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Mycoflora and Biodiversity of Black Aspergilli in Vineyard Eco-Systems

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1. Introduction

Environmental conditions in vineyard eco-systems are of particular interest because they can influence the fungal populations associated with grapes, fungal–plant interactions, and production of secondary metabolites, including mycotoxins. Some fungal species are pathogenic to grapevines, infecting the roots, canes, leaves and fruit (Hewitt, 1988; Tournas & Katsoudas, 2005). Grape contamination by different moulds occurs during vineyard pre-harvesting, harvesting and grape processing (Magnoli et al., 2003). Moulds commonly isolated from grapes are *Alternaria*, *Cladosporium* and *B. cinerea*, the latter causing bunch rot. Pathogenic and opportunistic species of *Fusarium*, *Penicillium* and *Aspergillus* can also colonize inducing grape disease.

In heavily infected fruit, moulds alter chemical composition and mould enzymes adversely affect wine flavor and colour as well as yeast growth during alcoholic fermentation (Fleet, 1999; Fleet, 2001). Some vineyard fungal species are capable of producing toxic secondary metabolites (mycotoxins) in infected tissue, which may contaminate grapes and grape products such as wine, grape juice and dried vine fruit (reviewed in Nielsen et al., 2009). The mycotoxins of greatest significance include aflatoxins, citrinin, patulin and ochratoxin A (OTA) and recently fumonisin B_2 (FB_2) (Frisvad et al., 2007; Logrieco et al., 2010; Morgensen et al., 2010a, 2010b; Susca et al., 2010). The most important mycotoxin in grapes and the grape-wine chain is OTA first reported by Zimmerli and Dick (1996). It has nephrotoxic, carcinogenic (2B group) (IARC, 1993), teratogenic and immunotoxic effects (Abarca et al., 2001; Castegnaro & Pfohl-Leszkowicz, 2002, Da Rocha et al., 2002; Pfohl-Leszkowicz et al., 2002; Petzinger & Weidenbach, 2002; Vrabcheva et al., 2000). Thereafter, several authors reported OTA contamination in wine and the presence of OTA-producing fungi in grapes in different wine-growing areas around the world (Battilani et al., 2006; Leong et al., 2007; Medina et al., 2005; Sage et al., 2004; Tjamos et al., 2004). The contamination of grapes with OTA can occur in the field, even without visible symptoms, while the grapes are still on the vine (Serra et al. 2006).

2. Occurrence and biodiversity of black aspergilli from grapes

2.1 Role in Ocratoxin A contamination

Several surveys conducted in the Mediterranean, South America, and Australia reported fungal species belonging to *Aspergillus* section *Nigri* (also known as Black Aspergilli-BA) as

the major responsible for OTA contamination in grape (reviewed in Perrone et al., 2007). Several BA have been isolated from grape or from vineyard soil/air such as *A. niger* aggregate (namely *A. niger* sensu stricto, *A. tubingensis, A. foetidus,* and *A. brasiliensis)*, *A. carbonarius* and the uniseriate species *A. aculeatus, A. japonicus,* and *A. uvarum* (Medina et al., 2005; Perrone et al., 2008). Only a few species produce OTA among the *A. niger* aggregate, *A. carbonarius*, and *A. japonicus* (Battilani et al., 2003a). *A. ochraceus* (belonging to section *Circumdati),* although able to produce OTA, have only occasionally been isolated from grape. The most frequently occurring species are *A. niger* aggregate and *A. carbonarius*, respectively, although the highest percentage of OTA-producing strains has been detected in the latter species (Serra et al., 2005).

OTA contamination of dried vine fruit was also found to be due to black aspergilli in Europe, including Spain, Hungary and other parts of the world such as Argentina and Australia (Varga & Kozakiewicz, 2006). In spite of the higher incidence of species belonging to the *A. niger* aggregate found in vineyards, only 5-10 % of *A. niger* OTA-producing strains were detected, whereas more than 50% and, in some studies up to 100%, in *A. carbonarius* (Battilani et al. 2006; Heenan et al,. 1998 Perrone et al., 2006a; Serra et al., 2005). Other *Aspergillus* species, such as *A. helicotrix*, *A. ellipticus* and A. *heteromorphus, A. ochraceus* are rare (Bau et al., 2005)

2.2 Isolation and Identification tools

Black aspergilli are isolated and identified at genus and species level by morphological criteria: colour, density and colony appearance (layer colour, wrinkled, umbilical, thick or flat) and microscope observation (conidial head, conidiophore and conidia characters) in accordance with appropriate keys (Klick, 2002; Klick and Pitt, 1988; Pitt & Hocking, 1999, 2009; Samson et al., 2004, 2007). The taxonomy of *Aspergillus* section *Nigri* is widely studied but although identification at section level is quite easy, at species level it is much more complex since morphologically taxa differences are very subtle requiring taxonomic expertise.

