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Managing ochratoxin A risk in the grape-wine food chain

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ABSTRACT

The main source of ochratoxin A (OTA) in the wine food chain is the infection of grapes by “black aspergilli” occurring in the field. OTA producing black aspergilli include mainly *Aspergillus carbonarius*, followed by *A. niger* and possibly *A. tubingensis*. They are opportunistic fungi that may develop massively on damaged berries at ripening, although they may occur and form OTA on grapes from veraison to harvest. Climatic conditions (high humidity and temperature) and geographical location are important factors favouring OTA accumulation in grape berries. The severity of aspergillus rot is influenced by excessive irrigation and rainfall prior to harvest causing berry splitting. Berry wounds caused by insect attacks provide preferential entries for black aspergilli. High OTA levels occur in grapes severely damaged by the grape moth *Lobesia botrana*, particularly in the Mediterranean areas.

Some grape varieties display greater susceptibility to aspergillus rots due to intrinsic genetic characteristics and bunch conformation (compact > sparse). Control measures for toxigenic mycoflora in the vineyards must consider these critical control points. Proper fungicidal and insecticidal treatments can reduce OTA contamination. Knowledge about the fate of OTA and its distribution in wine and winery by-products is important to manage OTA risk in contaminated stock. In our wine-making experiments, only 4% of the OTA present in grapes remained in the wine whereas most is retained in pressed grape pomaces. OTA concentration remained unchanged in wine after one year aging as well as in all liquid fractions collected during vinification (must, free run wine, and wine after first and second decantation).

Activated carbon can reduce OTA levels in wine but negatively affects wine quality.

KEYWORDS: ochratoxin A, black aspergilli, mycotoxins, grape, wine, food safety
INTRODUCTION

Ochratoxin A (OTA) is a major mycotoxin produced by several species of *Aspergillus* and *Penicillium* naturally occurring in a variety of food commodities prior to harvest or more commonly during storage. Numerous animal studies have shown that OTA is a potent nephrotoxin with the degree of renal injury depending on both toxin, dose and exposure time; decreasing nephrotoxic sensitivity was observed from pig to rat, to mice. OTA is immunotoxic, neurotoxic *in vitro* and *in vivo* in rats, teratogenic in mice, rats and rabbits (JECFA 2001). Based on renal carcinogenicity shown in rats and mice, the IARC (International Agency for Research on Cancer) has classified OTA as a Group 2B carcinogen, i.e. carcinogenic to animals and possible carcinogenic to human (IARC 1993). Studies on the genotoxicity of OTA remain controversial. Recent scientific evidence indicates that the site-specific renal toxicity as well as the DNA damage and genotoxic effects of OTA, measured in various *in vivo* and *in vitro* studies, are most likely attributable to cellular oxidative damage (EFSA 2006). Various studies in humans have associated OTA with an endemic kidney disease observed in the Balkans (Balkan Endemic Nephropathy and related Urinary Tract Tumors), but convincing epidemiological evidence associated with OTA exposure is currently lacking. It has been frequently found in human blood, urine and milk and has been demonstrated a widespread individual exposure to low levels of OTA in Europe and other continents (EFSA 2006).

The widespread human exposure to OTA is well documented by a number of surveys reporting the occurrence of OTA in a variety of food products. An assessment of the dietary intake of OTA by the population of the European Community has been performed showing that the main contributors to OTA exposure are cereals and cereal products (European
Commission 2002). Wine, coffee and beer were identified as significant contributors to human OTA exposure. Dried vine fruit and grape juice contribute to a significant extent to the OTA exposure of vulnerable groups of consumers such as children. The EFSA Scientific Panel on contaminants in the food chain has recently adopted an updated scientific opinion relating to OTA in food, taking into account new scientific informations and derived a tolerable weekly intake (TWI) of 120 ng/kg body weight (EFSA 2006).

Based on the available scientific toxicological and exposure data, the European Union established maximum permitted limits for OTA in a variety of food products that have been updated with the EC Regulation 1881/2006 (European Commission 2006). Maximum levels have been set at 2 µg/kg for wine, fruit wine, grape juice, grape nectar and grape must intended for direct human consumption, and at 10 µg/kg for dried vine fruit (currants, raisins and sultanas). OTA was detected in wine for the first time in 1996 (Zimmerli and Dick 1996).

