumed fresh, and only a minor portion (2%) are consumed as dried fruits. Dried vine fruits include raisins, currants and sultanas, according to the colour of the berries: white for sultanas and white or red for raisins; and to the origin: i.e., currants from Greece, sultanas from Turkey, raisins from the USA, Turkey, Greece and Australia. Italy is the world's leading producer of grapes and wine, with a production of about 8.2 and 5 million tons, respectively (FAOstat, 2008). The major grape-producing countries, i.e., Italy, China, United States of America (USA), France, Spain, Turkey, Chile and Argentina, are also the major wine-producing countries, except Turkey, for which the importance is tied to sultanas production (Figure 1).

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Mould contamination over the grape chain

Economic losses resulting from pathogenic fungi are significant because they can induce diseases, such as grape rot, and contaminate products with mycotoxins. Although many reports on the worldwide occurrence of ochratoxin A in wine are available, data on the economic impact of mycotoxins in grapes and wine are not available or are not in the public domain. In the vineyard, many fungi can occur and infect berries, depending on the environmental conditions (Pitt and Hocking, 1997). When the moisture of grapes is high and the temperature ranges between 20–30°C, the most common fungi occurring on grapes are *Alternaria*, *Aspergillus*, *Botrytis*, *Penicillium*, *Epicoccum*, *Cladosporium* and *Rhizopus*

ISSN (print): 0031-9465
ISSN (online): 1593-2095

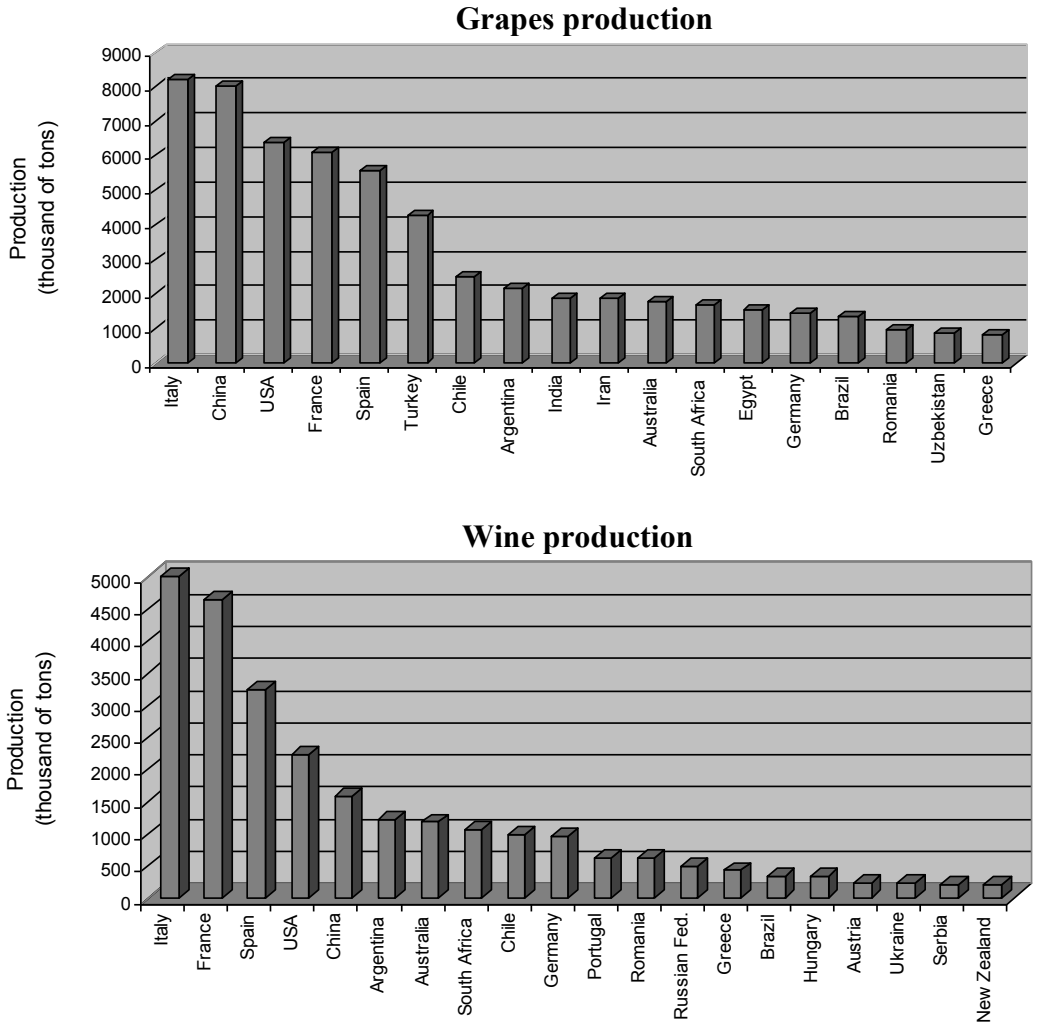

Figure 1. Grapes and wine production, expressed in thousand of tonnes, of the major producer countries of the world (FAOstat, 2008).

(Belli *et al*., 2004; Sage *et al*., 2002). During maturation, the spoilage agents, *Aspergillus*, *Botrytis*, *Penicillium* and *Rhizopus*, increase their incidence. When the temperature is higher than 37°C, species in *Aspergillus* section *Nigri*, usually called "black Aspergilli", predominate (Valero *et al*., 2005). At harvest time the conditions are optimal for fungal invasion, especially if physical damage has occurred on berries.

After harvest, grapes are subjected to different processes, depending on the intended use. Grapes can be eaten fresh, pressed for making wine, squeezed to make grape juice or dried by sunlight for raisin or sweet wine production. Each of these treatments is characterized by contamination from different fungal species. Grape berries, both for table consumption or wine making, are mainly contaminated in the field by *Aspergillus, Botrytis*, and *Penicillium* species, which often can be isolated from symptomless berries (Battilani and Pietri, 2002), and successively by black Aspergilli and *Botrytis cinerea* in post-harvest cold storage (Guzev *et al*., 2008). On dried fruits as well, *Aspergillus* and *Penicillium* species are often present (Valero *et al*., 2005); in particular the predominance of *Aspergillus* species on dried fruits is reported worldwide, including Italy, Spain (Abarca *et al*., 2003), Brazil (Iamanaka *et al*., 2005), Ar-

gentina (Da Rocha Rosa *et al*., 2002), and California (Palumbo *et al*., 2011).

Determination of the mycoflora occurring on grapes at the different stages of growing and processing is important to establish an adequate program of treatments for the prevention of fungal contamination in the vineyard and in storage. Some of the fungal species occurring on grapes and grape products can produce mycotoxins, so species identification is critical to predict the potential mycotoxin contamination of grapes and wine. Certainly the *Aspergillus* species are present worldwide, in all the grape products and under all environmental conditions.