For macromorphological observations, Czapek yeast autolysate (CYA), malt extract autolysate (MEA), Czapek yeast autolysate with 5% NaCl (CYAS) agar, yeast extract-sucrose (YES) agar, oatmeal agar (OA) and Czapek agar (CZA) are used (Samson et al., 2004). Some differential growth media e.g. DYSG Agar, Coconut Cream Agar (Heenan et al., 1998) and MEA-B (Pollastro et al., 2006) may facilitate the recognition of ochratoxigenic black aspergilli (Samson et al., 2007). Useful physiological features are very good growth and sporulation at 37 °C as well as growth and acid production on CREA agar (Samson et al., 2004). Species can be identified by micromorphological analysis of the fungal structures by light microscopy. Scanning Electron Microscopy (SEM) is helpful for vesicle observation which is necessary for distinguishing between uniseriate (i.e. *A. aculeatus*, *A. japonicus*) and biseriate species (ie. *A. carbonarius*, *A. ibericus*, *A. niger*) and conidia ornamentation which can distinguish between *A. niger* aggregate, *A. carbonarius* and *A. ibericus* (Serra et al., 2006; Varga et al., 2000) (Table 1).

As for other fungal species studies based on molecular sequence analysis of ribosomal and ubiquitous genes (ITS, IGS, calmodulin, ß-tubulin, elongation factor) and polymorphisms by obtained Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD) and microsatellites, have been performed for Aspergilli isolated from grapes. These studies have provided useful information on the taxonomy of BA and methods for their detection, identification and monitoring (reviewed in Abarca et al., 2004; Geiser et al., 2007; Niessen et al., 2005; Perrone et al., 2007, 2009; Samson et al., 2007).

Species	Conidiophore	Conidial size (μm)	OTA production
A. japonicus/A. aculeatus	Uniseriate	4–5	Negative
A. niger aggregate	Biseriate	3–5	Positive (low %)
A. sclerotioniger	Biseriate	5–6	Positive
A. carbonarius	Biseriate	7–9	Positive (high %)
A. ibericus	Biseriate	5–7	Negative

Table 1. Some characteristics of the main black *Aspergillus* species (from Serra et al., 2006)

PCR primers for detecting BA target the generic sequences for identifying or characterizing the fungus taxonomy at the intraspecific level or the sequence of genes involved in mycotoxin biosynthesis which are not necessarily able to distinguish fungal species (Bau et al., 2005; Dao et al., 2005; Perrone et al., 2007; Sartori et al., 2006; Schmidt et al.. 2003, 2004; Serra et al., 2005).

Species-specific primers based on ITS sequence differences were developed for *A. ellipticus, A. heteromorphus, A. japonicus*, *A. niger* (Gonzales-Salgado et al., 2005), *A. carbonarius* and *A. ochraceus* (Patiño et al., 2005). Some others were developed for *A. carbonarius* identification based on SCAR primers (Pollastro et al., 2003; Pelegrinelli-Fungaro et al. 2004), whereas PCR-RFLP analysis is necessary to distinguish the strains of *Aspergillus niger* aggregate into two groups: *A. niger* and *A. tubingensis* (N and T type) (Accensi et al., 2001, Gonzalez-Salgado et al., 2005).

More primers have been developed for the genes encoding the polyketide synthases (PKSs) involved in OTA biosynthesis in both *Aspergillus* and *Penicillium* (Ayoub et al., 2010; Atoui et al., 2006; Dao et al., 2005; O'Callaghan et al., 2003).

Recent studies have reported the use of degenerate primers targeting the ketosynthase domain (KS) which identified a new pks gene from *A. carbonarius* (AC*pks*) (Atoui et al., 2006; Gallo et al., 2009). By screening *Aspergillus* isolates with AC*pks* specific primers, AC*pks* homologues appeared to be present in *A. sclerotioniger* and *A. ibericus* which are closely related to *A. carbonarius*.

A duplex real-time PCR assay for simultaneously detecting members of the *Aspergillus niger* aggregate and *A. carbonarius* was developed by López-Mendoza et al. (2009) and Selma et al. (2009). They targeted the beta -ketosynthase and acyl transferase domains of the poliketide synthase of *A. carbonarius* and the *A. niger* aggregate, the assay allowing preferential amplification at greater concentrations providing a fast and accurate tool to monitor, OTA-producing species in grapes in a single reaction. These approaches gave rise to molecular diagnostic assays based on expression profiling and which determine the molecular triggers controlling OTA biosynthesis in *Aspergillus* spp.

More recently real-time/quantitative PCR (qPCR) protocols have detected and quantified ochratoxigenic fungi, developed using constitutive genes (González-Salgado et al., 2009; Morello et al., 2007; Mulè et al., 2006) or genes involved in toxin biosynthesis (Atoui et al., 2007; Schmidt et al., 2004; Selma et al., 2009).

2.3 European biodiversity monitoring

DNA-based fingerprinting techniques such as AFLP, RFLP. RAPD, ap-PCR, and the sequencing of subgenomic DNA fragments have drastically improved the understanding of the occurrence and biodiversity of *Aspergillus* spp. in grapes and vineyards worldwide.