Thereafter several surveys have been conducted, mainly in Europe, on the occurrence of the toxin in wine and related products, showing it as a problem mainly for Southern Europe. The results of several reports from different countries are reported in Table 1 for a total of 3512 samples. A high incidence of contamination (from 40% to 87%) was reported in all surveys but the Australian one having an incidence of 15% (Hocking et al. 2003), with maximum level at 15.6 µg/kg recorded in Italy (European Commission 2002). OTA levels showed a decreasing gradient from red to rosé, to white wines, and the same trend was observed for the grape juice. Wines from southern and warmer regions of Europe showed incidence and levels of contamination (72.3%, mean value 0.64 µg/kg, n = 635) higher than those from northern European areas (incidence 50.3%, average 0.18 µg/kg, n = 835). Wines produced in Southern Italy, where the climatic conditions favour the growth of OTA producing fungi in grapes, generally show incidence and levels of contamination higher than wines produced in Northern
and Central Italy. Recently, a survey of retailed wine samples from Southern Italy showed a positive correlation between high levels of OTA and resveratrol-related compounds (Perrone et al. 2007b). Considering that a significant reduction of hepatic and renal damage caused by OTA was reported in mice fed with grape juice contaminated with OTA (Jeswal 1998), there may be some possibility that toxic levels of OTA in red wine are, at some extent, counterbalanced by the beneficial effects of resveratrol derivatives.

In the following headings different aspects of OTA formation in grapes and strategies for prevention and control in the field are reported together with OTA distribution and possible corrective actions during wine processing. Good agriculture and manufacturing practices are currently under discussion by Codex Alimentarius for the adoption of a code of practice for the prevention and reduction of OTA contamination in wine, based on a code of sound vinicultural practices adopted by the Organisation Internationale de la Vigne et du Vin (OIV) in 2005 (FAO/WHO 2007).

**Fungi responsible for OTA accumulation in grapes: taxonomy and ecology.**

Fungi responsible for OTA accumulation in cereals, i.e., *Aspergillus ochraceus* and *Penicillium verrucosum*, were firstly thought to be involved also in OTA formation in grapes. However, a number of studies performed during the last decade provide evidence of a quite different situation showing that all fungi responsible for OTA in grapes belong to *Aspergillus* section *Nigri*, the so called “black aspergilli” (Battilani et al. 2003). Most epidemiological surveys, performed in Mediterranean, Australian and South American countries, have shown that within the black aspergilli the biseriate species, *Aspergillus carbonarius* and *A. niger* “aggregate”, and the uniseriate species, *A. aculeatus* and *A. japonicus*, are the prevalent ones occurring on grapes (Battilani et al. 2003; Da Rocha Rosa et al. 2002; Leong et al. 2006a).
The taxonomy of this Section is still not completely resolved, especially within the *A. niger* “aggregate” (a group of morphologically indistinguishable species), leading often to misidentification of the species distribution in food. A comprehensive molecular characterization of the black aspergilli occurring in grape in Europe was performed within the EU project Wine-Ochra Risk (QLK1-CT-2001-01761) using representative strains isolated from 107 vineyards of the Mediterranean basin (Bau et al 2006; Perrone et al 2006a,b). These studies led to the identification of four main populations, namely *A. carbonarius*, *A. tubingensis*, *A. niger*, and a group of *Aspergillus* “uniseriate”, that could be separated by using molecular methods including AFLP, RFLP and sequences analysis. The *Aspergillus* “uniseriate” group was clearly separated from *A. japonicus* and *A. aculeatus* by molecular techniques but was morphologically indistinguishable (Perrone et al. 2006a,b). Ecological and morphological differences between these species are summarized below.

*A. carbonarius* was easily distinguished from other biserial species due to its big and spiny conidia; a high percentage of strains of this species (98-100%) have been shown to produce OTA. Spore germination of *A. carbonarius* is very rapid, and occurs within 24 hours with water activity ($a_w$) 0.90-0.99 and temperature 25-35°C. Optimal growth is at 32-35°C and $a_w$ 0.95-0.98 (min 10°C and max 45°C). Optimal conditions for OTA production by *A. carbonarius* are at 20-25 °C and $a_w$ 0.95/0.98 (Belli et al. 2005).