Aspergillus black rot of grape

Aspergillus black rot is among one of the many bunch rots occurring on grapes. The disease appears on the berries as a black rot due to prolific fungal sporulation after it has invaded and consumed the berries which look completely empty and dry (Figure 2). Colonies of these fungi are present on the berry skin from fruit setting and increase in amount from early veraison to harvest, with a peak at ripening; however the incidence of colonised berries is highly related to climatic conditions during the ripening stage and to the geographical location (Cozzi *et al*., 2007; Visconti *et al*. 2008). The principal pathway of infection for black Aspergilli is damage to berry skins, caused by many factors including fungal diseases (downy mildew, powdery mildew), pests (grape berry moth, bunch mites) and environmental factors (wind, hail, rain or sunburn injury, berry splitting). As already mentioned, the incidence of *Aspergillus* rot of grapes is related to environmental conditions that influence fungal population growth, interaction with the plants and mycotoxin production. This disease has received more attention since, in the last decade, it was associated with contamination of grapes and wine by ochratoxin A (OTA), a strong nephrotoxic compound which we will discuss thoroughly (Battilani *et al*., 2003; Serra *et al*., 2003;

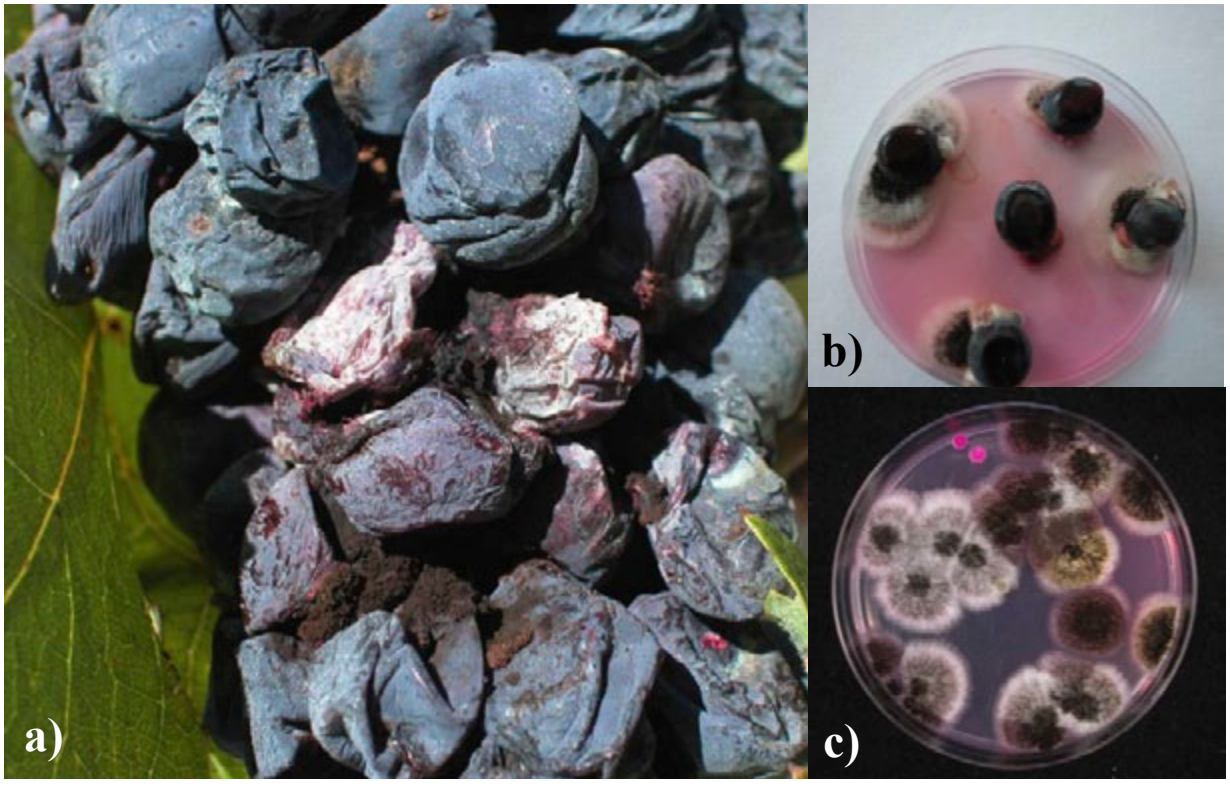

Figure 2. Black Aspergilli on grapes: a) black rot of berries caused by black Aspergilli; b) direct plating of berries on DRBC agar; c) different black *Aspergillus* colonies from berries' homogenate diluted and plated on DRBC.

Belli *et al*., 2004). Molecular characterization of fungal populations is needed to better identify the species involved in black rot of grapes, so that improved assessment of the toxigenic potential in food and better mycotoxin management can be possible.

Diversity and molecular detection of black Aspergilli

This section deals with several studies that were carried out and are in progress to deepen the understanding of genetic diversity among and within black *Aspergillus* species. Indeed the taxonomic situation of *Aspergillus* section *Nigri* is not yet clarified, and this means that the actual occurrence and toxicological potential of *Aspergillus* species may need to be reconsidered. Each species of black Aspergilli can produce a unique combination of mainly polyketide-derived secondary metabolites and other compounds of mixed biosynthetic origin (Nielsen *et al*., 2009). Since each species is characterized by a specific profile, a chemophylogeny could be defined (Frisvad *et al*., 2007). Moreover, *A. niger* is used in fermentation and biotechnology industries for the production of organic acids, enzymes, vitamins and antibiotics. The possibility that harmful mycotoxins, such as OTA and fumonisins, might contaminate the compounds used in biotechnological processes for food use should be investigated, and a correct species identification is very important from this perspective.

The classification of this section was traditionally based on morphological identification (Dalcero *et al*., 2002; Chulze *et al*., 2006), which is very difficult and can lead to misidentification, especially within the *A. niger* species aggregate (a group of morphologically indistinguishable species). This method was then integrated with physiological characters, i.e. extrolite production data (Frisvad *et al*., 2007), that often provide similar results obtained with phylogenetic analyses (Geiser *et al*., 2000). In Table 1 the morphological traits and the biochemical diversity of the black *Aspergillus* species occurring on grapes are reported. Different molecular tools have been used to differentiate taxa within section *Nigri*. These tools have included Western blotting, DNA hybridization, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), multilocus sequence analysis, PCR and Real Time PCR as reviewed by Samson *et al*. (2007). For example, RFLP analysis was used by Accensi *et al*. (1999) and Martinez-Culebras and Ramon (2007), to distinguish *A. japonicus* from *A. aculeatus* and to differentiate 5 species, respectively. *Aspergillus niger* was distinguished from *A. tubingensis* by Parenicova *et al*. (2001) by using PCR-RFLP, a technique more recently used to differentiate OTA-producing from OTA non-producing strains among the *A. niger* "aggregate" (Zanzotto *et al*., 2006). Also real-time PCR assays were developed, able to detect *A. carbonarius,* the main producer of OTA in grape (Mulè *et al*., 2006; Atoui *et al*., 2007; Selma *et al*., 2008; Gonzalez-Salgado *et al*., 2009). In order to analyze the diversity of black Aspergilli strains from Italian grape, Perrone *et al*. (2006a) carried out AFLP analysis by which 4 main clusters were clearly formed: the *A. carbonarius* cluster, the *A. niger* cluster, the *A. tubingensis* cluster, and a uniseriate cluster, formed by strains phylogenetically different from *A. japonicus* and *A. aculeatus*, but morphologically indistinguishable from them; this group was later identified as a new species, *A. uvarum* (Perrone *et al*., 2008). However, the most commonly used molecular tool has been PCR with species-specific primers, based on AFLP markers (Schmidt *et al*., 2004), RAPD sequences (Fungaro *et al*., 2004), calmodulin gene (Perrone *et al*., 2004; Susca *et al*., 2007a), internal transcribed spacer (ITS) regions (Gonzalez-Salgado *et al*., 2005; Patino *et al*., 2005), or polyketide synthase (PKS) sequence (Dao *et al*., 2005; Spadaro *et al*., 2011).