Sequencing techniques were primarily useful at the species identification level, whereas fingerprinting techniques were exploited at the intraspecific level (Ferracin et al., 2009; Dachoupakan et al., 2009; Perrone et al., 2007). Although all these studies contribute to analysing the species composition and genetic diversity of grape mycobiota, no genotypical differences could be established between OTA producers and non producers (Ilic et al., 2001, 2004; Martinez-Culebraz et al., 2009; Chiotta et al., 2011). Moreover, there was no correlation between genotype, the ability to produce OTA and geographical origin (Niessen et al., 2005). Surveys conducted in Europe during the four-year EU project 'Wine-Ochra Risk' (QLK1-CT 2001-01761) indicated a significant correlation between the incidence of grape infected by black aspergilli, as potentially OTA producer, at harvest and in climatic conditions and geography (latitude and longitude); there was increasing incidence from West to East and North to South (Battilani et al., 2006). Aspergilli in vineyards varied depending on years and geographic areas: France, Greece and Israel were the areas with the highest incidence, followed by South Italy, Spain and Portugal (Abarca et al., 2001; Battilani et al., 2006; Guzev et al., 2006; Logrieco et al., 2007; Otteneder & Majerus., 2000; Sage et al., 2002). In countries with colder temperate climates such as Germany, Northern Hungary, the Czech Republic as well as the northern parts of Portugal, France and Italy, BA has not often been isolated from grapes, although sometimes OTA has been detected in wines. The identification of OTA producing *Penicillium* species from grapes in Northen Italy and France suggests they could be responsible for contamination in these regions (Battilani et al., 2001; Rousseau, 2004).

Surveys in 107 vineyards in the Mediterranean basin have identified four main *Aspergillus* populations: *A. carbonarius*, *A. tubingensis*, *A. niger*, and a group of *Aspergillus* 'uniseriate' isolates morphologically indistinguishable from *A. japonicus* and *A. aculeatus.* The latter could be clearly distinguished by molecular tools such as AFLP, RFLP and sequence analyses (Bau et al. 2006; Perrone et al. 2006a, 2006b). Highest genetic variability was observed in the *A. niger* group due to its complexity and the difficulty of identifying it at species level by both AFLP and the sequencing of calmodulin and β-tubulin subgenomic fragments (Perrone et al., 2007). In Australian vineyards, Leong et al. (2007) documented the dominance of *A. niger* over *A. carbonarius* and *A. aculeatus.* Polyphasic studies using macro- and micromorphology, secondary metabolite profiles, partial sequences of β-tubulin, calmodulin and ITS genes, and AFLP analysis led to the description of a new *Aspergillus* species: *A. ibericus*, which is closely related to *A. carbonarius* but unable to produce OTA (Serra et al. 2006) and *A. brasiliensis* belonging to the *A. niger* aggregate (Varga et al., 2007), both isolated from grapes in the Iberian Peninsula; *A. uvarum,* morphologically very similar to *A. japonicus* and *A. aculeatus*, but clearly distinct by the molecular analysis of grapes samples in Portugal, Italy, France, Israel, Greece and Spain.

In Italy, field surveys studied the fungi associated with grapes and their ability to produce OTA in different grape-growing areas (as regards grape variety and farming methods) in the north and south of the country (Battilani et al., 2002, 2006). Analysis of these grape samples revealed that *A. niger* aggregate was the prevalent species and *A. carbonarius* was mostly found in Southern Italy and Sicily (Lucchetta et al., 2010; Oliveri, 2007). *A. carbonarius* was never dominant at different growth stages, or in different geographical areas and years, but it was confirmed as the key fungus because of the high percentage of strong OTA producing isolates in the population.

In sixteen vineyards located in 13 provinces (including Modena, Imola, Ravenna, Brindisi and the warmest places such as Trapani and Ragusa), the effect of geographic area on fungal flora was confirmed, even though a major role was played by meteorological conditions,

both on fungal colonisation and the OTA content in bunches. BA were present in bunches from setting, colonising most berries at early veraison (Battilani et al., 2006). The detection of isolates belonging to the *A. niger* aggregate, A. *carbonarius* and uniseriate varied with growth stage. At setting and berry pea-sized stages, more than 50% of isolates belonged to uniseriate; starting from early veraison, the *A. niger* aggregate became dominant (about 50%) whereas A. *carbonarius* was around 20% from pea-size to harvesting. The region with the highest percentage of grapes berries colonised by the *A. niger* aggregate was Veneto, while the lowest was in central Emilia Romagna. The highest incidence of *A. carbonarius* was detected in Puglia and the lowest in Emilia Romagna and Veneto. The number of OTA-producing strains among BA, isolated in each vineyard at different growth stages, was generally very limited (an encouraging result) (Battilani et al., 2006).