The *A. niger* “aggregate” comprised four different species indistinguishable by morphological characteristics. The most frequent species isolated from grapes were *A. niger* and *A. tubingensis*, while *A. foetidus* and *A. brasiliensis* were detected at minor extent (Perrone et al. 2007c). *A. niger* aggregate optimal growth conditions were 35–37 °C and $a_w$ 0.93-0.98 (min
6–8 °C and max 47 °C). This is one of the most common group of species in a wide range of fresh and dry fruits, cereals, etc., and is used in food processing as “GRAS” (generally regarded as safe). OTA production by *A. niger* “aggregate” normally occurs at 20-25 °C, and a_w 0.95/0.98 (Esteban et al. 2004). A low percentage of OTA producing strains (5-10%) were detected among the *A. niger* “aggregate” (Perrone 2006a).

Among the uniseriate group, *A. aculeatus* and *A. japonicus* were often isolated from grapes, but were not proven to produce OTA although they grow at similar conditions as for *A. carbonarius*. Recently the *Aspergillus* “uniseriate” population from grapes in Europe were characterized as a population molecularly quite different from *A. japonicus* and *A. aculeatus* (Perrone et al. 2006b). This population is being described as a new species to be called *A. uvarum* (Perrone et al. 2007 submitted).

Several reports from South America claiming the production of OTA by strains of *A. japonicus* or that *A. niger* is the major responsible for OTA accumulation in grapes are based on morphological identification of the producing strains (Dalcero et al. 2002; Chulze et al. 2006; Ponsone et al. 2007). These data have not been confirmed by molecular identification of the species and should be regarded with ultimate care to avoid confusion in the literature.

In our survey of about 600 strains of black aspergilli, representative of 3-year sampling, 5% of *A. niger* “aggregate” strains (360) resulted OTA producers, while all *A. carbonarius* strains (200) and none of the A. “uniseriate” strains (50) were positive to OTA production (Cozzi et al. 2007).

**Black aspergilli and OTA occurrence in the vineyards: role of environmental, ecological and agronomical factors.**
Black aspergilli cause a black rot disease of grape due to high fungal sporulation on berries which renders them completely deprived and dry. The incidence of colonised berries is related more closely to seasonal conditions during the year of cultivation than to the grape growth stage (Battilani et al. 2003; Cozzi et al. 2007; Leong et al. 2006a). Fungal conidia are usually present on berry skin from setting and increase in number from early veraison to harvest, with a peak at ripening. Black aspergilli overwinter in soil, and frequent soil cultivation can favour fungal infection in vineyard. The severity of aspergillus rot is influenced by excessive irrigation prior to ripening causing berry splitting. Rain prior to harvest is a common cause of berry damage favouring Aspergillus infection (Cozzi et al. 2007; Leong et al. 2006a). Berry damage caused by insects, birds or other fungal infection, is the primary factor affecting the disease development and OTA accumulation in berries.

OTA is produced in vineyards and is normally absent up to early veraison. Bunches without visible symptoms can also contain OTA although berries with visible black moulds normally show higher contamination levels. The absence of OTA at early growth stages can be explained by major difficulties encountered by fungi in berry penetration (Cozzi et al. 2007). The distribution of black aspergilli in vineyards can be summarized as follows: i) A. niger “aggregate” is the principal group at all growth stages; ii) A. carbonarius incidence is 2-3 times less than A. niger “aggregate” and increases from ripening to harvest; iii) Aspergillus uniseriate is the least represented group, sporadically occurring in Portugal, Greece and Spain, and more frequently in Italy, France and Israel (Battilani et al. 2006).

Based on the results of the Wine-Ochra Risk project carried out in six Mediterranean countries, the incidence of berries infected by black aspergilli at harvesting is significantly correlated with the latitude and longitude, with a positive West – East and North – South
gradient (Battilani et al. 2006). Following the geostatistical approach described by Battilani et al. (2006), data on incidence of *A. carbonarius* were run with ArcView and a predictive map was drawn. The incidence of *A. carbonarius* was significantly correlated with geographic coordinates showing a positive gradient going towards the South of Europe. Based on the combination of the degree-day and rainfall parameters in late August - early September in several countries of the Mediterranean basin, discriminant analysis gave promising perspectives for predicting OTA presence in vineyards by the development of thermo-wetness maps (Battilani et al. 2006).

