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Review

The Route of Mycotoxins in the Grape Food Chain

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Abstract: Grapes are consumed throughout the world in different ways, from fresh fruit to processed products. Regardless of the product, risk management starts in pre-harvest stages to control initial development of mycotoxigenic fungi and to avoid consequent problems in the whole chain. The main concern in grapes and grape products is the presence of black *Aspergillus* species, and the subsequent production of ochratoxin A. Even so, other mycotoxigenic fungi have been detected and might need further attention. The adoption of proper crop management strategies, such as choosing proper varieties, training system and soil management, can be effective in reducing fungal proliferation. Biological methods can also be employed to inhibit fungal contamination. These methods can substitute chemical approaches and their application can be done in later phases of grape processing to allow safe storage. Due to the wide range of products

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that can be obtained from grapes, different fungal species can be responsible for post-harvest deterioration. Taking this into account, the aim of this work is to review strategies for mitigation of mycotoxin risk in the whole grape chain, considering data on the occurrence and development of mycotoxigenic fungi and mycotoxin biosynthesis.

Key words: aflatoxin B1, dried grapes, grape juice, mycotoxigenic fungi, ochratoxin A, wine

1. Introduction

Yeasts and lactic acid bacteria are considered to account for the majority of grape microbiota, mainly because of their role during storage and processing; however, filamentous fungi should also be considered. These microorganisms are a threat to the quality of grapes, cause the deterioration of sensorial properties, and several species can be responsible for mycotoxin production. Mycotoxins are toxic secondary metabolites that can cause several health problems when ingested even at low concentrations.

In general, assessing the risk of contamination in a food product chain needs to consider external conditions at field such as: environment; the associated fungal load of the soil; pests; possible interactions with other microorganisms or plant diseases; intrinsic factors, related to substrate itself (variety differences, nutritional composition…); and practices at field and processing levels (Khalesi and Khatib 2011).

Fungal contamination begins in the field and is in the field where it is more relevant. The susceptibility of berries to fungal parasites is in general a result of conductive environmental conditions and unappropriated phytochemical applications (Barata et al. 2012). Additionally, berries may bear microfissures and soften with ripening, increasing nutrients availability, which

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allow further contamination by fungi. The genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium* are the most often reported as grape contaminants (Rousseaux et al. 2014). Cropping management will allow a proper control of contaminations and, posteriorly, good manufacturing practices during handling and processing can be crucial to the safety of final products. Nevertheless, the occurrence of fungi and their mycotoxins is unavoidable, and for this reason regulations in the EU set limits for mycotoxins, namely aflatoxins (AFs), ochratoxin A (OTA) and patulin (PAT), in different grape products (Table I).

Processing of grapes includes several steps and these can be adapted to reduce contamination risk. In the case of mycotoxins, and due to their thermal stability, thermal processing cannot assure a proper elimination or reduction. The combination of temperature with physical treatments, such as pressure, can be effective in eliminating mycotoxins, however it can have a negative impact on the organoleptic characteristics of the final product (Shukla et al. 2017).

In fact, the strategies to control mycotoxins can be applied in several stages of processing and can consist in a number of different methods. The demand for more natural products gathers attention to the application of naturally occurring antifungal compounds. Biological approaches that take advantage of competitive abilities of antagonistic microorganisms in field or during processing can be used to inhibit mycotoxin production.

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2. Mycotoxin producing fungi in grapes.

2.1 *Aspergillus* spp. in grapes and mycotoxin production.

Fumonisin B2 (FB2) and OTA and are the mycotoxins most often detected in grapes, however, AF produced by *Aspergillus flavus* can also be present (Rousseaux et al. 2014). The main species responsible for OTA production in grapes is *Aspergillus carbonarius*, but *Aspergillus ochraceus* and *Aspergillus niger* can also be found, with the last being the most frequently isolated (Serra et al. 2003, Battilani et al. 2006, Amezqueta et al. 2012, Lasram et al. 2012, Somma et al. 2012, Rousseaux et al. 2014). The biosynthesis of OTA involves polyketide synthase genes present in producers and is largely correlated with the size of fungal colony and biomass amount, and can be stimulated by the presence of other fungal species (Magan et al. 2010, Storari et al. 2010, Lappa et al. 2015). Studies on the influence of maturation stage of the grape on OTA levels demonstrated that OTA accumulation starts at the beginning of maturation (Serra et al. 2006), probably as a result of a reduction on skin thickness that enables fungi to grow inside the berries (Amezqueta et al. 2012, Lasram et al. 2012, Somma et al. 2012). Regarding the presence of FB2 in grapes, its production in mainly related to *A. niger* contamination (Logrieco et al. 2011). Abrunhosa et al. (2011) found that among the 597 strains of *A. niger* aggregate previously isolated from wine grapes, nearly 29 % were FB2 producers, while only 5 % were OTA producers. However, the amount of FB2 produced was low. Also, the same study showed that no significant correlation exists between OTA and FB2 production, with both toxins being produced by only 1.7 % (10 strains) of the studied 597 strains.

The presence of *A. carbonarius* and *A. niger* in grapes results from their presence in the soil, under grapevines, and can be triggered by agronomic practices that allows them to reach the

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plant (Khalesi and Khatib 2011, Amezqueta et al. 2012). The infection of grapes by *Aspergillus* species can cause *Aspergillus* black rot, a severe disease that causes significant crop losses. Since the fungal load is often present on the surface of grapes, the infection process starts with the entrance of the fungi into the fruit, promoted by damages in the skin caused by other fungal attacks, pests or climate actions, which provide also the appropriate moisture and sugar levels for mycotoxin production (Khalesi and Khatib 2011, Amezqueta et al. 2012, Somma et al. 2012). This assumption was confirmed since skin inoculation of grape berries with *A. carbonarius* was not able to cause rot symptoms or OTA production, unless a previous damage of the surface of fruits occurred (Jiang et al. 2013). Besides that, experiments aiming to compare the fungal development on grape-based culture medium, grape skins or flesh have concluded that the latter is the most suitable for spore germination of *A. carbonarius*, indicating that when the interior parts of the fruit is exposed the fungal attack will be much more effective (Camardo Leggieri et al. 2014).

Differences between grape varieties influence the spread of fungal contamination. Bunches more or less compact can play a role in fungal disease, since infection can spread more easily when the fruits are contacting with each other (Chiotta et al. 2013). During infection, a reduction in sugar and soluble solids levels is observed, contrarily to the acidity, confirming the assumption that OTA biosynthesis is higher at reduced pH (Lasram et al. 2012, Jiang et al. 2013). Battilani et al (2004) tested the susceptibility of the main Italian grape varieties to *A. carbonarius* infection and OTA production. Some varieties were found to be more susceptible to contamination – 'Cabernet Sauvignon', 'Trebbiano' and 'Verdeca' – than others – 'Bianco d'Alessano, 'Pampanuto' and 'Uva di Troia' – but, the susceptibility was not correlated with

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each bunch characteristics. Instead, a natural defense mechanism against infection may take place, and influence fungal growth and OTA production. Berries of *Vitis vinifera L.* cv. Barbera (disease susceptible) and the interspecific resistant variety Castor were infected at veraison with *A. carbonarius*; stilbene-synthase gene expression was induced by the fungus and stilbenes were produced in both varieties, but at a significantly higher value in the resistant variety (Vezzulli et al. 2007). The induction of trans-resveratrol is common to other black Aspergilli species, and this production is concomitant with fungal growth inhibition (Bavaresco et al. 2003). However, in synthetic must medium, OTA production was induced, and a much higher trans-resveratrol concentration was required for OTA inhibition (Bavaresco et al. 2003). The production of trans-resveratrol seems to be a defense mechanism against *A*. *carbonarius* contamination, after fungal infection is settled, since when proper levels of this compound are produced at the initial stage of infection there is an inhibition of OTA production (De Rossi et al. 2012). In fact, the plant produced the phytoalexins as a response to fungal attack. The interaction with the pathogen includes the production of enzymes by the latter to degrade phytoalexins (Flamini et al. 2016).

The composition of berries also influences colonization and mycotoxin production. Malic and tartaric acids are the major acids in grape musts and Atoui et al. (2007) studied their influence on OTA biosynthesis by *A*. *carbonarius* and found that the first favors mycotoxin production, while the latter has the opposite effect (Atoui et al. 2007).

Role of environmental conditions/climate change.

Generally, contamination of vineyards by *Aspergillus* spp. is associated with hot and wet environmental conditions but it is important to have in mind that the conditions for fungal

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growth and mycotoxin production are different (Rousseaux et al. 2014). While studying the influence of minimum and maximum temperatures (to illustrate field conditions during day and night), it has been demonstrated that hot days promote fungus to grow, while cooler nights benefit OTA production by *Aspergillus* species, with the range 20 °C to 30 °C resulting in the highest rate of both parameters for *A. carbonarius* (Chiotta et al. 2013, M. Barberis et al. 2014). In a general way, 30 °C seems to be the optimum temperature for *A. carbonarius* to produce OTA, while incidence of fungal contamination in field is dependent mostly of temperature and irrigation, and less of precipitation (Jiang et al. 2013, M. Barberis et al. 2014). The differences in temperatures between regions can influence OTA contamination, as it has been demonstrated in a study in Italy, which concluded that the incidence of the toxin decreases with the progression to the north of the country (Lucchetta et al. 2010). In Spain, the incidence of black Aspergilli species in two different regions demonstrated that hotter climate favors its presence, as well as OTA contamination (Garcia-Cela et al. 2015); also, in Portugal, it was found an higher incidence of ochratoxigenic strains in regions with Mediterranean climate, characterized by hot and dry summers, although with insignificant increase in OTA incidence (Serra et al. 2003).

Considering OTA presence under climate change scenarios, 30 °C is the limit of temperature above which there is an increased risk, while below 21 °C the conditions can be considered safe. For moisture, both wet and dry conditions can pose a threat because of the different water activity (a_w) requirements of OTA producing species (Paterson and Lima 2011). Since global warming may shift the distribution of temperature and precipitation patterns, with an increase of exceptionally unfavorable years for several crops, modelling can be useful for the construction of a prediction tool, as long as meteorological data are available, helping in

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grapevine planning and management. Therefore, a predictive system for OTA risk in grapes – OTA-Grapes – was already proposed (Battilani and Camardo Leggieri 2015).