Finally, sequence analyses of ITS, cytochrome oxidase subunit 1 (cox1), β-tubulin and calmodulin genes have been widely used (Yokoyama *et al*., 2001; Samson *et al*., 2004; Geiser *et al*., 2007; Varga *et al*., 2007; Perrone *et al*., 2008, 2011). Molecular investigations revealed several cryptic species within the commonly recognized morphological species, thus complicating the identification of black *Aspergillus* species (Samson *et al*., 2004). A new species named *A. ibericus*, from atypical *A. carbonarius* strains isolated in Spain and Portugal that do not produce OTA, was identified by using ITS and calmodulin sequences and AFLP analyses (Serra *et al*., 2006). More recently, single-stranded conformational polymorphism (SSCP) analysis led to rapid and efficient differentiation of 11 species (Susca *et al*., 2007b): *A. aculeatus*, *A. brasiliensis*, *A. carbonarius*, *A. ellipticus*, *A. foetidus*, *A. heteromorphus*, *A. ibericus*, *A. japonicus*, *A. niger*, *A. tubingensis* and *Aspergillus* "atypic uniseriate" (Perrone *et al*., 2006b). A polyphasic approach, integrating multilocus sequence analysis data with mor-

Table 1. Morphological and biochemical diversity of the black Aspergilli species occurring on grapes.

Species	Conidial size (µm)	Color and size of sclerotia (mm)	Main source	OTA[a]	FB_2[b] FB_4	Extrolites produced[c]
Biseriates						
A. brasiliensis	3.5–4.5	White, 1–1.5	Soil, grape	-	-	Naphtho-γ-pyrones (including aurasperone B), pyrophen, tensidol A and B, **dihydrocarolic acid**, **aflavinine**
A. niger - *A. awamori*	3.5–5	-	Grape, cocoa, coffee, cereals, soil, paper, date palm	+ 5–20%[d]	+ 60–70%	**Funalenone (kotanins),** naphtho-γ-pyrones, pyranonigrin A, **pyrophen**, tensidol A and B
A. tubingensis	3–5	White to pink, 0.5–0.8	Grape, cocoa, coffee, soil, cereals	+/- 5–20%	-	**Asperazine**, funalenone, naphtho-γ-pyrones, pyranonigrin A, tensidol A and B
A. carbonarius	5.5–8	Pink to yellow, 1.2–1.8	Grape, cocoa, coffee, spices, palm oil, soil, air	+ 90–100%	-	Pyranonigrin A, naphtho-γ-pyrones
A. ibericus	5–7	-	Grape	-	-	Naphtho-γ-pyrones (including aurasperone B), pyranonigrin A
Uniseriates						
A. aculeatus	4–5	-	Grape, papaya, pistachio, rice, tomato	-	-	Secalonic acid D and F
A. japonicus	4–5	White to cream, 0.5	Grape, green coffee berries, pineapple, sesame seed	-	-	Cycloclavine, festuclavine
A. uvarum	3–4	Dark brown to black, 0.5–0.8	Grape	-	-	**Asterric acid, geodin, erdin,** secalonic acid D and F

[a] OTA, ochratoxin A.
[b] FB_2 - FB_4, fumonisin B_2 and B_4.
[c] Extrolites in bold indicate the difference with the other species.
[d] Percentage of positive strains.

phological and physiological characters, was recommended to delineate new *Aspergillus* species (Geiser *et al*., 2007; Samson *et al*., 2007). In this way 9 new species have been recently identified within the Section *Nigri*: *A. ibericus* (Serra *et al*., 2006); *A. brasiliensis* (Varga *et al*., 2007); *A. uvarum* (Perrone *et al*., 2008); *A. aculeatinus* and *A. sclerotiicarbonarius* (Noonim *et al*., 2008), *A. eucalypticola*, *A. fijensis*, *A. indologenus* and *A. neoniger* (Varga *et al*., 2011). Moreover, *A. awamori* was revalidated by Perrone *et al*. (2011) as a cryptic species within *A. niger*, as they showed that this closely related species is distinguishable only through molecular tools, and no differences in extrolite profiles were found. In addition Varga *et al*. (2011) revalidated *A. violaceofuscus* and *A. acidus* (previously known as *A. foetidus* var. *pallidus* and *A. foetidus* var. *acidus*), while *A. foetidus* was declared synonymous with *A. niger*. At this time *Aspergillus* section *Nigri* is considered to comprise 24 defined species (Varga *et al*., 2011), although it remains under investigation, which may result in further changes. A phylogenetic tree based on calmodulin, beta-tubulin and ITS sequence analysis, representative of the black *Aspergillus* species, is presented in Figure 3.

Ochratoxigenic fungi in grapes

Black *Aspergilli* are considered the primary source of OTA on grapes (Logrieco *et al*., 2007), produced on the berries during the growing season mainly from