Meteorological conditions as well as closeness to the sea have been shown to play a major role in determining OTA occurrence in grapes (Cozzi et al. 2007). A 3-year survey (2004-2006) performed on 8 vineyards located in the Salento peninsula of Southern Italy, showed a wide variability of OTA levels between different cultivation years. In particular, the 2005 crop was the most conducive to black aspergilli contamination due to the higher relative humidity and rainfall levels, that were associated with hot temperatures at ripening and harvest time (late August-September). Figure 1 shows the occurrence of black aspergilli and OTA in 8 vineyards of the two major varieties (Negroamaro and Primitivo) cultivated in the area during the three grape-harvest seasons (Cozzi et al. 2007). *Aspergillus niger* aggregate was predominant from early veraison to ripening representing 80-85% of contamination. *A. carbonarius* increased from veraison to ripening. OTA contamination of processed berries was assessed and results were correlated with the incidence of black aspergilli population, in particular with the increasing CFU values of *A. carbonarius*. The incidence of *A. carbonarius* increased from ripening to harvest when vineyard relative humidity usually increased, with high risk of OTA accumulation always associated with hot temperature (Cozzi et al. 2007).
Despite the widespread occurrence of OTA in various types of wine, there is limited information on the ability of black aspergilli to infect berries and produce OTA in different grape varieties (Battilani et al. 2004). Grape varieties were shown to affect the incidence of black aspergilli and the level of OTA contamination in \textit{in vitro} experiments. Three out of the twelve tested varieties, namely “Bianco di Alessano”, “Pampanuto” and “Uva di Troia”, showed low OTA contamination after artificial infection with a mixture of 5 OTA producing strains, whereas the most susceptible variety (Cabernet Sauvignon) contained over 200 µg/kg OTA and about 80% incidence of colonized berries (Battilani et al. 2004).

The role of the cropping system was monitored in a 2-year survey carried out on four different cropping systems, namely spur-pruned cordon, bower system, head (or small tree) system and espalier, in eight vineyards in Apulia. In both years the espalier cropping system produced the most contaminated grapes in terms of \textit{A. carbonarius} infection and OTA accumulation, as shown in Figure 2. This can be explained by the closeness of bunches to the soil, which is the most important source of inoculum of \textit{A. carbonarius}, as compared to spur pruned cordon and bower system. The higher humidity occurring in the espalier cropping system with respect to the head system can explain the different contamination level despite the similar distance of bunches from the soil for both systems. (Cozzi et al. 2007).

All the above ecoagronomical factors play individual roles in developing \textit{A. carbonarius} on grapes and the consequent OTA accumulation although the final result in relation to OTA risk is better represented by a combination of all these factors. Developing of risk maps based on critical control points can help to prevent and control OTA accumulation in grapes.

\textit{Lobesia botrana} - an OTA risk factor in grapevine management
Black aspergilli are opportunistic fungi (saprophytes) mainly responsible for secondary rot of grape berries developing through entry sites favoured by berry wounds or splitting caused by either biotic or abiotic factors (insects, fungi, birds, rainfall, hail). *Lobesia botrana* (*Lepidoptera: Tortricidae*) is the major grape berry moth in vineyards of Southern Europe, and can complete three to four generations a year, depending on weather conditions during late summer. Generally the first generation larvae of *L. botrana* damage flowers, while the following generations damage berries at different ripening stages. A good correlation between pest damage and OTA content has been found in grape berries, due to the contribution of *L. botrana* to berry wounds and fungal spore dissemination (Cozzi et al. 2006). Larvae can either contribute to spore dispersal or act as spore vectors, by trapping conidia in the cuticle ornamentation, then facilitating a rapid fungal penetration by tunnelling into berries as demonstrated for *Botrytis cinerea* (Fermaud et al. 1989; Cozzi et al. 2006). Grape berry damage by *L. botrana* has been shown to increase considerably the contamination level of black aspergilli and the consequent OTA accumulation in grapes. In Figure 3 a comparison between groups of intact berries, black rot berries with and without *L. botrana* damages is reported. All samples of berries damaged by *L. botrana* showed considerable levels of OTA contamination (up to 1000 µg/kg OTA) and black aspergilli infection higher than $10^6$ CFU. OTA was not detected in the group of intact berries, while it was found at levels below 1 µg/kg in ca. 42 % of black rot berries without grape moth damages (Cozzi et al. 2006). Field trials, performed in 2004 and 2005 using both biological and conventional insecticidal treatments, confirmed that a successful control of the third generation of *L. botrana* reduces the inoculum of *Aspergilli* and the formation of OTA in grapes (Perrone et al. 2007a; Kappes et al. 2005). It is therefore important to ensure an adequate insect control in combination with fungicide treatment in order to obtain an effective pest management.
Chemical and biological control of OTA producing fungi.