With climate change, infection of grapes by black Aspergilli, under low water availability and higher temperatures, will be dominated by *Aspergillus tubingensis* and *A. niger* in detriment of *A. carbonarius,* and also *A. ochraceus* in places where the latter are now predominant species (Garcia-Cela et al. 2012, 2014). This scenario may lead to a reduction on OTA contamination of grapes, but higher FB2 incidence in Europe (Garcia-Cela et al. 2015). In temperate regions, AF may also become a higher concern (Paterson and Lima 2010). In cooler regions, the presence of black Aspergilli in general is expected to increase (Storari et al. 2012). However, research on the effect of the predicted climate change in mycotoxin occurrence is still scarce, and more knowledge needs to be acquired to construct better models that can help to predict the variations in mycotoxin contamination and take action more efficiently (Paterson et al. 2018).

2.2 Other mycotoxigenic fungi in grapes and mycotoxin production

The genera *Penicillium* appears also to be responsible for OTA contamination. Several *Penicillium* species can contaminate grapes, with *Penicillium verrucosum* playing a more active role regarding the presence of OTA (Amezqueta et al. 2012). *Penicillium* spp. in grapes are generally associated to dry climates with lower temperature, with the particular requirements of *P. verrucosum* for OTA biosynthesis being associated to cooler or more temperate regions (Amezqueta et al. 2012, Rousseaux et al. 2014). Even so, the *Penicillium* spp. populations are diverse, and vary between different regions, with *Penicillium brevicompactum* and *P. verrucosum* being the most frequent contaminants of vineyards (Rousseaux et al. 2014). Also,

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PAT and citrinin producing species can be found to contaminate grapes, at a lesser extent. In fact, the presence of *Penicillium expansum* has already been reported in Portugal, Chile, Slovakia and Hungary (Abrunhosa et al. 2001, Diaz et al. 2011, Felsociova et al. 2015a,b, Tancinova et al. 2015).

The interaction of *P. expansum* with other fungal species can affect its development, as well as PAT production. *In vitro* experiments indicate that *Botrytis cinerea*, a frequent presence in grapes, is a strong competitor with *P. expansum*, being able to decrease its own fungal development time, and to decrease PAT contamination by metabolizing the toxin (Morales et al. 2013). However, several authors reported that, in the presence of specific *B. cinerea* strains, *P. expansum* produces geosmin, that is thought to have a role on the competition with other fungal species, besides causing organoleptic deterioration. Geosmin increases *P. expansum* colonization and, consequently, mycotoxin production (Paterson et al. 2007, Barata et al. 2012). In fact, Morales-Valle et al. (2011) found that 46 % of the assessed *B. cinerea* strains induced geosmin production by *P. expansum*, and that its ability to do so was dependent on the medium (white grape broth medium or red grape broth medium).

Despite not representing the major problem in grapes, contamination with *Fusarium* has been confirmed, with the development of fruit rot disease caused by *Fusarium avenaceum* in *Vitis amurensis* after 7 days of conidial inoculation (Wang et al. 2015). When studying the infection process of *Fusarium* spp. during the withering of grapes and after a period of inoculation of 24 days, half of the total grape area appeared to have necrotic lesions and, under favorable conditions, an aerial growth of the mycelium was observed (Lorenzini and Zapparoli 2015). The infection of grapes with *Fusarium* spp. constitutes a risk due to the wide range of

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mycotoxins produced by the species of this genus. *Fusarium proliferatum*, *Fusarium sporotrichioides*, *Fusarium oxysporum* and *Fusarium verticillioides* are among the species most often found in assessments of Slovakian grapes (Mikusova et al. 2013, Maskova et al. 2014). The ability of these species to produced mycotoxins like beauvericin or fumonisins, HT-2 toxin or T-2 toxin is relevant when considering possible contaminations.

Despite the absence of regulation on the levels of *Alternaria* mycotoxins, their presence in grapes is a matter of concern; with numerous observations of mycotoxigenic *Alternaria* species in grapes and wine (at trace levels) being reported (Prendes et al. 2015, Trinidad et al. 2015, Tancinova et al. 2016).

3. Minimizing the risk of mycotoxin contamination along the grape chain

3.1 Cropping system management

All factors involved in farming practice should be assessed prior and during vineyards growth. The main parameters affecting mycotoxin occurrence at pre-harvest stage are summarized in Figure 1. Choosing a proper variety and its expected longevity is crucial to ensure quality products. However, an Argentinian study demonstrated that the age and variety of the vineyard and its predominant and total microbial population did not seem to have influenced *A*. *carbonarius* presence (M. Barberis et al. 2014).

Aspects like training system and soil management must be taken into account to control all the microbial populations present in vineyards, including mycotoxigenic fungi. These contaminations can also be controlled by exploring the host resistance or with the application of agents, chemical or biological based, that act against pests and disease proliferation.

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Trellising and training.

Trellising should be considered when planting a vineyard to assure the final quality of products. The trellis system should avoid sunburn or stuck of bunches during the growth period, since too much vegetative growth and vigor can limit proper development of bunches (Hocking et al. 2007, Somma et al. 2012). Trellising should also promote a good aeration of bunches (Somma et al. 2012). An Uruguayan study regarding the use of trellis system, planting density and cordon height impact on *Aspergillus* spp. presence in vineyards, showed that high densities and vegetative growth were beneficial to *Aspergillus* spp. presence (Ferrari et al. 2017).

The presence of *Aspergillus* spp. can be correlated with the positioning of the trellis. *A. carbonarius* and ochratoxigenic strains of *A. niger* incidence were higher in parral system, while globally *A. niger* prevailed in high vertical shoot positioned trellis (Chiotta et al. 2013). Since *Aspergillus* species are often found in the soil, the use of an espalier type system that places the vineyards near the soil is associated with higher levels of OTA contamination (Somma et al. 2012).

Soil management.

One of the prime parameters when choosing a proper cropping system is the type of soil. Vineyards can be planted in sandy, clay or silty soils. However, the impact of these variables was not proven to be relevant in the incidence of *Aspergillus* section *Nigri* isolates; yet, combining clay soils, reduced tillage practices and constant water availability, can reduce *A. carbonarius* contamination (Hocking et al. 2007, Chiotta et al. 2013). Tillage and other practices that involve soil movements promote fungal contamination because the soil is the source of inoculums of the

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majority of fungi, and tillage can help them reach plant and fruits, with negative impacts, especially if it is done close to the ripening stage (Meyvaci et al. 2012).

Irrigation.

Reduced tillage practices, and the use of irrigation systems where the water availability is maintained, particularly in furrow system instead of a drip system, can minimize the incidence of *Aspergillus* species in grapes, including *A*. *carbonarius* and ochratoxigenic *A*. *niger* strains (Hocking et al. 2007, Chiotta et al. 2013). The preparation of the irrigation system, mainly in case of furrow, should be made early to avoid soil dust to spread fungal presence from the soil. This knowledge is in line with the results obtained in vineyards, where incidence of *A*. *carbonarius* was associated to drip irrigation system (M. Barberis et al. 2014).

Host resistance and defense.

Resistance to the common mildew and *Botrytis* infection is a way of achieving *Aspergillus* spp. protection, since those contaminations can favor *Aspergillus* species to spread (Hocking et al. 2007). The interactions between the different microbial populations have a great impact on the spreading of a specific species by activating or inhibiting host defense mechanisms. Apaliya et al. (2017) found that by using trehalose pre-treated *Hanseniaspora uvarum*, the defense mechanisms of table grapes could be stimulated in case of *A*. *tubingensis* infection, which is related to enzyme activity. Similarly, *Sporidiobolus pararoseus* Y16 strain seemed to activate defense mechanisms in grapes against *A*. *niger* (Li et al. 2017). The grapevine should be resistant not only against diseases, but also against meteorological events, like damages caused by intense rain, with varieties with a thicker skin being less susceptible to physical deterioration.

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Pest and disease control.

The presence of pests is a threat to the integrity of the fruit, and is a priority in fungal control, due to the high increase of fungal contamination as a result of grape surface damage. Infection of grapevines, and consequent damage of grape skin by *Lobesia botrana* or *Planococcus ficus* is associated to higher fungal development (Chiotta et al. 2010; Cozzi et al. 2013). *L. botrana* is known to trigger black Aspergilli infection and consequent OTA contamination by causing high sugar content in the exudates. Protection against this moth with insecticide and bio insecticide (*Bacillus thuringiensis* and *Beauveria bassiana* formulates) was proven to prevent high levels of OTA in vineyards (Cozzi et al. 2009,2013; Meyvaci et al. 2012).

Fungicide treatments are the main tool to control fungi in crops. This practice needs to be adjusted to the predicted risk of fungal contamination of vineyards. In some cases, due to higher risks, the combined use of two chemical compounds is advised, and two sets of application can be necessary, starting some days before harvest (Somma et al. 2012). Cyprodinil, fludioxonil, tebuconazole and trifloxistrobin are some of the main fungicides with broad application.

A study with Switch (Syngenta, Switzerland), a 37.5 % cyprodinil and 25 % fludioxonil fungicide, and Flint® Max (Bayer, Germany), a fungicide with 500 g/kg tebuconazole and 250 g/kg trifloxistrobin, demonstrated that both decreased *A. carbonarius* and *A. ochraceus* contamination and OTA production in grapes (Garcia-Cela et al. 2012). Even so, Tjamos et al. (2004) found that Chorus® (Syngenta, Switzerland), a cyprodinil-based (500 g/kg) fungicide, was ineffective in controlling *Aspergillus* species, contrarily to what was reported with Switch (registered to control *Botrytis* and *Aspergillus* spp. infection in grapes). This could indicate that the antifungal activity of Switch is associated to the presence of fludioxonil. Cyprodinil, an

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anilinopyrimidine fungicide, has systemic properties, and is absorbed from the cuticle waxy layers of the plant, while fludioxonil is a phenylpyrrole fungicide and exerts antifungal activity by contact, when it is present on the external surfaces of the commodity. In case of trifloxistrobin and tebuconazole, respiration and sterol biosynthesis that takes place in membranes are affected, respectively (Garcia-Cela et al. 2012).

The efficacy of a pyrimethanil-based fungicide (Scala® (Bayer, Germany)) and a natural plant extract (Stifénia® - consisting of a homogenized fenugreek seed powder) as pre-harvest fungicides against *A. carbonarius* growth and OTA production was also reported in vines (Ahmed et al. 2015). Carbendazim, a benzimidazole antifungal agent, and difenoconazole, a triazole antifungal agent, have also shown their ability to inhibit growth of black Aspergilli species and OTA production in synthetic medium (Techarat et al. 2012). Contrarily, in Greek experiments in raisin vineyards, Carbendazim was not proven to be effective against *Aspergillus* infection (Tjamos et al. 2004).