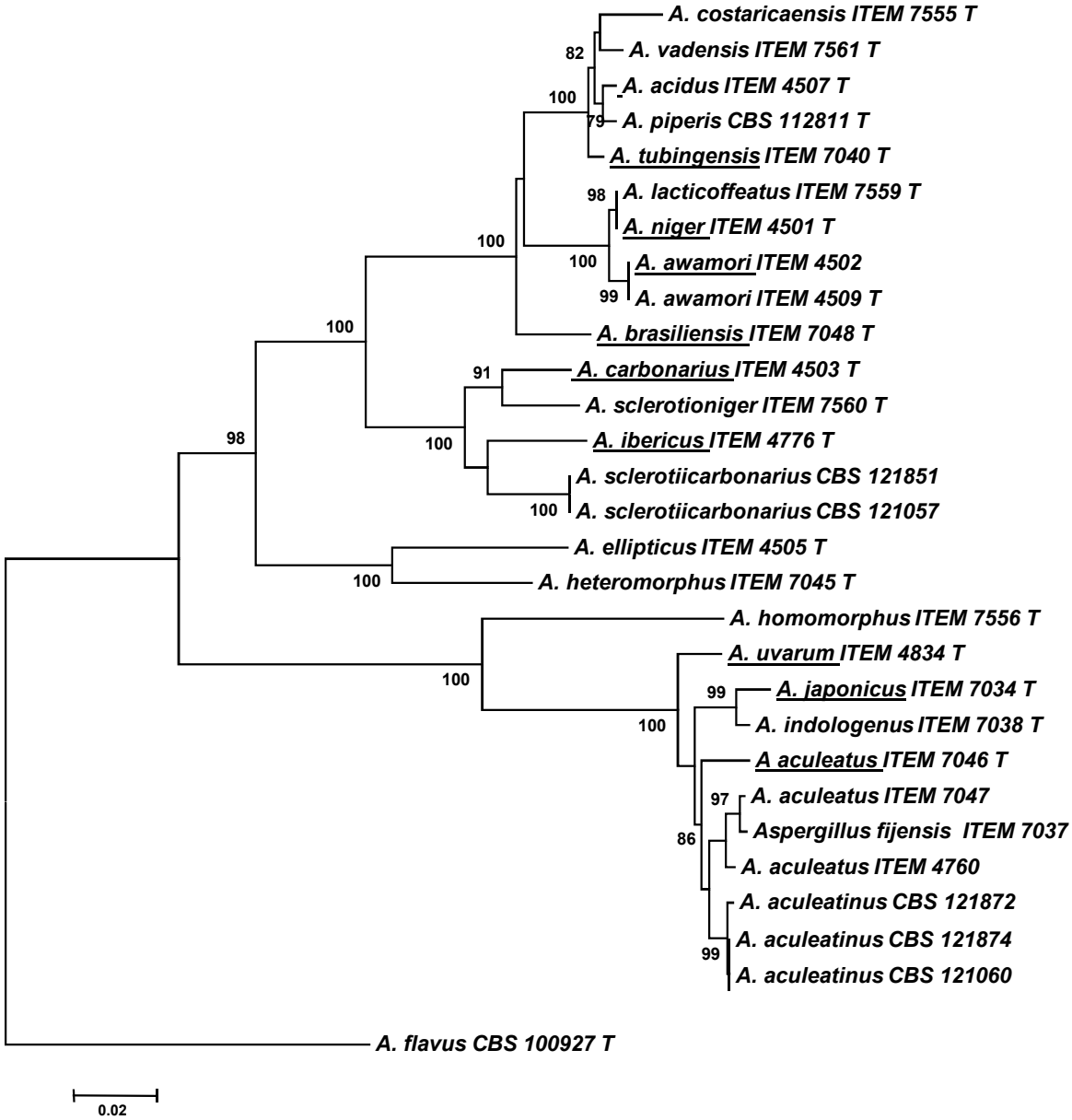

Figure 3. Phylogenetic tree based on analysis of calmodulin, betatubulin and ITS sequence data of *Aspergillus* section *Nigri* strains. Bootstrap values above 70% are indicated above branches. The species occurring on grapes are underlined.

veraison to ripening. In particular *A. carbonarius* is the most important producer of OTA; however *A. niger*, *A. awamori* and *A. tubingensis* can contribute to some extent in the vineyard (Medina *et al*., 2005; Perrone *et al*., 2011). Over the past five years several surveys and reports were published dealing with the epidemiology, ecology and distribution of black Aspergilli occurring in wine grapes and dried vine fruits. Most of the surveys were from Mediterranean and South American countries and Australia, as shown in Figure 4. This figure represents a map of the world in which the published data available on the occurrence of the black Aspergilli species are shown for different countries. These studies clarified that the biseriate species *A. niger* "aggregate" and *Aspergillus carbonarius*, and the uniseriate species *A.*

aculeatus, *A. japonicus* together with the new species *A. uvarum,* are the prevalent species occurring on grapes (Da Rocha Rosa *et al*., 2002; Leong *et al*., 2006; Perrone *et al*., 2008). In particular the most common species on grapes in Europe are *A. niger*, *A. tubingensis*, *A. carbonarius*, and *A. uvarum*; the first two are dominant in all the countries, *A. carbonarius* more frequent in southern Mediterranean areas (Greece, Portugal, South Italy and South France), with *A. uvarum* occurring more frequently in Italy, France, Greece, Israel (Perrone *et al*., 2008). *A. brasiliensis* and *A. ibericus* were detected only occasionally, in Spain and Portugal (Perrone *et al*., 2006a, 2007). In light of the recent identification (Perrone *et al*., 2011), *A. awamori* also has been reported as an OTA- and fumonisin-producing species occurring on dried fruits from different countries (Varga *et al*., 2010). Probably its distribution has been underestimated owing to the similarity with *A. niger*. Occurrence of black *Aspergillus* species in Australia is similar to Mediterranean countries (Figure 4), with a prevalence of *A. niger* and *A. carbonarius* (Leong *et al*., 2006; Battilani *et al*., 2006); the former is the most common species in South America on grapes, the latter in Argentina on dried vine fruits (Chulze *et al*., 2006).

Among section *Nigri*, *A. carbonarius* is the major OTA-producer (Battilani and Pietri, 2002; El Khoury and Atoui, 2010). *A. carbonarius* strains showed the highest percentage of OTA producers, near 100%, at consistent amounts (Sage *et al*., 2002; Abarca *et al*., 2003; Perrone *et al*., 2006b); *A. niger* "aggregate", although the most common, showed a low percentage of OTA producing strains, from 4 to10% (Serra *et al*., 2003; Perrone *et al*., 2006b); none of the strains belonging to the uniseriate group, further identified as *A. uvarum*, was able to produce OTA (Perrone *et al*., 2008). Two new species of section *Nigri*, *A. lacticoffeatus* and *A. sclerotioniger* are also reported as