The following chemicals have been shown to be active in reducing at different extent both fungal growth and OTA levels in grape bunches: mepanipyrim, pyrimethanil, fluazinam, iprodione and the mixture cyprodinil and fludioxonil. The latter mixture was confirmed as effective in several field trials carried out in several Mediterranean countries including France, Spain, Greece and Italy (Belli et al. 2007; Kappes et al. 2005; Tjamos et al. 2004). 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 applied against black aspergilli with the same combination and schedule, both in dosage and timing, is effectively used against grey mould, caused by Botrytis cinerea. Moreover, the 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 (Kappes et al. 2005). Promising results were also obtained by using yeast as biological control agents which were isolated from grapes in Greece and in Italy. In particular, good results were obtained with two strains of Cryptococcus laurentii and Aureobasidium pullulans, respectively, in Greece (Dimakopoulou et al. 2005), and with a strain of Hanseniaspora uvarum in Italy using weekly or two-weekly treatments.

Distribution of OTA in wine and winery by-products and its fate during vinification of red grapes.

OTA occurring in grapes is transferred to wine and relevant by-products during vinification. Therefore, the availability of reliable analytical methods for OTA determination in must, wine and relevant by-products is important for an adequate risk management of OTA contamination in the wine food chain. The availability of rapid methods would be necessary in winery for screening the whole production in order to take prompt corrective actions.
Several rapid methods are available for OTA analysis in food products that need to be adapted for their application to wine and by-products (Visconti and De Girolamo 2005). The AOAC official method 2001.01 for OTA determination by HPLC in wine (Visconti et al. 2001) can also be used for must providing that solid fraction is previously separated by centrifugation (Solfrizzo et al. 2006). The fate of OTA during vinification of grape has been studied by different authors with contrasting results. Fernandes et al. (2003) observed an increase of OTA concentration in must during maceration of crushed grapes and a consistent reduction of OTA during pomaces and lees separations. Grazioli et al. (2006) found little or no reduction of OTA concentration in wine after the first racking (separation of lees) while a significant reduction of OTA concentration was observed after spontaneous malo-lactic fermentation occurring between the first and the second racking. In contrast, Rousseau (2004) reported that OTA content in must increases after crushing grapes, and reaches maximum levels during malo-lactic fermentation. Leong et al. (2006b) reported that 24% of OTA originally present in crushed red grape passed into free run wine (must) and a 72% OTA reduction was recorded in wine after the first racking.

The different approaches used by these authors to conduct these studies could explain the controversial results. Due to unavailability of naturally contaminated grape some of these studies were performed either by artificially inoculating grape with toxigenic A. carbonarius (Leong et al. 2006b) or by spiking uncontaminated grapes with OTA (Fernandes et al. 2003). These materials are different from naturally contaminated grapes which comprise both contaminated and uncontaminated berries. Spiking uncontaminated grapes with OTA produces an apparent reduction of OTA concentration in the resulting wine since most of the spiked OTA is adsorbed by the grape pomaces and biomass (grape skins, pulp, yeasts released in must) (Fernandes et al. 2003). On the contrary, when using naturally contaminated berries...
for vinification, grape pomaces and solid biomass have high OTA concentration and represent the source of OTA in wine (Solfrizzo et al. 2007). Moreover, in most of these studies OTA was only monitored in liquid fractions and no measurements were performed on pressed pomaces and lees thus the distribution of OTA between solid and liquid fractions obtained during vinification was not measured (Grazioli et al. 2006; Fernandes et al. 2003; Rousseau 2004). Another critical point is the sample preparation of liquid fractions (must and wines before racking), which contain suspended biomass, before OTA analysis. The separation or inclusion of the biomass in the sample to be analysed has a significant effect on the measured OTA concentration due to the high amount of OTA reversibly bound to the biomass. Indeed Leong et al. (2006b) included the biomass when unsedimented liquid fractions (must and wines before racking) were analysed for OTA. Consequently the OTA concentrations found in these fractions were much higher than that found in the same liquid fraction analysed after spontaneous sedimentation of biomass (first racking).