An aspect to consider when assessing fungicides' efficacy is temperature. A study with chlorothalonil, mancozeb, cooper hydroxide, cooper oxychloride and tebuconazole based commercial fungicides demonstrated that the *in vitro* activity of these compounds against *A. carbonarius* growth and OTA accumulation was higher at the lowest temperature tested (15 °C, over 20 °C or 30 °C) (Terra et al. 2016). Also, the same study highlights the differences between *in vitro* experiments (Czapek yeast extract agar and semisynthetic grape medium) and the effects on inoculated grapes, since the positive results of inhibition observed *in vitro* were not confirmed when using grapes.

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Fungal inhibition through competition with other microorganisms can take place at different stages of the grape chain. In the field, the use of biological approaches can complement chemical fungicides and overcome the increasing problems of fungicide resistance. Even knowing that the presence of other species can cause competition for vital resources, assessing potential biocontrol agents (BCA) is important, though it can be a complex issue. In fact, mycotoxin production can be stimulated during interactions between mycotoxigenic fungi and other species (Magan et al. 2010).

Table II lists some of the microorganisms found to have potential application in controlling mycotoxin-producing fungi in grapes. Among the different possibilities, yeasts are referred as very adaptable to use as BCA because they can easily spread on grape surface and compete effectively for nutrients (Sarrocco and Vannacci 2018).

Environmental conditions influence the contamination of grapes by spoilage fungi, but also the colonization of the BCA. A study with *Metschnikowia pulcherrima* and *Aureobasidium pullulans* was focused on the effect of temperature and relative humidity on the biocontrol of *A. carbonarius* contamination in grapes. It was shown that lower temperatures (20 °C, 25 °C versus 30 °C) and higher relative humidity (100 % versus 60 %) favor antagonists' colonization and inhibition of *A. carbonarius* from day 2. OTA concentration was reduced at 5th day in all the conditions tested (De Curtis et al. 2012).

A study with 55 fungal isolates obtained from grape berries, found that 28 of them, namely *A. pullulans*, *Cryptococcus magnus* and *Candida sake* strains, were able to inhibit *A. tubingensis* infection in grapes, with *A. pullulans* showing the higher efficacy, reaching up to 96 % inhibition (Pantelides et al. 2015). Previously, Dimakopoulou et al. (2008) demonstrated

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the activity of 17 yeasts on *A. carbonarius* control, of which *A. pullulans* was the most effective, in detached berry tests and in field conditions, being the latter experiment compared to the application of a commercial fungicide (fludioxonil and cyprodinil based). The results of the field study, with two different grape varieties, showed reduction of OTA in the produced must, and no significant difference between the commercial fungicide and the BCA in reducing *A. carbonarius* in field.

Kluyveromyces thermotolerans (*Lanchancea thermotolerans*) strains have also been considered as BCA, being observed that two strains were able to reduce OTA production and growth rates of *A. niger* aggregate species and *A. carbonarius in vitro*, and that OTA presence in musts from contaminated Cabernet Sauvignon grapes was reduced in 100 % (Ponsone et al. 2011). A more recent study has shown that the same species was a suitable BCA for *Aspergillus* section *Nigri* and OTA contaminations in grapes, with inhibitions of 27 % to 100 %, respectively, in both greenhouse and field experiments (Ponsone et al. 2016).

Cubaiu et al. (2012) confirmed the ability of strains of *Saccharomyces cerevisiae* and *Kloeckera apiculata* to control *A. carbonarius* and *A. ochraceus* development and OTA production *in vitro* and on grapes. The mechanisms involved in the inhibition was the regulation of mycotoxin biosynthesis by a reduction on the transcriptional activity of the polyketide synthase genes involved in the process (Cubaiu et al. 2012).

When studying the antagonistic ability of yeasts, Prendes et al. (2018) found that 14 strains of *Metschnikowia* (*pulcherrima* or spp.), *Starmerella bacillaris* and *H. uvarum* were able to act against *Alternaria alternata* infection of wine grapes and consequent tenuazonic acid

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353 production. As mentioned before, *H. uvarum* also seemed to reduce *A. tubingensis* infection by
354 activating grape defense mechanisms (Apaliya et al. 2017).
355 *In vitro* experiments with 30 strains, assessed their ability to inhibit OTA production by *A.*
356 *carbonarius*, it was observed that non-toxic strains of *A.* section *Nigri*, *Trichoderma* spp.,
357 *Clasdosporium* spp., *Acremonium* spp., *Geotrichum* spp. and one strain of *A. flavus* could reduce
358 OTA concentration from 40 % to 100 % (C. Barberis et al. 2014).
359
360 **3.2 Harvest management**
361 During the collection of mature grapes, and because of their thinner skin at this stage, the
362 control of the harvest process is essential to avoid damages that can favor the entrance of fungi in
363 the fruit and lead to mycotoxin contaminations (Hocking et al. 2007, Amezqueta et al. 2012).
364 The period in which harvest occurs should be controlled, in order to reduce the time
365 window until the initial processing step, which will depend on the final product (*e.g.*, table
366 grapes, dried vine fruits, juice or wine). Processing should occur as fast as possible and proper
367 sanitation procedures need to be adopted in order to ensure the quality of the product required to
368 the next steps (Hocking et al. 2007).
369
370 **3.3 Storage management**
371 The essential parameters to control grapes' storage are temperature and water activity,
372 which must be below 5 °C and 0.8, respectively (Amezqueta et al. 2012). Before any processing
373 step, grapes can be stored for shorter or longer periods, so it is mandatory to have proper control
374 in this stage. The storage of grapes is often made by just using plastic materials to cover the

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boxes, however, the choice of the material used may impact fungal contamination. A study on seedless grapes proved that the simple use of polyamide bags, instead of the more common high-density polyethylene ones, could reduce *A. niger* contamination (Camargo et al. 2012).

In table grapes, a temperature of 0 °C combined with exposure to sulphur dioxide seems appropriate to control black Aspergilli species, for a period of up to 1 month (Guzev et al. 2008). The application of modified atmosphere and the use of bioactive packages may give an additional protection to table grapes. Coatings with chitosan and two different mentha essential oils (*Mentha piperita* L. or *Mentha villosa* Huds) have shown a reduction on *A. niger*, *B. cinerea*, *P. expansum* and *Rhizopus stolonifer* growth, showing its potential application for fungal contamination control (Guerra et al. 2016).

In wine, the storage of grapes is of particular relevance to guarantee not only the safety of the final product, but also its required sensorial qualities. Ozone fumigation is a method with potential application for this purpose, since it was found to reduce fungal (including yeast) contamination by 50 %, and, if made before the dehydration step of wine making, it will not have negative effects on the levels of polyphenols and carotenoids (Botondi et al. 2015).

Biological approaches can also be adopted at this stage. When studying the effect of *Bacillus subtilis*, *Trichoderma harzianum* and *Trichoderma viride* under storage conditions, Senthil et al. (2011) showed reductions of disease incidence caused by spoilage fungi, both *in vitro* and *in vivo*.

*Application of natural compounds***.**

Applying natural compounds to control fungal development is an approach with growing interest due to the increasing rejection of synthetic fungicides by consumers. This application can

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be done at different stages, with more studies focusing on applications to control spoilage in harvested grapes during storage, but also some successful *in vitro* studies at pre-harvest stages. Table III presents results on effective natural compounds, indicating the fungal species inhibited and the type of assay conducted.

A great interest has been given to the potential use of essential oils (EOs) as antifungal agents. In fact, research on this field is diverse with a wide range of EOs already tested against different microorganisms. Lemon (*Citrus lemon* L.), eucalyptus (*Eucalyptus globulus* LABILL.), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare* L.), sage (*Salvia officinalis* L.) and lavender (*Lavandula angustifolia* MILLER.) EOs were tested to inhibit *A. niger* and *A. tubingensis*, with the higher fungal inhibition being observed for *O. vulgare* L. and *T. vulgaris* L., followed by *L. angustifolia* MILLER. and *S. officinalis* L. (which only caused a slower growth), and with no inhibition occurring when using *C. lemon* L. and *E. globulus* LABILL. EOs (Cisarova et al. 2016).

Sonker et al. (2014) tested 15 EOs for their *in vitro* efficacy in reducing *A. flavus*, *A. niger* and *A. ochraceus* growth. EOs obtained from *Ageratum conyzoides* Linn., *Ocimum canum* Sims, *Piper methysticum* Frost., *Putranjeeva roxburghii* Wall and *Cymbopogon citratus* (DC.) Stapf were the most promising ones, with inhibitions above 54 % for the three fungal isolates, with *Cymbopogon citratus* (DC.) Stapf causing an inhibition of 100 %. The essential oil of *C. citratus* also inhibited aflatoxin B1 (AFB1) and OTA production (Sonker et al. 2014).

After testing 20 different EOs, Sonker et al. (2015) found that the Indian wormwood (*Artemisia nilagirica* (Clarke) Pamp.) EO was the most effective *in vitro*, inhibiting AFB1 and OTA production and fungi contamination (including reduction of *A. flavus*, *A. niger* and *A.*

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ochraceus by 100 %) on grapes. This EO showed fungistatic and fungicidal activities, at 0.29 µL/mL and 0.58 µL/mL, respectively. Moreover, the EOs from *Blumea membranacea* DC., *Hyptis suaveolens* (L.) Poit. and *Ocimum gratissimum* Linn inhibited the three fungal species by more than 46 % (Sonker et al. 2015).

Basil (*Ocimum basilicum*) EO was also tested for its inhibition of *A. flavus* when added to 1 kg of *Vitis vinifera* grapes stored in plastic containers at 1 µL/mL, resulting in a reduction of *A. flavus* by 62.5 %. Basil EO showed a minimum inhibitory concentration of 1 µL/mL, and inhibition of AFB1 production at 0.5 µL/mL (Kumar et al. 2011).

Several authors have studied the mode of action of essential oils in the inhibition of mycotoxin biosynthesis. A reduction on the expression of *AcOTAnrps* gene in *A. carbonarius* was observed when using *E. cariophyllus*, *C. citratus*, *C. cassia* and *C. reticulate* EOs, which is in line with previous studies that found downregulation of *acOTApks*, *acOTAnrps*, *acpks*, *lae*A and *vea* genes involved in OTA biosynthesis in the presence of *R. sofficinalis*, *P. anisum*, *C. nobile*, *F. vulgare*, *E. cardamomum* and *A. graveolens* EOs (El Khour et al. 2016, Lappa et al. 2017).