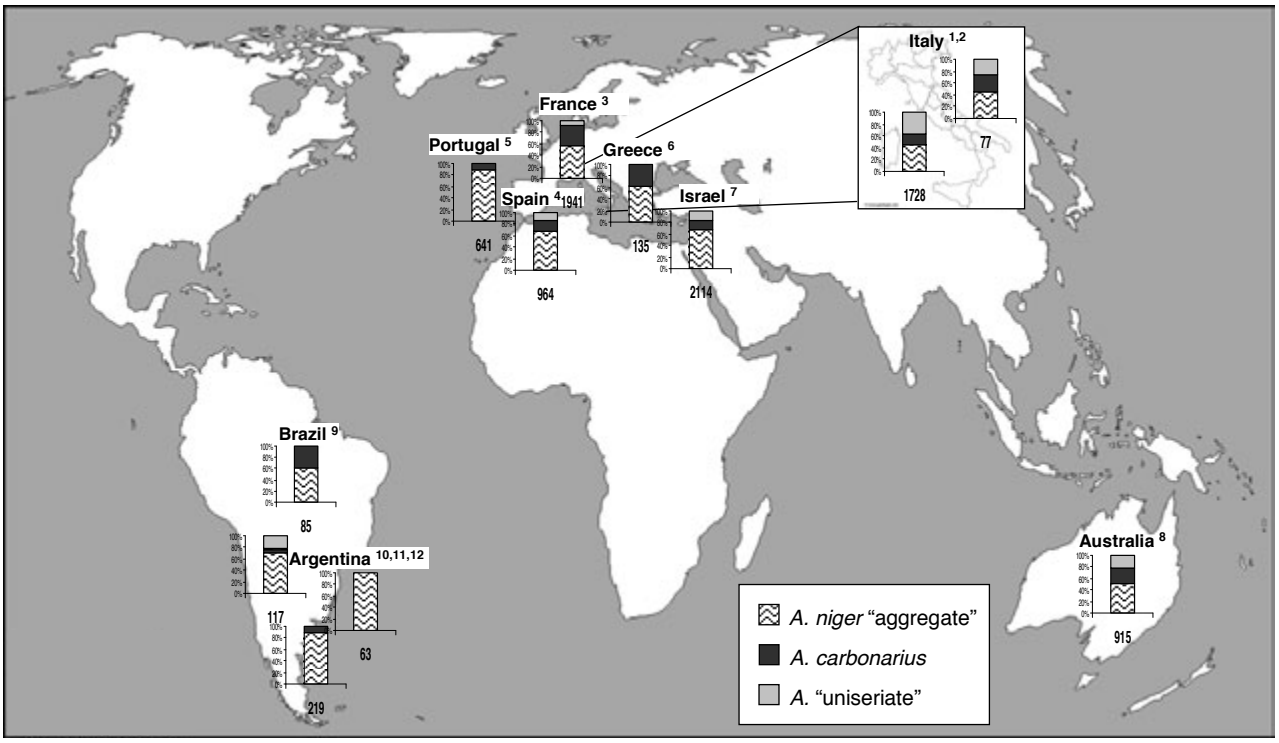

Figure 4. Distribution of Black Aspergilli species worldwide. Under each graph the number of analyzed strains was reported. *A. niger* "aggregate" includes *A. tubingensis*, *A. awamori* and *A. foetidus*; *A.* "uniseriate" includes *A. japonicus*, *A. aculeatus* and *A. uvarum*. The numbers near the country name indicate the literature references: [1] Perrone *et al*., 2006b; [2] Battilani *et al*., 2006; [3] Bejaoui *et al*., 2006; [4] Belli *et al*., 2006; [5] Serra *et al*., 2006; [6] Tjamos *et al*., 2006; [7] Guzev *et al*., 2006; [8] Leong *et al*., 2006; [9] Da Rocha Rosa *et al*., 2002; [10] Ponsone *et al*., 2007; [11] Magnoli *et al*., 2003; [12] Magnoli *et al*., 2004.

OTA producers, both isolated from coffee (Samson *et al*., 2004), but recently morphologically detected in raisin samples (Hakobyan *et al*., 2010). Nevertheless, occurrence of ochratoxigenic fungal species other than *Aspergillus* section *Nigri* has been found on grapes but not correlated to OTA contamination in berries. *Aspergillus* species belonging to sections *Circumdati* and *Flavi* are also reported as OTA producers (Bayman *et al*., 2002; Frisvad *et al*., 2004). A high percentage of OTA-positive strains, although in lower frequency then black Aspergilli, was reported for *A. ochraceus* (Belli *et al*., 2004; Frisvad *et al*., 2004). OTA production was also linked to *Penicillium* species occurring in vineyards of Italy, Argentina and France, but their presence was never correlated with OTA contamination of grapes and wine (Magnoli *et al*., 2003; Battilani *et al*., 2004; Frisvad *et al*., 2004).

Mycotoxins occurring in grapes and grape products

OTA regulation and occurrence

Mycotoxin contamination of food and feed is an important concern for human health. The most common mycotoxin detected in grapes, wine and dried vine fruits is OTA (Aksoy *et al*., 2007; Visconti *et al*., 2008). OTA is one of the three most important and harmful mycotoxins in the world (Palencia *et al*., 2010). OTA is a very strong nephrotoxin, with carcinogenic, teratogenic and immunosuppressive properties, classified as Group 2B by the International Agency for Research on Cancer (IARC, 1993). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established 100 ng kg^{-1} bw as the tolerable weekly intake (PTWI) recommended for OTA (JECFA, 2007), which is also regulated by the European Commission. The regulation levels in food and feed products are established at 10 μg kg^{-1} in dry grapes (EC No 472/2002), 2 μg kg^{-1} in grape juice, must and wine, and 0.5 μg kg^{-1} in food for babies and infants (EC No 123/2005). On the contrary OTA levels are not regulated in United States of America.

OTA was first detected in 1996 in wine (Zimmerli and Dick, 1996), which is considered a major source of daily OTA intake, second only to cereals (European Commission 2002). OTA presence in wine has been confirmed in several studies worldwide, as shown in Figure 5, which reports the percentage of wine samples contaminated by OTA, from the Mediterranean area, South America, and Australia. It is evident that the risk of OTA contamination in the Mediterranean basin is highest, because of the relatively high percentage of contaminated samples and the mean level of OTA. On the contrary, in Australia and South America, lower OTA occurrence and levels of contamination (except for data from Ponsone *et al*., 2010, in Argentina) were observed. Visconti *et al*. (2008) highlighted that OTA levels showed a decreasing gradient from red to rosé, to white wines, and the same trend was observed for grape juice; also that wines from southern and warmer regions of Europe showed incidence and levels of contamination higher than those from northern European areas. The same trend was observed for wines produced in Southern Italy which showed incidence and levels of contamination higher than wines produced in Northern and Central Italy (Pietri *et al*., 2001; Lucchetta *et al*., 2010).

Very high levels of OTA contamination have been reported on dried vine fruits (e.g., sultanas, raisins) worldwide, showing frequently around 100% of the samples contaminated (Meyvaci *et al*., 2005; Aksoy *et al*., 2007; Palumbo *et al*., 2011), as shown in Figure 6. In most reports, the high percentage of contaminated samples was associated with an average OTA level over 2 μg kg^{-1}, with maximum values up to 100 μg kg^{-1} (Magnoli *et al*., 2004; Aksoy *et al*., 2007). Sweet wines, typical of Mediterranean countries, demonstrated an average OTA content higher than that of common wine, because of extended exposure to fungal contamination during the drying process (Gomez *et al*., 2006; Valero *et al*., 2008). Since grape juice is consumed primarily by children, OTA levels in this product were investigated and often were found to be higher than allowed (Miraglia and Brera, 2002; Chulze *et al*., 2006). OTA contamination of vinegar also was observed, and higher levels were reported in red vinegar than in white (Varga and Kozakiewicz, 2006).