The fate of OTA and its distribution in wine and winery by-products during vinification of naturally contaminated Negroamaro and Primitivo grapes has been recently reinvestigated at laboratory (microvinification) and industrial level by Solfrizzo et al. (2007). Samples of must (before and after maceration), grape pomaces, wine and lees (after the first and second racking) were analysed for OTA in order to evaluate its levels at each step of vinification. Before analysis the liquid fractions were centrifuged in order to separate the biomass and measure the soluble OTA. Results of microvinification experiments showed that only 4% of the OTA present in grapes remains in the wine whereas 95% of OTA originally present is retained on pressed grape pomaces (98% in the skin and 2% in the seeds) and 1% is retained on the lees. Leong et al (2006b) found that 9% of OTA originally present in grapes passed into wine. The use of these wine by-products as food ingredients should be therefore avoided.
or checked for OTA contamination. OTA concentration in must remained nearly constant after maceration, pressing, juice clarification, alcoholic fermentation and lees separations (after first and second racking). The same OTA concentrations were found in wine samples analysed after one year. The results obtained with the microvinification were also confirmed at industrial level. An increase of OTA concentration in must was observed during maceration of Primitivo crushed grapes highly contaminated with OTA. This increase could be explained by the high concentration of OTA in the grapes that required a longer time for toxin to equilibrate between must and grape pomaces.

**Removal of OTA**

Several fining agents have been tested for their ability to remove OTA from contaminated must/wines (Castellari et al. 2001; Leong et al. 2006c). Oenological decolourising carbon has been reported to remove the highest amount of OTA, although carbon also removes anthocyanins and other coloured polyphenols from wine. The effectiveness of the treatment with oak wood fragments depended upon the quantity of wood chips and powder used (Savino et al. 2007). Removal of OTA from grape juice, must and wine by using oenological yeast strains has been reported (Bejaoui et al. 2004; Garcia Moruno et al. 2005; Cecchini et al. 2006). The removal of OTA during fermentation is based on adsorption mechanism other than degradation. However, the efficacy of yeasts for OTA reduction at industrial level as well as their impact on wine quality parameters (phenol compounds) has not been shown. In our laboratory we have confirmed OTA reduction by yeasts or inactivated yeast walls while a consistent reduction of colour index (expressed in terms of Folin Ciocalteu index) occurred.

The results obtained in our laboratory on the efficacy of selected adsorbent materials to remove OTA from contaminated red wine are reported in Table 2. The best results in terms of
OTA removal were obtained with carbon or commercial preparations containing carbon (Mikofree, Myco AD A-Z, Standard Q/FIS). On the other hand the efficacy in removing OTA was proportional to the reduction of polyphenol content of treated wines.

Conclusions
The main source of OTA in the wine production chain is the infection by “black aspergilli” occurring in the field. A. carbonarius is the main species responsible for OTA accumulation in grape berries occurring from early veraison to ripening. OTA production is influenced by: climatic conditions/geographic areas; grape varieties/crop systems; berries damage caused by insects, fungal infection or excessive irrigation/rainfall. Fungicidal and insecticidal treatments reduce OTA contamination. Susceptibility to infection can vary from different years and regions. Developing of risk maps based on critical control points can help to prevent and control OTA accumulation in grapes. Availability of rapid methods for OTA analysis is also important for preventive and corrective intervention at some critical control points. After maceration of (red) grapes OTA remains stable during vinification and after one year aging. During vinification of (red) grapes only 4% OTA remains dissolved in the wine, while 96% is retained by solid winery by-products (grape pomace and lees). Carbon reduces OTA concentrations in wines, but negatively affects wine quality. Good Agriculture Practices (balanced soil tillage, irrigation, nitrogen fertilization, pruning) and Good Manufacturing Practices (reduced harvest to vinification time, segregation of rot bunches) help considerably to reduce OTA contamination risk. Main critical control points as well as preventive and corrective actions are summarized in Table 3.