Based on the inhibition results obtained *in vitro* of *A. alternata*, *B. cinerea*, *Penicillium digitatum*, *P. expansum* and *A. niger*, Shemesh et al. (2016) tested a packaging material for grapes constituted by polyamide films incorporating volatile carvacrol molecules encapsulated with Halloysite nanotubes. It was demonstrated that 2 % and 4 % of carvacrol lead to 25 % and 27 % reduction on the decay of grapes, respectively (Shemesh et al. 2016).

After demonstrating the inhibition potential of chitosan and *O. vulgare* L. EO *in vitro*, dos Santos et al. (2012) studied their application as a coating for grapes. The use of coating

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441 solutions of chitosan at 5 mg/mL and EO at 2.5 µL/mL, resulted in no development of *Rhizopus*
442 *stolonifer,* for 24 h at 12 °C. However, infection of grapes by *A. niger* occurred in 33 % of grapes
443 after 12 days, and infection by *R. stolonifer* and *A. niger* reached, respectively, 25 % and 35 % of
444 fruits (at 25 °C) (dos Santos et al. 2012).

445 Antifungal potential of natamycin and pine-resin was confirmed on synthetic grape-juice
446 medium. A reduced growth and inhibition of OTA production was observed for three isolates of
447 *A. carbonarius* (a complete inhibition was achieved in case of pine-resin). The authors concluded
448 that these effects were correlated to environmental conditions, namely temperature and a_w
449 (Kogkaki et al. 2016).

450 Microbial metabolites have also been tested. *Lecanicillium muscar* cell-wall degrading
451 enzymes, which include mainly chitinases, reduced *A. carbonarius* contamination in white and
452 red grapes by 95 % and 89 %, respectively (Barghini et al. 2013). *Bacillus amyloliquefaciens*
453 1014 was proven to produce metabolites that can highly inhibit *A. niger* growth. The thermal
454 stability of these compounds showed that they have the potential to be applied in sterilized
455 products and preserve their properties during this treatment (Raut et al. 2014). Finally, the
456 application of isothiocyanates showed that after 20 days, *P. expansum* and *A. parasiticus* were
457 inhibited in solid medium, by concentrations higher than 50 mg and 5 mg, respectively (Manyes
458 et al. 2015).

459

460 **3.4 Processing management**

461 Grapes handling and processing will be further presented and discussed based on the final
462 grape product: table grapes, grape juice, dried vine fruits, and wine (including special wines). For

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each grape product, a simplified flow chart is presented (Figure 2) and relevant studies on strategies to reduce contaminations are reviewed.

Mycotoxins' reduction of the final products can be achieved by applying different methods, including biological and physical ones. The occurrence of OTA in grape products, will be the main focus, however, the presence of AF and PAT will also be mentioned. AF are of concern in dried vine fruits, because of the development of *A. flavus* strains during drying. *Penicillium* spp., particularly *P. expansum*, are known to contaminate apples and pears, and to produce PAT, and although less frequently, they can also contaminate grapes, with PAT being detected in grapes, grape juice and, occasionally, in wine (Diaz et al. 2011). In the grape chain, *P. expansum* can contaminate stored table grapes. In grapes for wine, the fungus has been isolated from apparently healthy grapes, and PAT may be detected in grape must, but rarely in wine due to its destruction by yeast during fermentation (Scott et al. 1977).

Table grapes.

For table grapes, which will not suffer further processing steps, looking for grapes suffering from discoloration can minimize OTA contamination risk, since this is a typical alteration resultant from black Aspergilli infection (Hocking et al. 2007). Besides the strategies mentioned for storage (as the application of natural compounds or of fumigants), dipping grapes into an alkaline solution may reduce OTA levels by degradation, as discussed in Serratosa et al. (2010). The use of irradiation to reduce spoilage of table grapes can be effective, and when used in combination with SO_2 can assure an appropriate storage until consumption. Although when applying it, the maintenance of organoleptic and nutritional properties needs to be assured (Barkai-Golan and Follett 2017).

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The inoculation of table grapes with *B. subtilis* liquid culture or its supernatant, but not with its volatile compounds, inhibited *A. carbonarius* growth, confirming previous results on its *in vitro* efficacy, and assessing a possible application during storage (Jiang, Shi, Liu, et al. 2014). *S. pararoseus* Y16 strain has also been studied for its possible application during storage of table grapes against *A. niger*. *S. pararoseus* Y16 was able to reduce infection and induce resistance of the fruits. A proper proliferation of *S. pararoseus* Y16 was observed, without affecting grapes' properties (Li et al. 2017).

Grape juice.

The interest in fruit juices for their nutritional value makes these products relevant for assessing contamination risks. Together with wine, grape juices are the second most frequent source of OTA intake, following grain products (Mandappa et al. 2018). Besides OTA, *Alternaria* toxins were already detected in grape juices (Asam et al. 2012, Liu and Rychlik 2015) and PAT presence is a cause of concern.

Dachery et al. (2017) studied the effect of grape processing steps in OTA. Comparing grape juice making and winemaking, it was found that the reduction in OTA level was 73 %, 66 % and 44 % for grape juice, red wine and white wine, respectively (Dachery et al. 2017).

In grape juice, where fermentation does not take place, yeast biocontrol strategies involve the application of microorganisms without or with low fermentative activity. Fiori et al. (2014) reported that two low-fermenting strains (*Candida intermedia* and *L. thermotolerans*) were more effective than two non-fermenting ones (*Cyberlindnera jadinii* and *Candida friedrichii*) in controlling *A. carbonarius* development, and in reducing OTA levels in grape juice, indicating that reduction of OTA possibly resulted from an adsorption activity (Fiori et al. 2014). In another

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study, including 21 different strains isolated from grapes, it was observed that *Candida famata* 17, *Kloeckera* sp. B2, *Cryptococcus laurentii* B4, *Candida guilliermondii* S1 and three strains of *Candida lusitanie* were the most effective in inhibiting OTA production by *A*. *carbonarius* in both YES medium and grape juice (Var et al. 2011).

Farbo et al. (2016) tested a prototype packed bed bioreactor with yeast cells (*C. intermedia*) immobilized in alginate beads and reported the immobilization did not prevent adsorption of OTA in grape juice. OTA levels decreased by more than 80 %, within 48 h of treatment (Farbo et al. 2016). Nevertheless, adsorption of OTA on yeast cell walls is a reversible physical-chemical phenomenon in which equilibrium is established between bound and free toxin (Petruzzi et al. 2015).

Treatments with ozone in fruit juices can be a method to provide the required safety of these products. This method can also be useful in reducing mycotoxins' levels, as it was reported by Cataldo et al (2008) and Asokapandian et al. (2018) for PAT content in apple juice. Gamma irradiation has also been considered to control mycotoxin contamination, and it was found to reduce OTA levels in grape juice and wine by around 20 % for a treatment at 30.5 kGy.

Dried vine fruits.

Dried vine fruits include raisins (naturally dried grapes), sultanas (dried grapes that are pretreated with a drying emulsion, which results in a light color and a slightly sweeter flavor) and currants (dried black seedless grapes). In dried vine fruits production, mycotoxin occurrence will depend on the initial content in grapes, the concentration effect due to drying, and further toxin production during drying and storage, if temperature and water activity conditions favor mycotoxin production (Covarelli et al. 2012). Therefore, depending on edafoclimatic conditions,

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529 OTA content in these products is usually higher than in other grape products. OTA accumulation
530 in dried vine fruits starts before harvest (during berry maturation), and continues during drying,
531 while conducive water activity conditions persist, yielding dried vine fruits with higher OTA
532 levels. The occurrence of AFs in dried vine fruits is also of concern, with AFB1 being detected
533 by several studies (Juan et al. 2008, Azaiez et al. 2015, Jeszka-Skowron et al. 2017).

534 Prior to drying, grapes may suffer a pre-treatment with an alkaline solution to clean and
535 increase the permeability of the skin, allowing a faster drying stage. Several studies were
536 developed to compare different dipping and drying conditions (Serratosa et al. 2010, Sen et al.
537 2016). Natural drying under the sun, without additional treatment, yields a higher risk of black
538 *Aspergillus* development and OTA contamination. Dipping grapes in an alkaline solution before
539 drying reduces the risk of contamination, and using a controlled drying chamber will further
540 minimize this risk. Serratosa et al. (2010) found using a drying chamber at 50 °C with a relative
541 humidity of 20 % prevented fungal growth and OTA accumulation. In addition, the study found
542 that the increase in OTA content was lower than expected, taking into account the concentration
543 factor due to drying (Serratosa et al. 2010). The latter finding implies a degradation of OTA
544 possibly due to the dipping solution.

545 A survey in southern Spain studied the evolution of the mycoflora of grapes during
546 dehydration (Valero et al. 2005). It was found an increase in *Aspergillus* spp. incidence in grapes
547 with time, with the dominance of black *Aspergillus* species, while the water activity in drying
548 grapes dropped from about 0.95 to 0.75. These conditions, associated with high temperatures and
549 the germicidal effect of ultraviolet rays, favors grow of xerotolerant *A*. section *Nigri* strains over
550 other species.

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Mitigation tools are used to reduce fungal load, reduce mycotoxin accumulation or, even, destroy mycotoxins. One of these approaches is the use of gamma radiation. Inactivation of fungal spores due to irradiation is achievable (Kanapitsas et al. 2015, Kanapitsas et al. 2016), with consequent reduction in the accumulation of AFB1 and OTA. However, the ability of irradiation to destroy mycotoxins is controversial, being dependent on the location in the berry (superficial or internal) and on water content (Calado et al. 2014). In grapes for raisins, Kanapitsas et al. (2015) found reductions on AFB1 levels of 29 % and 65 %, for naturally contaminated samples and *A. parasiticus* spiked raisins, respectively. For OTA, the reduction in naturally contaminated samples was of approximately 88 %, and in *A. carbonarius* spiked samples the toxin was not detected (Kanapitsas et al. 2016).

Also, the application of electron beam treatment (β-radiation) to decrease spore contamination (genera *Aspergillus*, *Byssochlamys*, *Eurotium* and *Penicillium*) in raisins was tested, resulting in reductions of more than 1 log when applying a dosage of more than 31.8 kGy (Etter et al. 2018). However, this is a much larger radiation dose than what is currently regulated in the European Union.

Wine.

Levels of OTA carried-over from grapes to wines will depend on the initial level in grapes, as well as on the technology of winemaking (Figure 3). Among the main steps included in vinification, the use of biological agents has a great potential in reducing mycotoxin contamination in wine. Its application can take place during fermentation, combining mycotoxin

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571 binding and fermentation abilities, or at other stages by the addition of non-viable cells (Hocking
572 et al. 2007).