Molecular techniques to investigate strain variation in toxigenic and non-toxigenic black *Aspergillus* spp. showed that isolates of A. *carbonarius* and *A. niger* clustered into species groups, however, within species, strains displaying similar degrees of toxigenicity did not cluster together when characterized by RAPD techniques (Ilic et al., 2001, 2004). The profile of the *Aspergillus terreus* species isolated from dried grapes, analysed by RAPD, indicated great genomic diversity (Narasimhan & Asokan, 2010). Martínez-Culebras et al. (2009) recently carried out a study on ochratoxigenic mycobiota in grapes by ap-PCR sequence analysis of the ITS and IGS regions and their ability to produce OTA. Based on ap-PCR profiles, derived from two microsatellite primers, three main groups were obtained by UPGMA cluster analysis corresponding to *A. carbonarius*, *A. niger* and *A. tubingensis*. The cophenetic correlation values corresponding to ap-PCR UPGMA analysis showed higher genetic variability in *A. niger* and *A. tubingensis* than in *A. carbonarius*. In addition, no genotypical differences could be established between OTA producers and non-producers in all the species analysed. Regarding uniseriate black aspergilli, low divergence was found between *A. aculetus* and *A. uvarum*. OTA-production seems to be strain related since it was found in different clusters, with either ap-PCR or IGS-ITS phylogenetic analysis.

2.4 Epidemiology in vineyard

Black aspergilli are affected by several factors in the vine environment, i.e., grape status , the number of damaged grape berries, meteorological conditions, vineyard location, the cropping system as well as chemical treatments (Battilani et al., 2003b, 2006; Belli et al., 2005; 2007a; Blesa et al., 2006; Clouvel et al., 2008; Hocking et al., 2007; Leong et al., 2006). Generally fungi have been detected in vineyards and on grapes from setting. However, grape aspergilli increase gradually, reaching their maximum values at the beginning of veraison and ripening (Battilani et al., 2002). As *Aspergillus* species are not considered primary pathogens, various grape damage, such as attack by other fungi or mechanical injury, dramatically increases the risk of fungal infection by these species and OTA contamination (Serra et al., 2006; Belli et al., 2007b). However, grape damage due to insects, birds or other fungal infections, is the primary factor affecting the development of the disease and OTA accumulation in grapes (Cozzi et al., 2006).

Some Australian studies have demonstrated that vineyard soil at a depth of 0–5 cm beneath the vines is the primary reservoir of black aspergilli (Clarke et al., 2003; Kazi et al. 2004; Leong et al. 2006). Concentrations were also higher in the soil directly beneath the vines compared to the inter-row area. It is postulated that air movement deposits spores from the soil onto the grapes berry surfaces, because BA spores in air samples were higher closer to

the soil (Kazi et al., 2003a). Soil temperature could also affect the incidence of *A. carbonarius* in the soil; the optimal temperature for spore survival was around 25 °C, with counts decreasing at 15 °C and 35°C. Survival at 40 °C was poor (Kazi et al., 2003b, 2004).

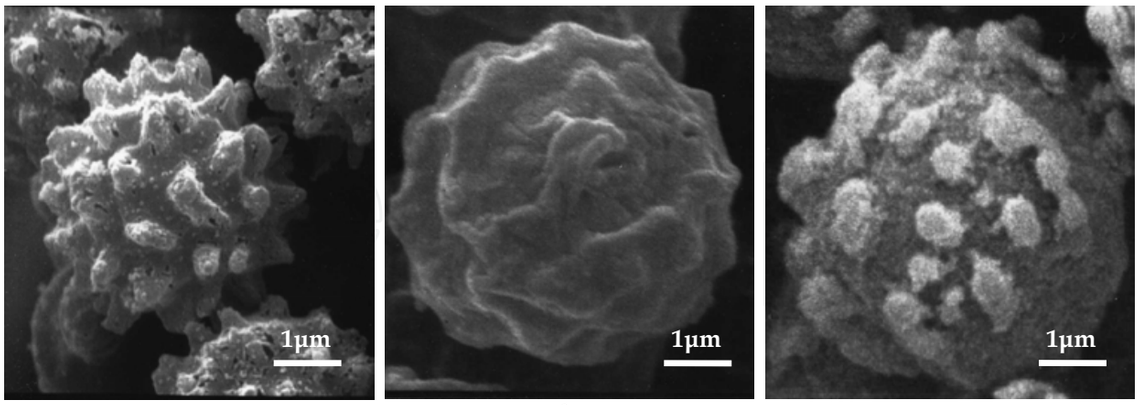
Agronomic practices and biological and chemical treatments have been found to reduce BA colonization and OTA levels in grapes and grape-derived products (reviewed in Varga & Kozakiewicz, 2006).

3. Biodiversity in a restricted geographical area: A case study from the Mount Etna Slopes

3.1 Vineyard mycobiota

Most studies address the biodiversity of grape mycoflora and above all of BA in countries and in wine producing areas. Recently we performed a two-year survey in a restricted geographical area to assess the population density and biodiversity of the mycoflora associated with the grapes, air and soil of the vineyards on the slopes of Mount Etna (eastern Sicily, Italy) where there is a long tradition of grape cultivation. This area is characterized by a temperate Mediterranean climate, with an average annual rainfall of 800 mm and high day/night temperature fluctuations. Moreover, the area is characterized by old and authoctonous wine-grape cultivars, i.e. Etna rosso DOC (Nerello mascalese, Nerello Cappuccio) and Etna Bianco (Carricante, Catarratto).