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32 grape and winery by-products by immunoaffinity column cleanup and


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Legenda to Figures

**Figure 1.** Occurrence of black aspergilli and OTA in 8 vineyards of Primitivo and Negroamaro varieties in Apulia during three grape-harvest seasons (2004-2006).

**Figure 2.** Influence of the training systems on the epiphytic black aspergilli CFU/g of grape berries samples and the OTA contamination in “Primitivo” during 2004/2005. OTA levels with same letters are not significantly different according to the Duncan test (P < 0.01).

**Figure 3.** Logarithmic graph of the distribution of OTA concentration in grape berries in relation to black aspergilli contamination levels in groups of berries: In: intact berries, Ar: *Aspergillus* rotten berries, and Lb: *Aspergillus* rotten berries with *L. botrana* larvae damages.
<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of analyzed samples</th>
<th>Main origin</th>
<th>Incidence of contamination</th>
<th>Range (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zimmerli &amp; Dick (1996)</td>
<td>118</td>
<td>Europe</td>
<td>70%</td>
<td>&lt;0.005 - 0.4</td>
</tr>
<tr>
<td>Majerus &amp; Otteneder (1996)</td>
<td>144</td>
<td>Europe</td>
<td>42%</td>
<td>&lt;0.01 - 7.0</td>
</tr>
<tr>
<td>Ospital et al. (1996)</td>
<td>30</td>
<td>France</td>
<td>50%</td>
<td>&lt;0.01 - 0.3</td>
</tr>
<tr>
<td>MAFF (1997, 1998)</td>
<td>60</td>
<td>Europe</td>
<td>56%</td>
<td>&lt;0.01 - 0.8</td>
</tr>
<tr>
<td>Ueno (1998)</td>
<td>46</td>
<td>Japan</td>
<td>41%</td>
<td>&lt;0.01 - 0.2</td>
</tr>
<tr>
<td>Burdaspal &amp; Legarda (1999)</td>
<td>192</td>
<td>Spain</td>
<td>82%</td>
<td>&lt;0.01 - 0.6</td>
</tr>
<tr>
<td>Visconti et al. (1999)</td>
<td>55</td>
<td>Italy</td>
<td>87%</td>
<td>&lt;0.01 - 7.6</td>
</tr>
<tr>
<td>Otteneder &amp; Majerus (2000)</td>
<td>420</td>
<td>Europe</td>
<td>48%</td>
<td>&lt;0.01 - 3.3</td>
</tr>
<tr>
<td>Pietri et al. (2001)</td>
<td>111</td>
<td>Italy</td>
<td>82%</td>
<td>&lt;0.001 - 3.8</td>
</tr>
<tr>
<td>European Commission (2002)</td>
<td>1470</td>
<td>Europe</td>
<td>59%</td>
<td>&lt;0.01 - 15.6</td>
</tr>
<tr>
<td>Soufleros et al. (2003)</td>
<td>35</td>
<td>Greece</td>
<td>63%</td>
<td>&lt;0.02 - 3.2</td>
</tr>
<tr>
<td>Hocking et al. (2003)</td>
<td>601</td>
<td>Australia</td>
<td>15%</td>
<td>&lt;0.02 - 1.0</td>
</tr>
<tr>
<td>Tateo &amp; Bonomi (2003)</td>
<td>80</td>
<td>Italy</td>
<td>85%</td>
<td>&lt;0.01 - 2.9</td>
</tr>
<tr>
<td>Finoli et al. (2004)</td>
<td>150</td>
<td>Italy</td>
<td>80%</td>
<td>&lt;0.01 - 5.2</td>
</tr>
</tbody>
</table>
Table 2. Percent removal of ochratoxin A (OTA) from red wine containing 10 µg/L OTA and treated with different amounts of adsorbent.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Adsorbent dosage (g/L)</th>
<th>0.1</th>
<th>0.2</th>
<th>0.4</th>
<th>0.5</th>
<th>1.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oenological decolourising carbon (Esseco)</td>
<td></td>
<td>80</td>
<td>(9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Activated carbon (SIGMA)</td>
<td></td>
<td>36</td>
<td>73</td>
<td>85</td>
<td>88</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>Mikofree (Perdomini)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>63</td>
<td>(4)</td>
<td>83</td>
</tr>
<tr>
<td>Standard Q/FIS (Feed Industry Service)</td>
<td></td>
<td>9</td>
<td>28</td>
<td>31</td>
<td>-</td>
<td>68</td>
<td>-</td>
</tr>
<tr>
<td>Myco AD A-Z (Special Nutrients Inc.)</td>
<td></td>
<td>11</td>
<td>25</td>
<td>28</td>
<td>-</td>
<td>53</td>
<td>-</td>
</tr>
<tr>
<td>Cholestyramine (SIGMA)</td>
<td></td>
<td>6</td>
<td>10</td>
<td>16</td>
<td>-</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>Bentonite (Vason)</td>
<td></td>
<td>1</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polygel (AEB Group)</td>
<td></td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oak wood chips (different types and sizes)(^c)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>26-42</td>
</tr>
</tbody>
</table>