Fumonisins

Fumonisins have been extensively studied as mycotoxins with cancer-promoting activity and are associated with a number of animal and human diseases. They are very harmful mycotoxins, causing equine leukoencephalomalacia and pulmonary edema in swine; they also are nephrotoxic, hepatotoxic and hepatocarcinogenic in rats. Moreover fumoni-

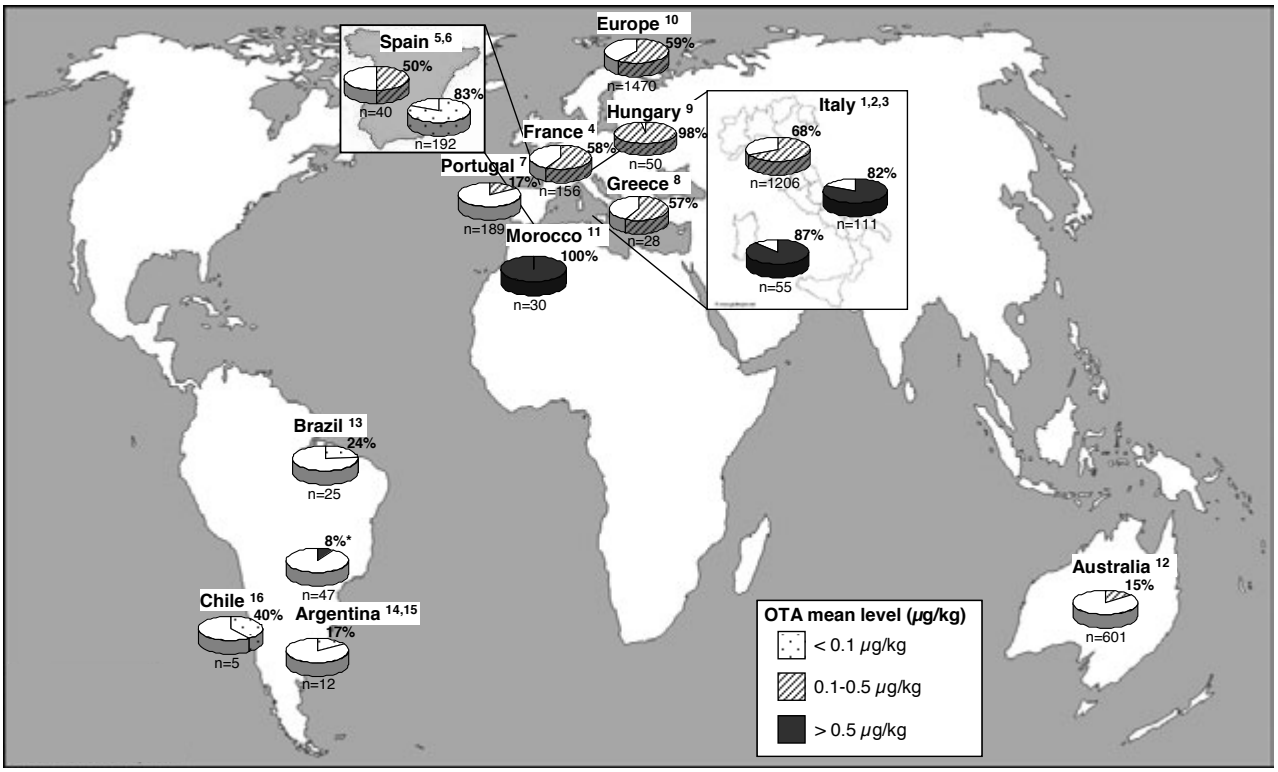

Figure 5. Ochratoxin A contamination of wine samples worldwide. Each graph shows the percentage of contamined samples on the total analyzed samples (number reported under the graph) and the mean level of ochratoxin A detected. The numbers near the country name indicate the literature references: [1] Spadaro *et al*., 2010; [2] Pietri *et al*., 2001; [3] Visconti *et al*., 1999; [4] Clouvel *et al*., 2008; [5] Lopez de Cerain *et al*., 2002; [6] Burdaspal and Legarda, 1999; [7] Peito *et al*., 2004; [8] Soufleros *et al*., 2003; [9] Varga *et al*., 2004; [10] Miraglia and Brera, 2002; [11] Zinedine *et al*., 2010; [12] Hocking *et al*., 2003; [13] Rosa *et al*., 2004; [14] Ponsone *et al*., 2010; [15] Rosa *et al*., 2004; [16] Rosa *et al*., 2004. * The OTA mean level is over 2.5 μg kg^{-1}.

sins are associated with human esophageal cancer in China and in South Africa, and involved in human neural tube defects (Marasas *et al*., 2001). Because of these findings, fumonisin levels in food and feed are regulated by the European Commission Regulation (EC No 1126/2007) and classified by IARC in Group 2B of the carcinogenic compounds.

Fumonisin B (FB) analogs are the most common fumonisins, among which FB_1 predominates, followed by FB_2 and FB_3, while FB_4 is usually detected in insignificant amounts (Rheeder *et al*., 2002). However FB_2 was reported as more cytotoxic than FB_1 (Gutleb *et al*., 2002). Fumonisin production was originally detected in *Fusarium verticillioides* (Gelderblom *et al*., 1988), then in fifteen other *Fusarium* species (Rheeder *et al*., 2002). FB_1 production also has been detected in *Alternaria alternata* (Chen *et al*., 1992; Abbas *et al*., 1996) and recently a putative gene cluster for fumonisin biosynthesis was identified in *A. niger* (Baker, 2006). Confirmation of fumonisin production by *A. niger* strains was firstly reported by Frisvad *et al*. (2007); in particular the researcher reported the production of FB_2 in all the analyzed *A. niger* strains. Further reports revealed production of FB_2 and FB_4 by *A. niger* and *A. awamori* strains from grape (Logrieco *et al*., 2009; Mogensen *et al*., 2010a; Varga *et al*., 2010; Chiotta *et al*., 2011), as well as a FB_1 isoform, named FB_6 (Mansson *et al*., 2010). Recently, Frisvad *et al*. (2011), when studied 180 strains of *A. niger* from various sources, found about 80% of the producing FB_2 strains. Although the percentage of fumonisin-producing strains of *A. niger* reported in the mentioned studies was very high, a discontinuous distribution of fumonisin-producing strains and the absence of at least part of the fumonisin biosynthetic gene cluster has been reported in *A. niger* (Susca *et al*., 2010).

With respect to these results, and because *A. niger* was widely detected on grapes and grape products, further studies were conducted to evaluate the contamination of these commodities by fumonisins. FB_2 contamination was detected in must (Logrieco *et al*., 2009), in wine (Logrieco *et al*., 2010; Mogensen *et al*., 2010b) and in dried vine fruits (Varga *et al*., 2010). These findings indicate a real risk for grape product consumers (Logrieco *et al*., 2011), also considering the risk of synergic effects between fumonisins and other toxic compounds, such as OTA, occurring in the products. Nevertheless, additional studies on natural occurrence of fumonisins in wine and dried vine fruits are needed for a better evaluation of toxin exposure risk.