Special emphasis was made on toxigenic fungal species, e.g. *Aspergillus* Sect. *Nigri* spp. and *Penicillium* spp. (Oliveri, 2006; Oliveri et al., 2008). It is postulated that air movement deposits soil fungal spores onto grapes, because their incidence in air samples increases closer to the soil. So, healthy grape soil beneath the vines was ecologically monitored and its air was sampled at two different heights at the pea stage, early veraison and ripening using plating methods. Spore-producing filamentous fungi were detected, identified at the genus level, and then the *Aspergillus* and *Penicillium* strains were isolated and identified at the species level (Fig. 1).



a b c

Fig. 1. Scanning electron microscopy pictures of a) *A. carbonarius*, *A. niger* b) and c) *A. japonicus* spores, where spore ornamentation differences are clearly seen (bar = 1 µm) (Zeiss, DSM 940).

The most frequent genera isolated from air, soil and grapes by increasing order were *Aspergillus, Penicillium, Cladosporium* and *Rhizopus*.

Fungi belonging to *Aspergillus* spp. were present in all the sampled matrices from the pea stage and they were predominant to *Penicillium* spp. from early veraison to ripening. Fungi from 6 genera were isolated on grapes at the ripening stage in eight different vineyards (Fig. 2).

Population densities of *Aspergillus* spp. in grape wash water ranged between 4.5×10^3 and 1.2×10^4 cfu ml^{-1}.

The most frequent *Penicillium* species isolated from the vineyard eco-system were *P. chrysogenum*, *P. expansum* and *P.olsonii* (Tab. 2). *P. verrucosum* was isolated from only one soil sample. Among the *Aspergillus* species, the most frequent were from section *Nigri*; the level of contamination by *A. ochraceus* and *A. flavus* was low.

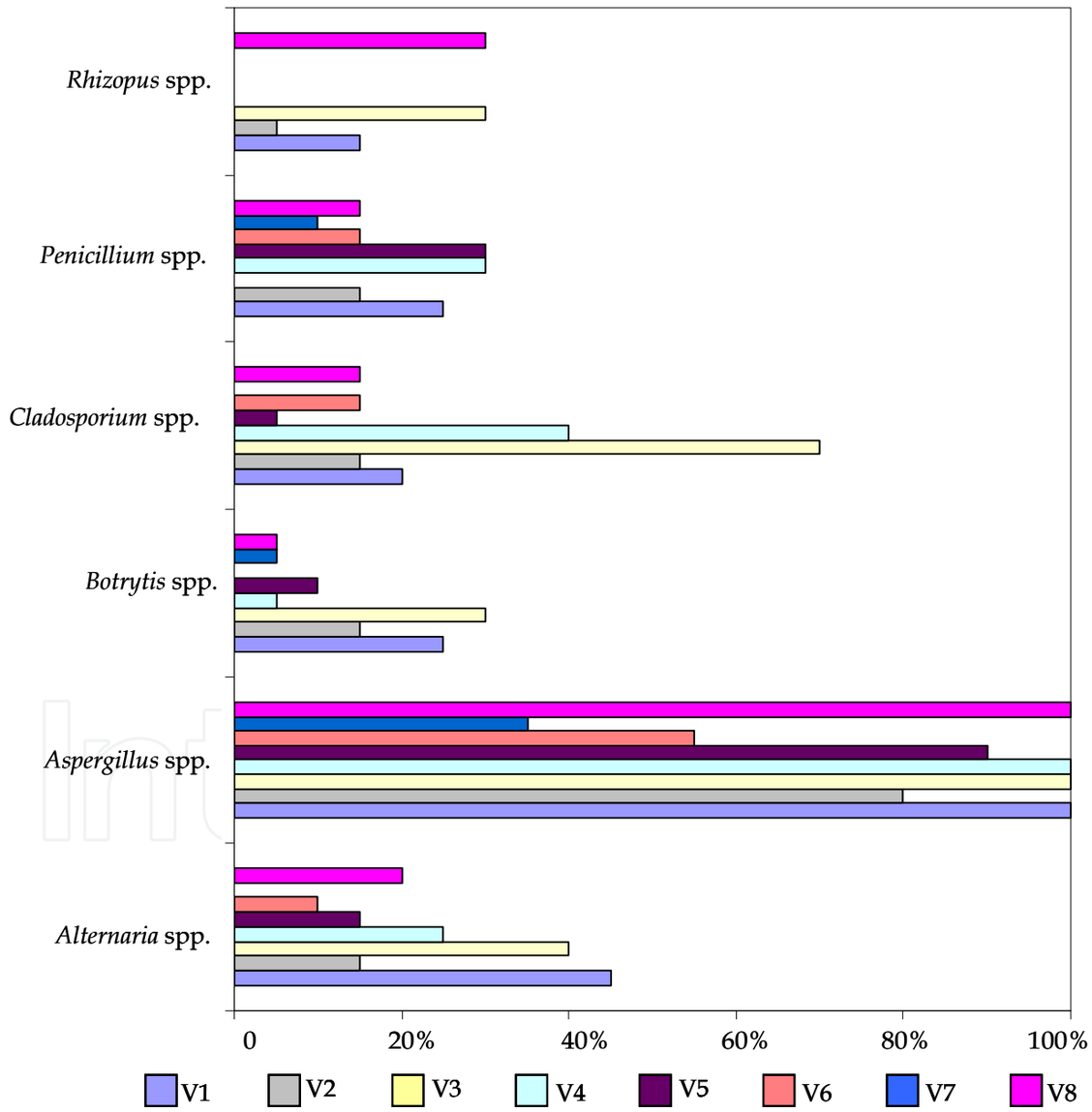


Fig. 2. Population composition (%) detected in 8 vineyards (V1-V8) from 20 grape samples, during the ripening stage and over a 2-year survey.