\(^a\) Percent reduction of polyphenol content (Folin Ciocalteu index) is reported in parenthesis

\(^b\) not tested

\(^c\) used on red wine containing 8.5 OTA µg/L
<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Preventive and corrective measures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Field and pre-harvest</strong></td>
<td></td>
</tr>
<tr>
<td>Mediterranean basin, closeness to the sea</td>
<td>Monitoring climatic conditions from veraison to harvest</td>
</tr>
<tr>
<td>High temperature and relative humidity from veraison to harvest</td>
<td>Monitoring with trap system the <em>Lobesia botrana</em> pressure in the vineyards</td>
</tr>
<tr>
<td>Rainfall during ripening period (berry splitting)</td>
<td>Monitoring black aspergilli rot berries from veraison to ripening</td>
</tr>
<tr>
<td>Berry damage (high risk with grape berry moth infestation)</td>
<td>Avoid excess of vigour and vegetation favouring aeration of bunches</td>
</tr>
<tr>
<td>Grape training system susceptibility (high risk with espalier)</td>
<td>Avoid tillage from veraison to harvest</td>
</tr>
<tr>
<td>High nitrogen fertilization, frequent tillage</td>
<td>Combined fungicide/insecticide treatments (1 or 2) when favourable climatic conditions occur</td>
</tr>
<tr>
<td>Grape variety susceptibility</td>
<td></td>
</tr>
<tr>
<td><strong>Harvest – Wine making</strong></td>
<td></td>
</tr>
<tr>
<td>Mechanical harvest without selection of bunches</td>
<td>Anticipate harvest time in high OTA risk areas when favouring conditions occur</td>
</tr>
<tr>
<td>High incidence of rot bunches</td>
<td>Segregate rot bunches at harvesting</td>
</tr>
<tr>
<td>Long grape storage after harvesting (&gt; 8 h)</td>
<td>Minimize storage time before processing</td>
</tr>
<tr>
<td></td>
<td>Control OTA contamination in must</td>
</tr>
<tr>
<td></td>
<td>Use carbon preparations to reduce OTA contamination during fermentation</td>
</tr>
</tbody>
</table>
Figure 1

The figure shows the growth of different species of black aspergilli and OTA levels from 2004 to 2006. The x-axis represents the years, and the y-axis represents the log (CFU+1)/g and OTA (µg/kg) levels. The species are marked by different symbols:
- A. niger
- A. carbonarius
- A. japonicus
- OTA

The black aspergilli levels are highest in 2005, with A. carbonarius showing significantly higher levels than the other species. OTA levels are minimal compared to the aspergilli levels.
Figure 2

![Graph showing black aspergilli and OTA levels across different systems]

- **A. niger**
- **A. carbonarius**
- **OTA**

System Comparison:
- **Spur-pruned Cordon**
- **Bower System**
- **Head System**
- **Espalier**

 OTA (µg/kg)

- 0.0
- 0.5
- 1.0
- 1.5
- 2.0
- 2.5
- 3.0
- 3.5
- 4.0
- 4.5
- 5.0
- 5.5
- 6.0
- 6.5
- 7.0
- 7.5
- 8.0
- 8.5
- 9.0
- 9.5
- 10.0

Log (CFU+1)/g
Figure 3

This figure illustrates a scatter plot with Log(CFU+1) on the x-axis and OTA (ng/g) on the y-axis. The plot shows data points for different conditions, indicated by markers: ▲ for In, ● for Ar, and ● for Lb. The x-axis ranges from 0.0 to 8.0, and the y-axis ranges from 0.0 to 1000.00.