573 Using the concept of Food Safety Objective (Pitt et al. 2013), most of the steps involved
574 in winemaking will contribute to a decrease in OTA content. The final carry-over of OTA will be
575 the result of the combination of all stages in the wine chain. Due to these, Dachery et al (2017)
576 observed a reduction of OTA contamination of 66 % and 44 % for red and white wine,
577 respectively.

578 Sweet, dessert or fortified wines tend to have a higher level of OTA due to its higher level
579 in grapes, while red wines will tend to have a higher level than rose or white wines, mainly due
580 to the initial steps of winemaking process (Covarelli et al. 2012, Quintela et al. 2013). After
581 crushing, winemaking starts with a maceration step consisting on the contact between must and
582 grape skin to increase the extraction of phenolic compounds (and other compounds) from the
583 grape skin. This maceration step is longer in red wines, and shorter in white wines, being well
584 established that the contact between grape and must will favor the solubility of OTA in musts
585 (Quintela et al. 2013).

586 After crushing and maceration, pressing will separate grape skins (pomace) from must.
587 Gambuti et al. (2005) studied the effect of pressure at this step, and found that increasing
588 pressure will increase the volume of must, but will also increase the amount of OTA extraction
589 from skins. Authors observed a four times increase of OTA in must when pressing pressure
590 increased 10 times (Quintela et al. 2013).

591 OTA has a high affinity for pomace, being suggested the use pomace to remove OTA
592 from contaminated musts after pressing (Solfrizzo et al. 2010). OTA will also bound to other

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593 solids present in the fermenting must (as yeast, small fragments of grape skins or seeds, and
594 other suspended solids), and this physical-chemical adsorption process can be explored to
595 remove OTA in clarification steps after alcoholic fermentation, malolactic fermentation and
596 fining.

597 Jiang, Shi, Cheng, and Liu (2014) studied the effect of *A*. *carbonarius* inoculum in grapes
598 on winemaking. Different amounts of *A*. *carbonarius* spores were added to grapes after crushing,
599 and their development, as well as OTA accumulation, was followed during winemaking. The
600 presence of this inoculum did not affect significantly the winemaking process, yielding wines
601 with similar properties. The number of *A. carbonarius* spores increased slightly in the first hours
602 after inoculation, but then decreased sharply, being detected at residual levels after 24 to 48 h.
603 OTA content increased after inoculation and tend to decrease continuously during winemaking,
604 however it was not clear if the fungus was producing OTA during the winemaking process. Most
605 OTA was removed from wine with pressing, where very high levels of OTA in pomaces were
606 obtained.

607 The accumulation of OTA during winemaking is also inhibited by the alcohol content. It
608 was found that a concentration of ethanol above 2 % (v/v) was enough to delay significantly
609 *Aspergillus* spp. growth and to inhibit OTA production (Jiang et al. 2015).

610 Several authors studied the fate of OTA during alcoholic fermentation with different
611 yeast species in synthetic medium, must and wine. Aiming to decrease OTA content, binding
612 strategies can be exploited in order to guarantee products' safety, being the case of wine
613 particularly relevant since fermentation starter cultures can also have binding potential. *S.*
614 *cerevisiae* commercial yeast strains were able to remove OTA by up to 68 % (Quintela et al.

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615 2013). These authors observed that OTA removal was mainly due to adsorption, but the
616 microbial degradation of OTA could not be excluded (Ciconova et al. 2010). Similarly, different
617 strains of *S. cerevisiae* were tested for OTA adsorption in grape must and resulted in a 20 % to
618 70 % adsorption of the toxin. However, since this is a reversible phenomenon, the stability of
619 these bonds is weak and the contact of these yeasts with an OTA free must will release more than
620 55 % of the bonded toxin, which can be a challenge for the practical application of this approach
621 (Petruzzi et al. 2015). During processing, selection of fermentative strains that can
622 simultaneously inhibit undesirable fungal incidences and consequent mycotoxin production is
623 promising. However, achieving inhibition during and after fermentation is a challenge,
624 considering the changes occurring during this process, mainly the high increase of medium
625 acidity (Cubaiu et al. 2012).
626 The evolution of OTA during alcoholic fermentation was also studied in a spiked must
627 (Esti et al. 2012). OTA depletion started at the beginning of fermentation with a constant
628 removal rate. In the study, slightly higher removal was achieved in the presence of grape skins.
629 Two phases have been identified: an initial phase were content of OTA varied due to
630 adsorption/desorption from grape skins and where OTA content may increase, if grape skins are
631 contaminated; and a second phase, where the presence of yeast surpasses the effect of grape
632 skins and adsorption phenomenon will govern OTA removal. OTA content found in lees, a solid
633 fraction obtained after alcoholic fermentation with most of the yeast cells, is much higher than
634 the concentration left in wine. After malolactic fermentation, a reduced effect on OTA content
635 was observed that was mainly attributed to the ethanol content of wine, as well as the lower
636 amount of solids (Fernandes et al. 2007, Mateo et al. 2010).

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637 Strategies to reduce mycotoxin levels through adsorption or degradation can rely on
638 biological agents, other than the mentioned fermentative strains. The application of antagonistic
639 yeasts can promote OTA removal during winemaking. Patharajan et al. (2011) found that some
640 yeasts might remove OTA, mainly by degradation. Among tested strains, *M. pulcherrima*
641 MACH1 and *Pichia guilliermondii* M8 were able to degrade up to 80 % of OTA, by an unknown
642 mechanism. Also, malolactic fermenting bacteria can be used to remove OTA. *Pediococcus*
643 *parvulus* strains isolated from wines that underwent spontaneous malolactic fermentation were
644 tested for OTA removal with promising *in vitro* results, but not as efficient in natural grape
645 musts (Abrunhosa et al. 2014).
646 Kapetanakou et al. (2012) studied several bacterial and yeast isolates on the inhibition of
647 *A. carbonarius* growth on synthetic grape medium, at different environmental conditions, and
648 observed that no inhibition occurred with the bacterial isolates (probably due to the pH 3); and
649 that OTA reductions, although observed in both cases, were also more significant with yeasts,
650 achieving a reduction of up to 65 %. In beverages (grape juice, red wine and beer), the higher
651 reduction in OTA was observed, also, with yeasts, most likely caused by adsorption to cell walls
652 (Kapetanakou et al. 2012). The best *in vitro* results were obtained with mixed cultures including
653 *Hanseniaspora guilliermondii*, *Issatchekia occidentalis*, *Issatchenkia orientalis*, *Kluyveromyces*
654 *dobzhankii*, *L. thermotolerans*, *Pichia fermentas*, *P. guilliermondii* and *S. cerevisiae*
655 (Kapetanakou et al. 2012).
656 In fact, OTA adsorption to yeast cell walls has been attributed to the presence of
657 mannoproteins and beta-glucans, and this is explored also in fining stages. Adsorption of OTA
658 improves with autoclaved cells, possible due to heat causing changes in the surface properties of

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659 the cells (Piotrowska et al. 2013). The same behavior has been observed for bacterial cells, with
660 adsorption to autoclaved cells of *B. subtilis* being higher than to viable cells (Shi et al. 2014).
661 This study showed also that a *B. subtilis* strain with inhibitory activity against *A. ochraceus* and
662 *A. carbonarius* could adsorb OTA, and that its cell-free supernatant degraded approximately 97
663 % of the mycotoxin.

664 The degradation of mycotoxins by yeasts during fermentation has also been tested for
665 AFB1. In beer fermentation and grape must fermentation, AFB1 concentration was found to
666 decrease slightly and significantly, respectively (Inoue et al. 2013). The decrease in beer was
667 marginal and due to adsorption, and in wine fermentation, a decrease of up to 30 % was observed
668 and attributed to the production of a hydrated derivative of the toxin. Differences in degradation
669 during beer and wine fermentation could be due to the use of different yeast strains and different
670 fermentation conditions (temperature and pH).

671 At fining stages, different oenological fining agents are used to aid wine clarification and
672 to remove several undesirable compounds. Many products are used at this stage, and most have
673 been tested for OTA adsorption in other comprehensive reviews (Quintela et al. 2013). However,
674 these products are not specific for OTA, and will remove other compounds responsible for the
675 flavor, taste and color of wines. For these reasons, the removal of OTA by these products should
676 be regarded as a collateral effect and not as their main role in wine making.

677 In high ethanol content wines (Figure 2), occurrence of OTA depends on its initial
678 content in grapes as well as on the wine being produced. Comparing with table wines, some
679 special wines production includes a drying stage and because of that will tend to have higher

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OTA content. This drying step can also impact the aromatic profile of the final product (Barata et al. 2012).

Bejarano et al. (2010) evaluated the effect of drying in musts, observing a much higher OTA content when grapes are dried naturally, when compared with drying in a climatic chamber at 40 °C and relative humidity of 10 % (28.8 µg/kg vs 6.9 µg/kg). However, a sensory analysis of grape musts revealed a slightly higher preference for the sun dried grapes (Bejarano et al. 2010). The effect of maceration and fermentation on OTA was also reported for sweet wine making. During aging, a progressive decrease in OTA content was observed, with the decrease being more accentuated when aging took place in oak barrels (up to 37 % after 90 days) (Bejarano et al. 2010).

High ethanol content wines produced from over-ripened and dried grapes are particularly susceptible to contamination with AFs as well. The co-occurrence of AFs and OTA was reported in special wines with both toxins occurring in 20 % of the samples (n=30), but at low levels (OTA < 2 µg/L and AFs < 0.1 µg/L) (Di Stefano et al. 2015).

4. Conclusions

The exposure of grapes to fungal contamination in field can result in health risks from the presence of mycotoxins. Although diverse fungal species can contaminate grapes, OTA producing species, mainly black *Aspergillus*, are by far the greatest problem in grape spoilage. OTA is mainly a pre-harvest problem, but the toxin is resilient and will occur in many grape products. Occasionally, other fungi may produce mycotoxins in grapes, such as PAT by *P. expansum* or AFs by *Aspergillus* section *Flavi* (El Khoury et al. 2008, Diaz et al. 2011). The

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occurrence of PAT is mainly a pre-harvest problem in table grapes and grape juices, but rarely occurs at concerning levels. On the other hand, AFs are mainly a post-harvest problem in dried vine fruits, and can also occur in special wines, due to improper drying conditions. In the past decades, it was shown that black *Aspergillus* spp. could also produce FB2; however, the levels of occurrence in grapes and in derived products (as wine) are not causes for concern (Logrieco et al. 2010, Knudsen et al. 2011).