Genus	Species	n. samples [a]		
		air (224)	Soil (112)	Grapes (160)
Aspergillus	*niger* aggregate	125	94	128
	carbonarius	43	49	38
	flavus	18	21	1
	ochraceus	8	20	1
Penicillium	*aurantiogriseum*	4	3	n.d.
	chrysogenum	50	55	n.d.
	expansum	63	22	15
	italicum	29	19	14
	olsonii	n.d.	7	n.d.
	verrucosum	n.d.	1	n.d.

n.d.= not detected

[a] in parenthesis the total number of analyzed samples for each source

Table 2. *Aspergillus* and *Penicillium* spp. isolated from air, soil and grape samples over a two year survey.

3.2 Black Aspergilli and OTA producers

Black aspergilli appeared in all the tested samples, their incidence being higher at early veraison and ripening (Oliveri et al., 2006; Oliveri, 2006) (Fig.3 a-c). According to macro- and micromorphological characteristics, they were identified and classified into two main groups, *A. niger* aggregate and *A. carbonarius* (Fig.3 g-n). *A. ochraceus* has occasionally been detected in grape samples (Fig.3 d-f).

A subset of 66 strains was selected for further analysis. PCR assays supported the morphological identification. *A. niger*, *A. carbonarius* and *A. japonicus* were identified by target sequences for each species according to assays described by González-Salgado et al. (2005) and Patiño et al. (2005). In order to characterise the species in *A. niger* aggregate, i.e. *A. niger* and *A. tubingensis,* very difficult to differentiate by classical morphological criteria, the RFLP analysis with *Rsa*I was performed. This differentiation is very important to as to avoid overestimating toxicological contamination and related risks. A primer annealing site or restriction nuclease cleavage site was further confirmed by ITS sequencing which also confirmed the identity of the isolates (Oliveri et al., 2008).

The OTA production of isolates belonging to *A. carbonarius*, *A. niger*, *A. tubingensis* and *A. japonicus* was assessed by enzyme-linked immunosorbent assay (Oliveri et al., 2006b, 2008). 56% of strains were shown to produce OTA. *A. carbonarius* isolates were the strongest OTA producers with some of them producing high concentrations of OTA (>40 ppb).

3.3 Intraspecific variability

A fAFLP protocol was used to assess specific and intraspecific variability (Oliveri et al., 2008). In agreement with other studies (Perrone et al., 2006a, 2006b), the AFLP technique generated enough polymorphism to differentiate between and within the species of black aspergilli. *A. niger, A. tubingensis*, *A. carbonarius* and *A. japonicus* strains were clearly differentiated, although *A. niger* strains clustered into two different groups. Intraspecific variability didn't correlate with the isolate origin. In fact isolates from different vineyards either of grape or the environment could also cluster in the same or in different clusters. Perrone et al. (2006a, 2006b) analyzed representative strains from the main wine producing