Other mycotoxins

Besides OTA and fumonisins, other mycotoxins have been reported in grapes and grape products, although in lower frequency and with no apparent risk for human health.

Patulin has been detected in grape juice and wine (Majerus *et al*., 2008; Scott, 2008), and patulin-producing strains of *Penicillium* have been isolated from grapes (Bragulat *et al*., 2008). This toxin has shown to be neurotoxic, immunotoxic, immunosuppressive, genotoxic, teratogenic and carcinogenic (Moake *et al*., 2005). Citrinin, a hepato-nephrotoxic compound, also was detected in grapes before storage (Aziz and Moussa, 2002) and citrinin-producing strains were isolated from grapes (Bragulat *et al*., 2008). However, because the occurrence of patulin- and citrinin-producing strains was very low (Bragulat *et al*., 2008), these compounds do not appear to represent human health risks.

Trichothecium roseum mycotoxins, such as trichothecin, may also occur in grapes and wine (Schwenk *et al*., 1989), but this appears to be rare.

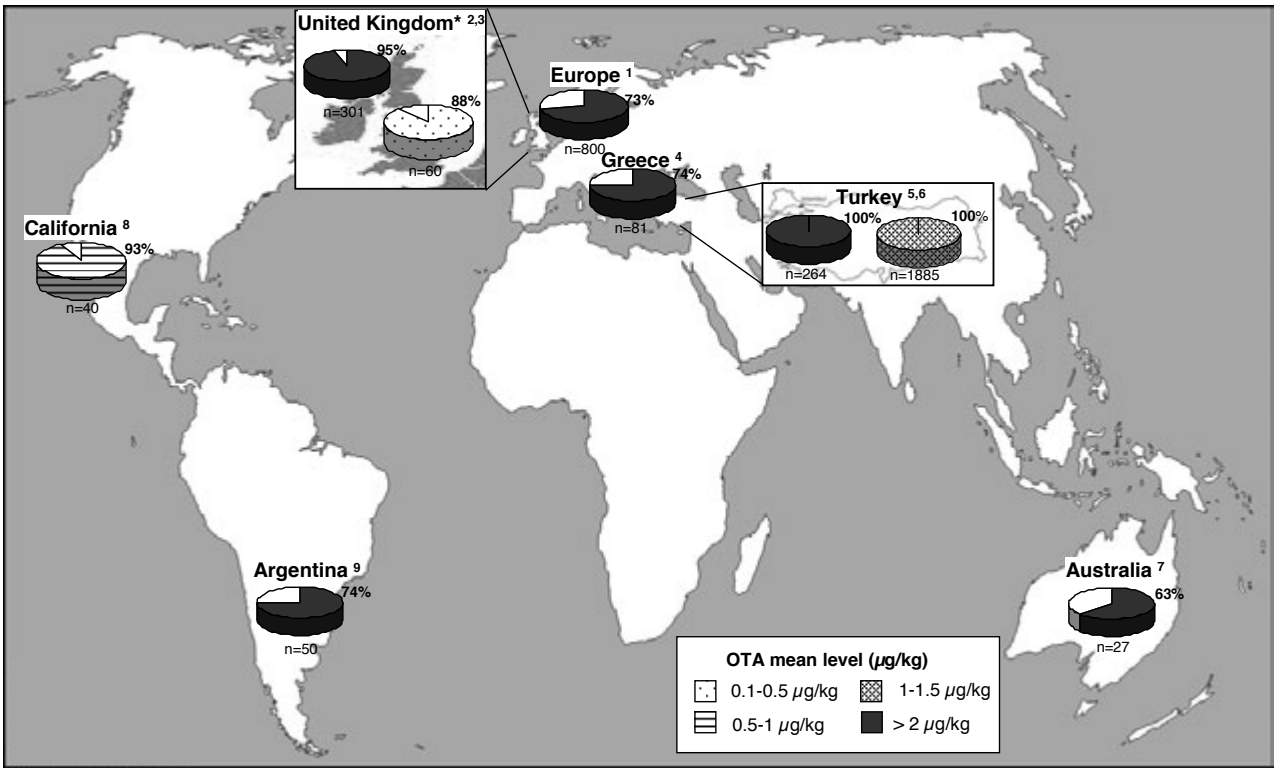

Figure 6. Ochratoxin A contamination of dried vine fruits samples worldwide. Each graph shows the percentage of contamined samples on the total analyzed samples (number reported under the graph) and the mean level of ochratoxin A detected. The numbers near the country name indicate the literature references: [1] Miraglia and Brera, 2002; [2] MAFF, 1999; [3] Mac Donald *et al*., 1999; [4] Stefanaki *et al*., 2003; [5] Meyvaci *et al*., 2005; [6] Aksoy *et al*., 2007; [7] Leong *et al*., 2006; [8] Palumbo *et al*., 2011; [9] Magnoli *et al*., 2004. * The United Kingdom data are based on market samples.

Aflatoxins and aflatoxin producing strains (Fredj *et al*., 2007) have been detected in wine and must occasionally, as recently reported in Lebanon and Turkey (El Khoury *et al*., 2008; Aydogdu and Gucer, 2009). So far, aflatoxin contamination in the grape and wine product chains does not seem to be a real risk for human and animal health. On the other hand, aflatoxins may occur as common contaminants of dried vine fruits in some countries, i.e. India (Saxena and Mehrotra, 1990), Egypt (Youssef *et al*., 2000) and Greece (Apergi *et al*., 1998) also at very high levels.

The presence of the *Alternaria* toxins, alternariol and alternariol monomethyl ether, in wine and grape juice was also reported (Scott, 2008; Asam *et al*., 2009), but their importance has not been established.

Furthermore, the occurrence of a "potential mycotoxin" in wine, mycophenolic acid, has been reported (Scott, 2008). This compound is an immunosuppressant drug derived from some *Penicillium* strains, recognized as an antibiotic substance for bacteria and dermatophytic fungi; its toxicity to mammals has been reported as low (Lafont *et al*., 1979).

Management of mycotoxin risk

Since the major mycotoxin risk in the grape product chain is represented by OTA, several systems could be applied to reduce OTA contamination of commodities. In general the development of risk maps to define risk areas or levels has been suggested as an effective decision-making tool for control strategies (Battilani *et al*., 2006).

In the vineyard

In vineyards the best way to reduce OTA production is to control the presence of ochratoxigenic fungi. For this purpose, knowledge of the ecological factors that affect occurrence of black Aspergilli in the vineyard plays an important role.

Leong *et al*. (2006) reported that soil and stubble were the primary sources of inoculum, so that keeping constant moisture of the soil and practices of minimal tillage were recommended, in particular to avoid tillage from veraison to harvest.