Plant-pathogen interactions are complex and, under climate change, fungal development in grapes might differ from what is expected under the actual scenario in each region, which can allow the proliferation of different species and the occurrence of other mycotoxins, challenging the safety of grapes and derived product. In fact, these aspects can raise the need for new efforts, besides all the already adopted and reviewed herein at cropping and processing levels. The diversity of conditions under which the different grape products are obtained can also potentially lead to fungal proliferation and mycotoxin production. Therefore, the adoption of an integrated vision on the pre- and post-harvest strategies is crucial to achieve an overall mitigation of risks.

Literature Cited

Abrunhosa L, Calado T and Venâncio A. 2011. Incidence of fumonisin B(2) production by *Aspergillus niger* in Portuguese wine regions. J Agric Food Chem 59(13):7514-7518.

Abrunhosa L, Inês A, Rodrigues AI, Guimarães A, Pereira VL, Parpot P, Mandes-Faia A and Venâncio A. 2014. Biodegradation of ochratoxin A by *Pediococcus parvulus* isolated from Douro wines. Int J Food Microbol 188:45-52.

Abrunhosa L, Paterson R, Kozakiewicz Z, Lima N and Venâncio A. 2001. Mycotoxin production from fungi isolated from grapes. Lett Appl Microbiol 32:240-242.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

Ahmed H, Strub C, Hilaire F and Schorr-Galindo S. 2015. First report: *Penicillium adametzioides*, a potential biocontrol agent for ochratoxin-producing fungus in grapes, resulting from natural product pre-harvest treatment. Food Control 51:23-30.

Amezqueta S, Schorr-Galindo S, Murillo-Arbizu M, Gonzalez-Penas E, Lopez de Cerain A and Guiraud JP. 2012. OTA-producing fungi in foodstuffs: A review. Food Control 26(2):259-268.

Apaliya MT, Zhang H, Yang Q, Zheng X, Zhao L, Kwaw E and Mahunu GK. 2017. *Hanseniaspora uvarum* enhanced with trehalose induced defense-related enzyme activities and relative genes expression levels against *Aspergillus tubingensis* in table grapes. Postharvest Biol Technol 132:162-170.

Asam S, Lichtenegger M, Liu Y and Rychlik M. 2012. Content of the *Alternaria* mycotoxin tenuazonic acid in food commodities determined by a stable isotope dilution assay. Mycotoxin Res 28(1):9-15.

Asokapandian S, Periasamy S and Swamy GJ. 2018. Chapter 25 - Ozone for Fruit Juice Preservation. Rajauria G and Tiwari BK, eds., Fruit Juices (Academic Press: San Diego, USA) pp. 511-527.

Atoui A, Mitchell D, Mathieu F, Magan N and Lebrihi A. 2007. Partitioning of ochratoxin A in mycelium and conidia of *Aspergillus carbonarius* and the impact on toxin contamination of grapes and wine. J Appl Microbiol 103(4):961-968.

Azaiez I, Font G, Manes J and Fernandez-Franzon M. 2015. Survey of mycotoxins in dates and dried fruits from Tunisian and Spanish markets. Food Control 51:340-346.

Barata A, Malfeito-Ferreira M and Loureiro V. 2012. The microbial ecology of wine grape berries. Int J Food Microbiol 153(3):243-259.

Barberis C, Pena G, Carranza C and Magnoli CE. 2014. Effect of indigenous mycobiota on ochratoxin A production by *Aspergillus carbonarius* isolated from soil. Mycotoxin Res 30(1):1-8.

Barberis M, Merlera GG, Reynoso MM, Chulze SN and Torres AM. 2014. Factors affecting distribution and abundance of *Aspergillus* section *Nigri* in vineyard soils from grapevine growing regions of Argentina. J Sci Food Agric 94(14):3001-3007.

Barghini P, Esti M, Pasqualetti M, Silvi S, Aquilanti A and Fenice M. 2013. Inhibition of the ochratoxin -A producer *Aspergillus carbonarius* on white and red grapes by crude cell-wall degrading enzymes from the Antarctic fungus *Lecanicillium muscarium* CCFEE 5003. J Environ Prot Ecol 14(4):1673-1679.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

Barkai-Golan R and Follett PA. 2017. Chapter 6 - Irradiation for Quality Improvement of Individual Fruits. Barkai-Golan R and Follett PA, eds., Irradiation for Quality Improvement, Microbial Safety and Phytosanitation of Fresh Produce (Academic Press: San Diego, USA) pp. 55-107.

Battilani P, Giorni P, Bertuzzi T, Formenti S and Pietri A. 2006. Black aspergilli and ochratoxin A in grapes in Italy. Int J Food Microbol 111:S53-S60.

Battilani P, Logrieco A, Giorni P, Cozzi G, Bertuzzi T and Pietri A. 2004. Ochratoxin A production by *Aspergillus carbonarius* on some grape varieties grown in Italy. J Agric Food Chem 84(13):1736-1740.

Bavaresco L, Vezzulli S, Battilani P, Giorni P, Pietri A and Bertuzzi T. 2003. Effect of ochratoxin A-producing Aspergilli on stilbenic phytoalexin synthesis in grapes. J Agric Food Chem 51(21):6151-6157.

Battilani P and Camardo Leggieri M. 2015. OTA-grapes: A mechanistic model to predict ochratoxin a risk in grapes, a step beyond the systems approach. Toxins 7(8):3012-3029.

Bejarano MJR, Dodero MCR and Barroso CG. 2010 Optimizing the Process of Making Sweet Wines To Minimize the Content of Ochratoxin A. J Agric Food Chem 58(24):13006-13012.

Botondi R, De Sanctis F, Moscatelli N, Vettraino Anna M, Catelli C and Mencarelli F. 2015. Ozone fumigation for safety and quality of wine grapes in postharvest dehydration. Food Chem 188:641-647.

Calado T, Venâncio A and Abrunhosa L. 2014. Irradiation for Mold and Mycotoxin Control: A Review. Compr Rev Food Sci Food Saf 13(5):1049-1061.

Camardo Leggieri M, Battilani P, Mitchell D, Aldred D and Magan N. 2014. Hydro- and thermotimes for conidial germination kinetics of the ochratoxigenic species *Aspergillus carbonarius in vitro*, on grape skin and grape flesh. Fungal Biol 118(12):996-1003.

Camargo RB, Terao D, Peixoto AR, Ono EO, Cavalcanti LS and da Costa RM. 2012. Modified atmosphere in preserving the quality of 'Thompson Seedless' grapes and reducing *Aspergillus* rot. Summa Phytopathol 38(3):216-222.

Cataldo F. 2008. Ozone Decomposition of Patulin - A Micotoxin and Food Contaminant. Ozone Sci Eng 30(3):197-201.

Chiotta ML, Ponsone ML, Sosa DM, Combina M and Chulze SN. 2013. Biodiversity of *Aspergillus* section *Nigri* populations in Argentinian vineyards and ochratoxin A contamination. Food Microbiol 36(2):182-190.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

789 Chiotta ML, Ponsone ML, Torres AM, Combina M and Chulze SN. 2010. Influence of
790 *Planococcus ficus* on *Aspergillus* section *Nigri* and ochratoxin A incidence in vineyards from
791 Argentina. Lett Appl Microbiol 51(2):212-218.

792 Ciconova P, Laciakova A and Mate D. 2010. Prevention of Ochratoxin A Contamination of Food
793 and Ochratoxin A Detoxification by Microorganisms - A Review. Czech J Food Sci
794 28(6):465-474.

795 Cisarova M, Tancinova D and Medo J. 2016. Antifungal activity of lemon, eucalyptus, thyme,
796 oregano, sage and lavender essential oils against *Aspergillus niger* and *Aspergillus*
797 *tubingensis* isolated from grapes. Potravinarstvo: Scientific Journal for Food Industry
798 10(1):83-88.

799 Covarelli L, Beccari G, Marini A and Tosi L. 2012. A review on the occurrence and control of
800 ochratoxigenic fungal species and ochratoxin A in dehydrated grapes, non-fortified dessert
801 wines and dried vine fruit in the Mediterranean area. Food Control 26(2):347-356.

802 Cozzi G, Haidukowski M, Perrone G, Visconti A and Logrieco A. 2009. Influence of *Lobesia*
803 *botrana* Field Control on Black Aspergilli Rot and Ochratoxin A Contamination in Grapes. J
804 Food Protect 72(4):894-897.

805 Cozzi G, Somma S, Haidukowski M and Logrieco AF. 2013. Ochratoxin A management in
806 vineyards by *Lobesia botrana* biocontrol. Toxins 5(1):49-59.

807 Cubaiu L, Abbas H, Dobson ADW, Budroni M and Migheli Q. 2012. A *Saccharomyces*
808 *cerevisiae* wine strain inhibits growth and decreases ochratoxin A biosynthesis by *Aspergillus*
809 *carbonarius* and *Aspergillus ochraceus*. Toxins 4:1468-1481.

810 Dachery B, Veras FF, Magro LD, Manfroi V and Welke JE. 2017. Exposure risk assessment to
811 ochratoxin A through consumption of juice and wine considering the effect of steam
812 extraction time and vinification stages. Food Chem Toxicol 109:237-244.

813 De Curtis F, de Felice DV, Ianiri G, De Cicco V and Castoria R. 2012. Environmental factors
814 affect the activity of biocontrol agents against ochratoxigenic *Aspergillus carbonarius* on
815 wine grape. Int J Food Microbol 159(1):17-24.

816 De Rossi P, Ricelli A, Reverberi M, Bello C, Fabbri AA, Fanelli C, De Rossi A, Corradini D and
817 Nicoletti I. 2012. Grape variety related trans-resveratrol induction affects *Aspergillus*
818 *carbonarius* growth and ochratoxin A biosynthesis. Int J Food Microbol 156(2):127-132.

819 Di Stefano V, Pitonzo R, Avellone G, Di Fiore A, Monte L and Ogorka AZT. 2015.
820 Determination of aflatoxins and ochratoxins in sicilian sweet wines by high-performance

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

liquid chromatography with fluorometric detection and immunoaffinity cleanup. Food Anal Methods 8:569-577.

Diaz GA, Yanez L and Latorre BA. 2011. Low occurrence of patulin -producing strains of *Penicillium* in grapes and patulin degradation during winemaking in Chile. Am J Enol Viticult 62(4):542-546.