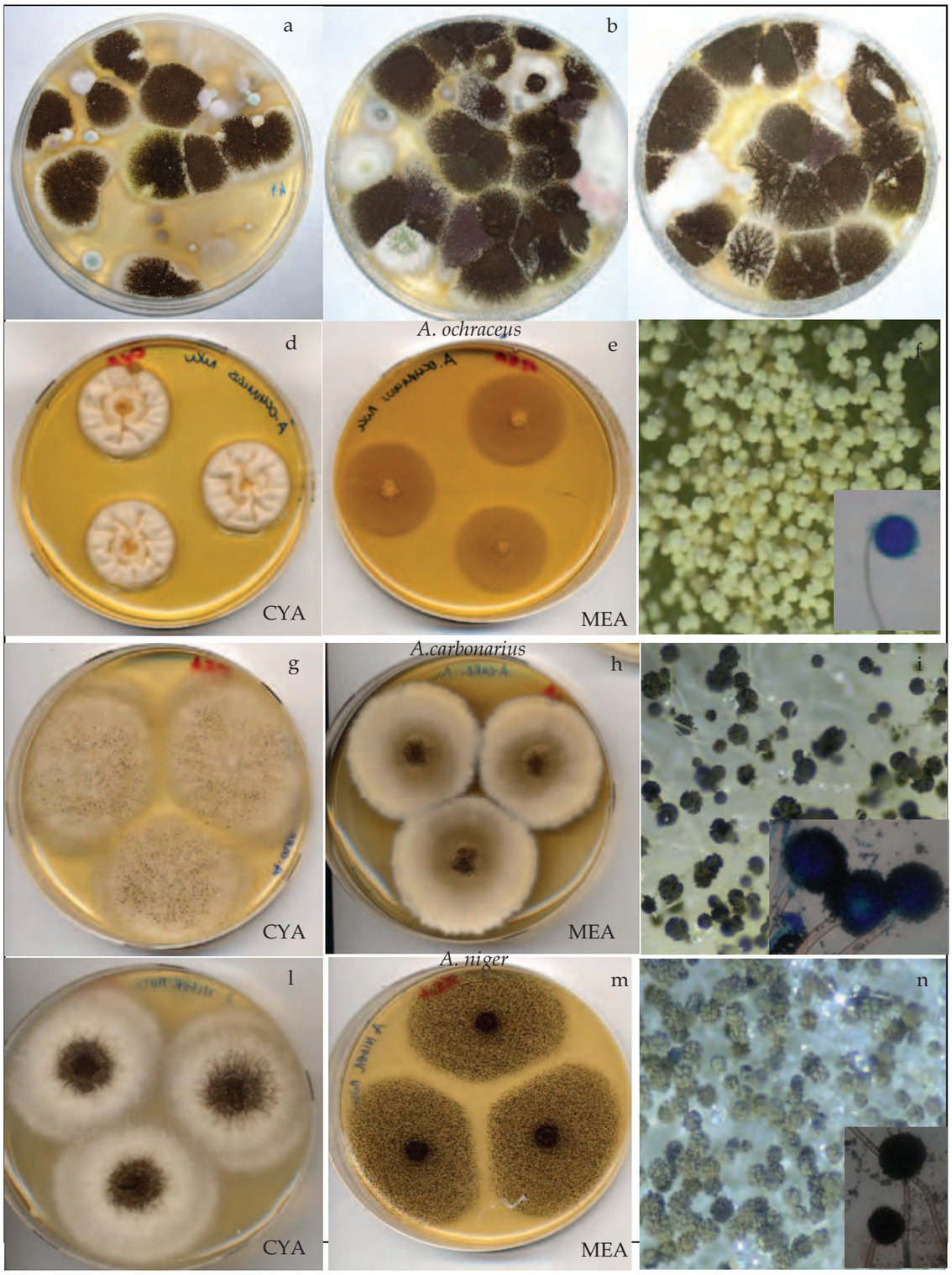
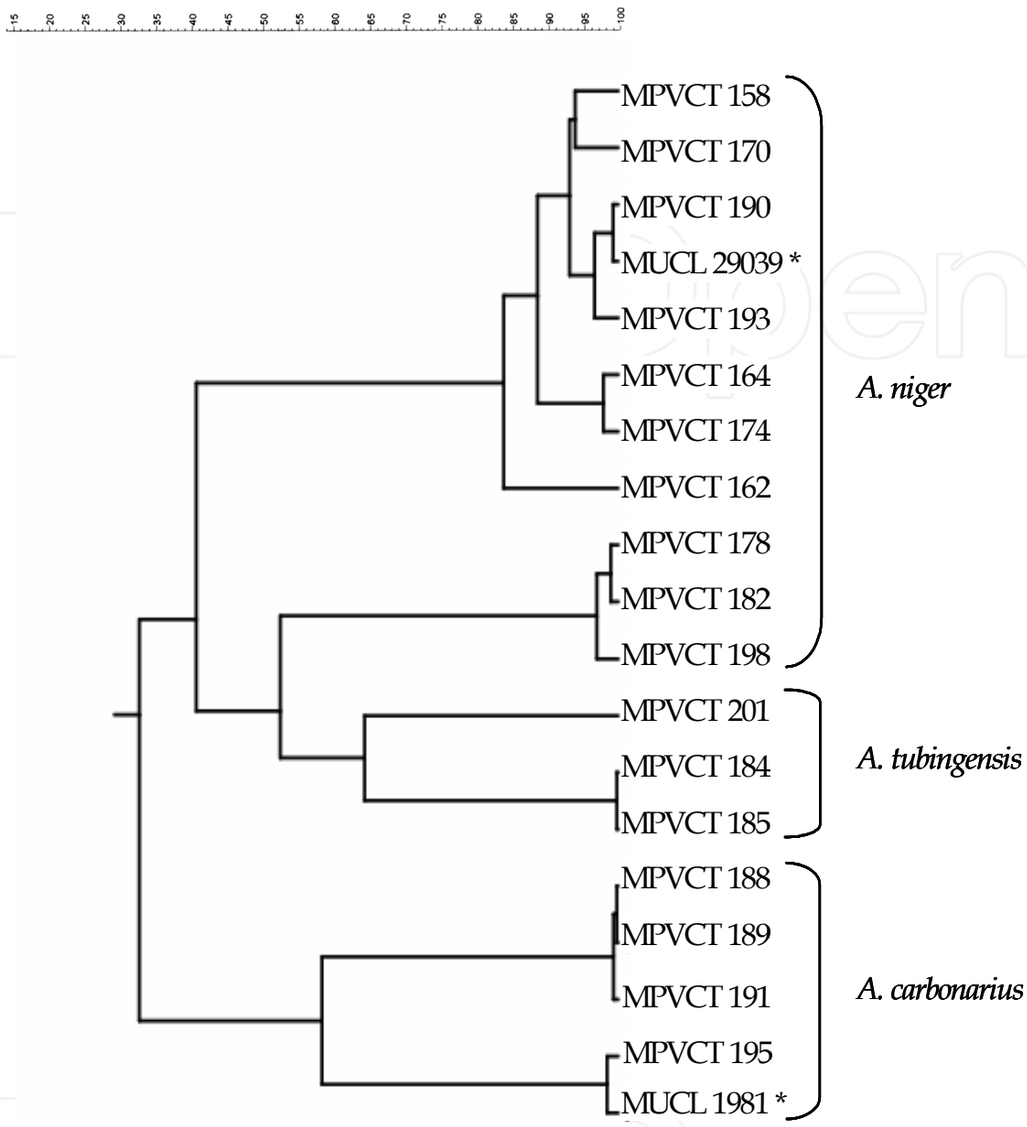