Warm weather conditions and rainfall favour the incidence of OTA production (Visconti *et al*., 2008; García-Cela *et al*., 2011), which also is affected by latitude and longitude, with a positive gradient West-East and North-South in Europe. Temperatures of 30–35°C are optimal for *A. carbonarius* and *A. niger* growth, respectively, while OTA production is favoured by 20–25°C (Belli *et al*., 2005; Astoreca *et al*., 2010). It is also useful to avoid excess vigour and vegetative growth and to promote the aeration of bunches. In general it is important to control the population of black Aspergilli in the vineyard in high risk conditions by applying 1–2 chemical treatments; the most effective mixture of chemical antifungal compounds has been reported as cyprodinil and fludioxonil (Tjamos *et al*., 2004; Belli *et al*., 2007). The most effective treatment was observed at 21 days before harvesting and a previous treatment at veraison was suggested in high risk conditions. This mixture of active ingredients in the same combination and schedule, both in dosage and timing, is effectively used against grey mould, caused by *Botrytis cinerea*. Moreover, insecticide treatment against *L. botrana* in combination with the fungicide contributes significantly to reduction of OTA level in the field, particularly in crop years at high contamination risk (Cozzi *et al*., 2009). Biological control strategies also were proposed for the prevention of the fungal growth and OTA formation. For example, Bleve *et al*. (2006) reported a strain of *Issatchenkia terricola* able to reduce *A. carbonarius* and *A. niger* colonization on grape berry. Recently, Ponsone *et al*. (2011) demonstrated the efficacy of two yeast strains of *Kluyveromyces thermotolerans* for reducing OTA accumulation (from 3 to 100%) and the growth rate of ochratoxigenic fungi (from 11 to 82.5%), in the field.

The espalier cropping system showed the highest incidence of OTA contamination, perhaps for the closeness to the soil, the source of fungal inoculum. Thus, among factors that affect OTA occurrence on grapes, cropping system and grape cultivar susceptibility should be taken into account (Visconti *et al*., 2008).

Post-harvest

Regarding wine-making, preventive actions are to harvest early in high OTA risk areas when favorable conditions occur, segregate rotted bunches at harvest and minimize/avoid storage time before processing the grapes for wine making. In the case of table grapes, which could be subjected to fungal infection during storage, it could be useful to reduce the storage time and to discard visibly rotted bunches. In addition, Lichter *et al*. (2005) showed that the incidence of black Aspergilli in post-harvest could be reduced with sulphur dioxide in cold storage (0°C).

For dried vine fruit production, a rapid drying at greater than 30°C is recommended (Hocking *et al*., 2007).

During wine-making

The actions already mentioned cannot completely prevent the ochratoxin A problem, and severe contamination of wine can occur especially for susceptible grape varieties in high risk regions or vintages with climatic conditions conducive to *A. carbonarius* infection. Red wines are nearly always more contaminated by OTA than white wines, probably for the different wine-making technique, since in red wine a longer maceration increases OTA content (Majerus *et al*., 2000; Battilani *et al*., 2003). Therefore corrective actions are necessary and should be adopted in the winery during the wine-making process. Several fining agents have been tested for their ability to remove ochratoxin A from contaminated musts or wines, with enological charcoal showing the highest adsorption capacity for ochratoxin A (Visconti *et al*., 2008). But charcoal for enological use has only recently been accepted as a corrective action, by using the lowest possible and most effective doses (Codex Alimentarius Commission, 2007). The efficacy of these fining agents, and in particular of the charcoal, is directly related to reductions in quality parameters of the treated wines including the polyphenol content; so this kind of treatment should be avoided if possible (Solfrizzo *et al*., 2010). In addition, effective OTA absorption was reported by using some lactic acid bacteria (Del Prete *et al*., 2007) during wine fermentation, and several studies showed that OTA removal was also possible by using yeast strains, both dead or alive (Scott, 2008; Ciconova *et al*., 2010; Ponsone *et al*., 2011; Var *et al*., 2011). However, the efficacy of yeasts for OTA reduction at the industrial level as well as their impact on wine quality parameters (phenol compounds) has not been shown, and in our studies we have evidence that the reduction of OTA by yeasts or inactivated yeast walls involved the loss of colour of wines and the efficacy of this treatment is low (Visconti *et al*., 2008). More recently, Solfrizzo *et al*. (2010), studying the fate of OTA during the vinification process, demonstrated that 95% of ochratoxin A originally present in grape remains adsorbed to grape pomaces. Then, they demonstrated that ochratoxin A can be effectively removed from contaminated wine by repassage of wine over uncontaminated pomaces obtained from the same grape variety or from different grape varieties. The experiment was evaluated also at an industrial scale and resulted in a useful and environmentally friendly technique for the wineries located in high risk regions for OTA (Solfrizzo *et al*., 2010).

Conclusions

In this review we wished to give an updated overview on the occurrence, biodiversity, toxigenic potential and detection of black Aspergilli, together with other potential toxicological risks, in grapes and dried vine fruits. Black Aspergilli are the causative agents of black rot of grapes, and they can contaminate grapes, dried vine fruits and grape products with OTA. OTA contents can reach high levels in wine in some parts of the Mediterranean basin and in dried vine fruits in South America, Australia and Europe. OTA production is influenced by various factors including climatic conditions/geographic areas; grape varieties/crop systems; berry damages caused by insects, fungal infection or excessive irrigation/rainfall. Fungicidal and insecticidal treatments can reduce infection by OTA-producing fungi and consequently, OTA contamination. Molecular analyses indicated several cryptic species within the section *Nigri* by identifying 24 different species of which 10 occur on grapes and dried vine fruits. Therefore, it is very important to have worldwide studies on biodiversity and population structure of the black Aspergilli on grapes and dried vine fruits, both to understand the toxigenicity and species composition, and to comprehend the biogeography and migration of black Aspergilli causing bunch rot of grapes at a global level. Several molecular methods have been developed for fast detection of black Aspergilli on grapes. The availability of these rapid methods for identification and quantification of OTA-producing fungi in the early stages of veraison, in combination with knowledge of the important environmental and biotic factors, can help to achieve the most effective chemical application against black Aspergilli in the field. Species belonging to black Aspergilli are reported as both OTA and fumonisins producers; therefore FBs could represent a new emerging mycotoxin risk in the grape products.

Attempts to reduce fungal colonization and OTA content of grapes including agronomic practices and biological and chemical treatments have met with

varying degrees of success, and the data obtained are sometimes controversial. Several attempts have been made to identify possible corrective actions for OTA decontamination of wine and grape juice. Only recently, the use of repassage of wine over uncontaminated pomaces was demonstrated as a possibly useful technique for wineries located in high risk regions for OTA. Finally, we described further potential risk related to other mycotoxins in grapes and dried vine fruits.

Acknowledgements

This work was supported by EC KBBE-2007-222690-2 MYCORED and by Puglia Region Project PS_008 - INNOWINE.

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Accepted for publication: October 27, 2011