Dimakopoulou M, Tjamos SE, Antoniou PP, Pietri A, Battilani P, Avramidis N, Markakis EA and Tjamos EC. 2008. Phyllosphere grapevine yeast Aureobasidium pullulans reduces *Aspergillus carbonarius* (sour rot) incidence in wine-producing vineyards in Greece. Biol Control 46:158-165.

dos Santos NST, Aguiar AJAA, de Oliveira CEV, de Sales CV, Silva SM, da Silva RS, Stamford TCM, and de Souza EL. 2012. Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (Vitis labrusca L.). Food Microbiol 32(2):345-353.

El-Samawaty AE-RMA, Moslem MA, Yassin MA, El-Shikh MS, El-Samawaty AE-RMA, Yassin MA and Sayed SRM. 2013. Control of grape blue molding Penicillia by *Allium sativum*. J Pure App Microbio 7(2):1047-1053.

El Khour R, Maroun R, El Khoury A, El Khour R, Verheecke C, Mathieu F and Atoui A. 2016. Essential oils modulate gene expression and ochratoxin a production in *Aspergillus carbonarius*. Toxins 8:2072-6651.

El Khoury A, Rizk T, Lteif R, Azouri H, Delia M-L and Lebrihi A. 2008. Fungal contamination and Aflatoxin B1 and Ochratoxin A in Lebanese wine–grapes and musts. Food Chem Toxicol 46(6):2244-2250.

Esti M, Benucci I, Liburdi K and Acciaro G. 2012. Monitoring of ochratoxin A fate during alcoholic fermentation of wine -must. Food Control 27(1):53-56.

Etter D, Rupp A, Prange A and Drissner D. 2018. Inactivation of mould spores in a model system and on raisins by low-energy electron beam. Food Control 92:357-361.

European Commission.2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union 15-16.

Farbo MG, Urgeghe PP, Fiori S, Marceddu S, Jaoua S and Migheli Q. 2016. Adsorption of ochratoxin A from grape juice by yeast cells immobilised in calcium alginate beads. Int J Food Microbiol 217:29-34.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

853 Felsociova S, Rybarik LU, Tancinova D, Maskova Z and Kacaniova M. 2015a Microfungi and
854 mycotoxins of grapes from eastern Slovak wine region. J Microbiol Biotechnol Food Sci
855 4:12-15.

856 Felsociova S, Rybarik LU, Tancinova D, Maskova Z and Kacaniova M. 2015b. Microfungi and
857 mycotoxins of grapes from Tokaj wine region. J Microbiol Biotechnol Food Sci 4:16-18.

858 Fernandes A, Ratola N, Cerdeira A, Alves A and Venâncio A. 2007. Changes in Ochratoxin A
859 Concentration during Winemaking. Am J Enol Viticult 58(1):92.

860 Ferrari V, Dellacassa E, Coniberti A and Disegna E. 2017. Role of grapevine vegetative
861 expression on *Aspergillus* spp. incidence and OTA accumulation in wines produced in a
862 temperate humid climate. Food Addit Contam Part A Chem Anal Control Expo Risk Assess
863 34(2):299-306.

864 Fiori S, Urgeghe Pietro P, Razzu S, Hammami W, Jaoua S and Migheli Q. 2014. Biocontrol
865 activity of four non- and low-fermenting yeast strains against *Aspergillus carbonarius* and
866 their ability to remove ochratoxin A from grape juice. Int J Food Microbiol 189:45-50.

867 Flamini R, Zanzotto A, de Rosso M, Lucchetta G, Dalla Vedova A and Bavaresco L. 2016.
868 Stilbene oligomer phytoalexins in grape as a response to *Aspergillus carbonarius* infection.
869 Physiol Mol Plant Path 93:112-118.

870 Gambuti A, Strollo D, Genovese A, Ugliano M, Ritieni A and Moio L. 2005. Influence of
871 Enological Practices on Ochratoxin A Concentration in Wine. Am J Enol Viticult 56(2):155.

872 Garcia-Cela E, Crespo-Sempere A, Gil-Serna J, Porqueres A and Marin S. 2015. Fungal
873 diversity, incidence and mycotoxin contamination in grapes from two agro-climatic Spanish
874 regions with emphasis on *Aspergillus* species. J Sci Food Agric 95(8):1716-1729.

875 Garcia-Cela E, Crespo-Sempere A, Ramos AJ, Sanchis V and Marin S. 2014. Ecophysiological
876 characterization of *Aspergillus carbonarius*, *Aspergillus tubingensis* and *Aspergillus niger*
877 isolated from grapes in Spanish vineyards. Int J Food Microbiol 173:89-98.

878 Garcia-Cela E, Ramos AJ, Sanchis V and Marin S. 2012. Ochratoxigenic moulds and
879 effectiveness of grape field antifungals in a climatic change scenario. J Sci Food Agric
880 92(7):1455-1461.

881 Guerra ICD, de Oliveira PDL, Santos MMF, Lúcio ASSC, Tavares JF, Barbosa-Filho JM,
882 Madruga MS, and de Souza EL. 2016. The effects of composite coatings containing chitosan
883 and *Mentha* (*piperita* L. or x *villosa* Huds) essential oil on postharvest mold occurrence and
884 quality of table grape cv. Isabella. Innov Food Sci Emerg Technol 34:112-121.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

885 Guzev L, Danshin A, Zahavi T, Ovadia A and Lichter A. 2008. The effects of cold storage of
886 table grapes, sulphur dioxide and ethanol on species of black *Aspergillus* producing
887 ochratoxin A. Int J Food Sci Technol 43(7):1187-1194.

888 Hocking AD, Leong SL, Kazi BA, Emmett RW and Scott ES. 2007. Fungi and mycotoxins in
889 vineyards and grape products. Int J Food Microbiol 119:84-88.

890 Inoue T, Nagatomi Y, Uyama A and Mochizuki N. 2013. Degradation of aflatoxin B1 during the
891 fermentation of alcoholic beverages. Toxins 5(7):1219-1229.

892 Irkin R and Guldas M. 2014. Chitosan Coating of Red Table Grapes and Fresh-Cut Honey
893 Melons to Inhibit *Fusarium oxysporum* Growth. J Food Process Pres 38(4):1948-1956.

894 Jeszka-Skowron M, Zgola-Grzeskowiak A, Stanisz E and Waskiewicz A. 2017. Potential health
895 benefits and quality of dried fruits: Goji fruits, cranberries and raisins. Food Chem 221:228-
896 236.

897 Jiang C, Shi J, Chen X and Liu Y. 2015. Effect of sulfur dioxide and ethanol concentration on
898 fungal profile and ochratoxin a production by *Aspergillus carbonarius* during wine making.
899 Food Control 47:656-663.

900 Jiang C, Shi J, Cheng Y and Liu Y. 2014. Effect of *Aspergillus carbonarius* amounts on
901 winemaking and ochratoxin A contamination. Food Control 40:85-92.

902 Jiang C, Shi J, Liu Y and Zhu C. 2014. Inhibition of *Aspergillus carbonarius* and fungal
903 contamination in table grapes using *Bacillus subtilis*. Food Control 35(1):41-48.

904 Jiang C, Shi J and Zhu C. 2013. Fruit spoilage and ochratoxin A production by *Aspergillus*
905 *carbonarius* in the berries of different grape cultivars. Food Control 30(1):93-100.

906 Juan C, Zinedine A, Moltó JC, Idrissi L and Mañes J. 2008. Aflatoxins levels in dried fruits and
907 nuts from Rabat-Salé area, Morocco. Food Control 19(9):849-853.

908 Kanapitsas A, Markaki P, Batrinou A and Aravantinos A. 2015. Effect of .gamma.-radiation on
909 the production of aflatoxin B1 by *Aspergillus parasiticus* in raisins (*Vitis vinifera* L.). Radiat
910 Phys Chem 106:327-332.

911 Kanapitsas A, Markaki P, Batrinou A, Sflomos C and Aravantinos A. 2016. Gamma radiation
912 inhibits the production of Ochratoxin A by *Aspergillus carbonarius*. Development of a
913 method for OTA determination in raisins. Food Biosci 15:42-48.

914 Kapetanakou AE, Kollias JN, Drosinos EH and Skandamis PN. 2012. Inhibition of *A.*
915 *carbonarius* growth and reduction of ochratoxin A by bacteria and yeast composites of
916 technological importance in culture media and beverages. Int J Food Microbiol 152(3):91-99.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

917 Khalesi M and Khatib N. 2011. The effects of different ecophysiological factors on ochratoxin A
918 production. Environ Toxicol Pharmacol 32(2):113-121.

919 Knudsen PB, Mogensen JM, Larsen TO and Nielsen KF. 2011. Occurrence of fumonisins B(2)
920 and B(4) in retail raisins. J Agric Food Chem 59(2):772-776.

921 Kogkaki EA, Natskoulis PI and Panagou EZ. 2016. Modeling the effect of natamycin, pine-resin
922 and environmental factors on the growth and OTA production by *Aspergillus carbonarius*
923 using response surface methodology. Food Res Int 79:19-28.

924 Kumar A, Shukla R, Singh P, Prakash B and Dubey NK. 2011. Chemical composition of
925 *Ocimum basilicum* L. essential oil and its efficacy as a preservative against fungal and
926 aflatoxin contamination of dry fruits. Int J Food Sci Technol 46(9):1840-1846.

927 Lappa I K, Kizis D, Natskoulis PI and Panagou EZ. 2015. Comparative study of growth
928 responses and screening of inter-specific OTA production kinetics by *A. carbonarius* isolated
929 from grapes. Front Microbiol 6:502.

930 Lappa IK, Simini E, Nychas G-JE and Panagou EZ. 2017. *In vitro* evaluation of essential oils
931 against *Aspergillus carbonarius* isolates and their effects on Ochratoxin A related gene
932 expression in synthetic grape medium. Food Control 73:71-80.

933 Lasram S, Barketi A, Mliki A and Ghorbel A. 2012. Growth and ochratoxin A production by
934 *Aspergillus carbonarius* at different pHs and grape maturation stages. Lett App Microbiol
935 54(5):418-424.

936 Li Q, Li C, Li P, Zhang H, Zhang X, Zheng X, Yang Q, Apaliya MT, Boateng NAS and Sun Y.
937 2017. The biocontrol effect of *Sporidiobolus pararoseus* Y16 against postharvest diseases in
938 table grapes caused by *Aspergillus niger* and the possible mechanisms involved. Biol Control
939 113:18-25.

940 Liu Y and Rychlik M. 2015. Biosynthesis of seven carbon-13 labeled *Alternaria* toxins including
941 altertoxins, alternariol , and alternariol methyl ether, and their application to a multiple stable
942 isotope dilution assay. Anal Bioanal Chem 407:1357-1369.