Fig. 3. Fungal colonies isolated from grape (a) , soil (b) and air (c) samples from vineyard. Colony morphologies and light microscopy pictures of conidiphores and conidia of representative isolates belonging to *Aspergillus ochraceus* (d–f), *A. carbonarius* (g - i), *A. niger* (l - n).



MPVCT: Micothèque of Institute of Plant Pathology, University of Catania, Italy; USA; MUCL: Micothèque de L'Universitè Catholique de Lovain, Belgium
* Reference strains

Fig. 4. UPGMA dendrogram obtained from fAFLP analysis with the selective primer pair E-AT (Cy5-labelled) and M-CT on 17 isolates (10 *A. niger*, 3 *A. tubingensis,* 4 *A. carbonarius*) and 2 reference strains isolated from grape samples in the same vineyard. Fragments between 50 and 600 bp were analysed with PHYLIP® v. 3.66 software

European countries (Italy, France, Spain, Portugal, Greece and Israel) and the four main groups were obtained by AFLP clustering analysis of the strains, three of them showing a well-defined homogeneous population/species with intraspecific homology higher than 48%: *A.carbonarius*, *A. tubingensis* and *Aspergillus* 'uniseriate'. The fourth cluster, called *A. niger* 'like', showed low homology with *A. niger* 'type strain' and high internal heterogeneity. The intra-population variability of *Aspergillus* Section *Nigri* strains isolated

from grape samples within the same vineyard proved that mixed populations of *A. niger* and *A. carbonarius* were present and most of them were OTA producers (Fig. 4). As for larger population studies, no correlation was found between genotypes and mycotoxin production (Martinez-Culebraz et al., 2009).

4. Conclusion

This chapter summarizes data on grape mycoflora, toxigenic fungi and mycotoxin contamination at the pre-harvesting, harvesting and processing stages. Grape rotting and spoilage can be caused by a variety of fungal species, including *Botrytis cinerea*, *Penicillium*, *Aspergillus*, *Alternaria* and *Cladosporium*. In recent years, black *Aspergillus* species (Section *Nigri*) and in particular *A. carbonarius* and *A. niger* aggregate have been described as the main source of grape contamination with the mycotoxin ochratoxin A. In this chapter, we highlighted how *Aspergillus* species distribution on European grapes may occur and vary in relation to meteorological conditions and geographical areas and several studies have shown an increase in the amount of OTA in warmer climates. The literature on various molecular methods used for species identification is reviewed and a critical evaluation of the usefulness of various techniques and genomic loci for the species identification of black aspergilli is presented. Reports of the occurrence of black aspergilli in vineyards and their potential toxigenicity must be reconsidered on the basis of the wide molecular biodiversity found within morphologically undistinguishable strains of this section. Mycotoxin production is a characteristic of the species, so by studying the species intraspecific biodiversity can predict potential mycotoxin hazards. Different isolates belonging to the black aspergilli species showed varying abilities to produce OTA so it becomes utmost importance to guarantee a quality control of the grapes and grape derived products, through accurate contaminant mycoflora identification.

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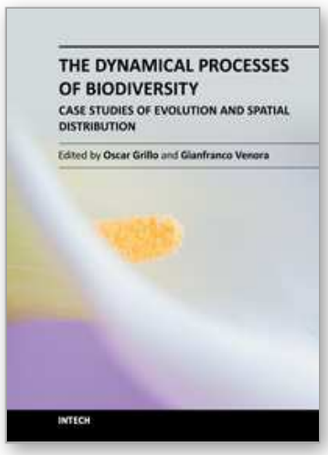

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Driven by the increasing necessity to define the biological diversity frame of widespread, endemic and threatened species, as well as by the stimulating chance to describe new species, the study of the evolutive and spatial dynamics is in constant execution. Systematic overviews, biogeographic and phylogenic backgrounds, species composition and distribution in restricted areas are focal topics of the 15 interesting independent chapters collected in this book, chosen to offer to the reader an overall view of the present condition in which our planet is.

How to reference

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