943 Logrieco A, Ferracane R, Cozzi G, Haidukowsky M, Susca A, Mule G and Ritieni A. 2011.
944 Fumonisin B2 by *Aspergillus niger* in the grape - wine chain: an additional potential
945 mycotoxicological risk. Ann Microbiol 61(1):1-3.

946 Logrieco A, Ferracane R, Visconti A and Ritieni A. 2010. Natural occurrence of fumonisin B2 in
947 red wine from Italy. Food Addit Contam Part A Chem Anal Control Expo Risk Assess
948 27(8):1136-1141.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

949 Lorenzini M and Zapparoli G. 2015. Occurrence and infection of *Cladosporium*, *Fusarium*,
950 *Epicoccum* and *Aureobasidium* in withered rotten grapes during post-harvest dehydration.
951 Antonie Van Leeuwenhoek 108:1171-1180.

952 Lucchetta G, Bazzo I, Cortivo Gianluca D, Stringher L, Bellotto D, Borgo M and Angelini E.
953 2010. Occurrence of black aspergilli and ochratoxin A on grapes in Italy. Toxins 2(4):840-
954 855.

955 Magan N, Aldred D, Hope R and Mitchell D. 2010. Environmental factors and interactions with
956 mycobiota of grain and grapes: effects on growth , deoxynivalenol and ochratoxin production
957 by *Fusarium culmorum* and *Aspergillus carbonarius*. Toxins 2(3).

958 Mandappa IM, Basavaraj K and Manonmani HK. 2018. Chapter 36 - Analysis of Mycotoxins in
959 Fruit Juices. Rajauria G and Tiwari BK, eds., Fruit Juices (Academic Press: San Diego, USA)
960 pp. 763-777.

961 Manyes L, Luciano FB, Manes J and Meca G. 2015. *In vitro* antifungal activity of allyl
962 isothiocyanate (AITC) against *Aspergillus parasiticus* and *Penicillium expansum* and
963 evaluation of the AITC estimated daily intake. Food Chem Toxicol 83:293-299.

964 Maskova Z, Tancinova D, Rybarik LU and Felsoeciova S. 2014. Colonization of grape berries by
965 the genus *Fusarium* and toxigenity of the most common representatives. J Microbiol
966 Biotechnol Food Sci 3:256-258.

967 Mateo EM, Medina Á, Mateo R and Jiménez M. 2010. Effect of ethanol on the ability of
968 *Oenococcus oeni* to remove ochratoxin A in synthetic wine-like media. Food Control
969 21(6):935-941.

970 Meyvaci KB, Aksoy U, Altindisli A, Eltem R, Askun T and Taskin E. 2012. Effect of yearly
971 conditions and management practices on ochratoxin A production in Sultana Seedless
972 vineyards. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 29(7):1157-
973 1167.

974 Mikusova P, Ritieni A, Santini A, Juhasova G and Srobarova A. 2010. Contamination by moulds
975 of grape berries in Slovakia. Food Addit Contam Part A Chem Anal Control Expo Risk
976 Assess 27(5): 738-747.

977 Mikusova P, Srobarova A, Sulyok M and Santini A. 2013. *Fusarium* fungi and associated
978 metabolites presence on grapes from Slovakia. Mycotoxin Res 29(2):97-102.

979 Morales-Valle H, Silva LC, Paterson R, Venâncio A and Lima N. 2011. Effects of the origins of
980 *Botrytis cinerea* on earthy aromas from grape broth media further inoculated with *Penicillium*
981 *expansum*. Food Microbiol 28(5):1048-1053.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

Morales H, Paterson RRM, Venâncio A, and Lima N. 2013. Interaction with *Penicillium expansum* enhances *Botrytis cinerea* growth in grape juice medium and prevents patulin accumulation *in vitro*. Lett Appl Microbiol 56(5):356-360.

Pantelides IS, Christou O, Tsolakidou M-D, Tsaltas D and Ioannou N. 2015. Isolation, identification and in vitro screening of grapevine yeasts for the control of black aspergilli on grapes. Biol Control 88:46-53.

Paterson RRM and Lima N. 2010. How will climate change affect mycotoxins in food? Food Res Int 43(7):1902-1914.

Paterson RRM and Lima N. 2011. Further mycotoxin effects from climate change. Food Res Int 44(9):2555-2566.

Paterson RRM, Venâncio A and Lima N. 2007. Why do food and drink smell like earth? Méndez-Vilas A, ed. Communicating Current Research and Educational Topics and Trends in Applied Microbiology (Formatex) pp. 120-128.

Paterson RRM, Venâncio A, Lima N, Guilloux-Bénatier M and Rousseaux S. 2018. Predominant mycotoxins, mycotoxigenic fungi and climate change related to wine. Food Res Int 103:478-491.

Patharajan S, Reddy KRN, Karthikeyan V, Spadaro D, Lore A, Gullino ML and Garibaldi A. 2011. Potential of yeast antagonists on *in vitro* biodegradation of ochratoxin A. Food Control 22(2):290-296.

Petruzzi L, Corbo MR, Baiano A, Beneduce L, Sinigaglia M and Bevilacqua A. 2015. *In vivo* stability of the complex ochratoxin A - *Saccharomyces cerevisiae* starter strains. Food Control 50:516-520.

Piotrowska M, Nowak A and Czyzowska A. 2013. Removal of ochratoxin A by wine *Saccharomyces cerevisiae* strains. Eur Food Res Technol 236:441-447.

Pitt JI, Taniwaki MH and Cole MB. 2013. Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of Food Safety Objectives. Food Control 32(1):205-215.

Ponsone ML, Chiotta ML, Combina M, Dalcero A and Chulze S. 2011. Biocontrol as a strategy to reduce the impact of ochratoxin A and *Aspergillus* section *Nigri* in grapes. Int J Food Microbiol 151:70-77.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

1012 Ponsone ML, Nally MC, Chiotta ML, Combina M, Koehl J and Chulze SN. 2016. Evaluation of
1013 the effectiveness of potential biocontrol yeasts against black sur rot and ochratoxin A
1014 occurring under greenhouse and field grape production conditions. Biol Control 103:78-85.

1015 Prendes LP, Merin MG, Andreoni MA, Ramirez ML and Ambrosini VIM. 2015. Mycobiota and
1016 toxicogenic *Alternaria* spp. strains in Malbec wine grapes from DOC San Rafael, Mendoza,
1017 Argentina. Food Control 57:122-128.

1018 Prendes LP, Merín MG, Fontana AR, Bottini RA, Ramirez ML and Ambrosini VIM. 2018.
1019 Isolation, identification and selection of antagonistic yeast against *Alternaria alternata*
1020 infection and tenuazonic acid production in wine grapes from Argentina. Int J Food Microbiol
1021 266:14-20.

1022 Quintela S, Villaran MC, Armentia ILd and Elejalde E. 2013. Ochratoxin A removal in wine: A
1023 review. Food Control 30(2):439-445.

1024 Raut I, Calin M, Lazaroaie MM, Roseanu A, Badea-Doni M, Oancea F and Jecu L. 2014.
1025 Inhibition of toxigenic *Aspergillus niger* by microbial metabolites. Rev Chim 65(7):779-783.

1026 Rousseaux S, Diguta CF, Radoi-Matei F, Alexandre H and Guilloux-Benatier M. 2014. Non-
1027 *Botrytis* grape -rotting fungi responsible for earthy and moldy off-flavors and mycotoxins.
1028 Food Microbiol 38:104-121.

1029 Sarrocco S and Vannacci G. 2018. Preharvest application of beneficial fungi as a strategy to
1030 prevent postharvest mycotoxin contamination: A review. Crop Prot 110:160-170.

1031 Scott PM, Fuleki T and Harwig J. 1977. Patulin content of juice and wine produced from moldy
1032 grapes. J Agric Food Chem 25(2):434-434.

1033 Sen L, Ocak I, Nas S and Sevik R. 2016. Effects of different drying treatments on fungal
1034 population and ochratoxin A occurrence in sultana type grapes. Food Addit Contam Part A
1035 Chem Anal Control Expo Risk Assess 33(9):1444-1455.

1036 Senthil R, Prabakar K, Rajendran L and Karthikeyan G. 2011. Efficacy of different biological
1037 control agents against major postharvest pathogens of grapes under room temperature storage
1038 conditions. Phytopathol Mediterr 50(1):55-65.

1039 Serra R, Abrunhosa L, Kozakiewicz Z and Venâncio A. 2003. Black *Aspergillus* species as
1040 ochratoxin A producers in Portuguese wine grapes. Int J Food Microbiol 88(1):63-68.

1041 Serra R, Mendonça C and Venâncio A. 2006. Ochratoxin A occurrence and formation in
1042 Portuguese wine grapes at various stages of maturation. Int J Food Microbiol 111:S35-S39.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

1043 Serratosa MP, Lopez-Toledano A, Millan C, Medina M and Merida J. 2010. Changes of
1044 ochratoxin A in grapes inoculated with *Aspergillus carbonarius* and subjected to chamber-
1045 drying under controlled conditions. J Agric Food Chem 58(22):11907-11912.

1046 Shemesh R, Krepker M, Nitzan N, Vaxman A and Segal E. 2016. Active packaging containing
1047 encapsulated carvacrol for control of postharvest decay. Postharvest Biol Technol 118:175-
1048 182.

1049 Shi L, Liang Z, Li J, Hao J, Xu Y, Huang K, Tian J, He X and Xu W. 2014. Ochratoxin A
1050 biocontrol and biodegradation by *Bacillus subtilis* CW 14. J Sci Food Agric 94(9):1879-1885.

1051 Shukla S, Kim DH, Chung SH and Kim M. 2017. Chapter 28 - Occurrence of Aflatoxins in
1052 Fermented Food Products. Frias J, Martinez-Villaluenga C and Peñas E, eds., Fermented
1053 Foods in Health and Disease Prevention (Academic Press: Boston, USA) pp. 653-674.

1054 Solfrizzo M, Avantaggiato G, Panzarini G and Visconti A. 2010. Removal of Ochratoxin A from
1055 Contaminated Red Wines by Repassage over Grape Pomaces. J Agric Food Chem 58(1):317-
1056 323.

1057 Somma S, Perrone G and Logrieco AF. 2012. Diversity of black Aspergilli and mycotoxin risks
1058 in grape, wine and dried vine fruits. Phytopathol Mediterr 51(1):131-147.

1059 Sonker N, Pandey AK and Singh P. 2015. Efficiency of *Artemisia nilagirica* (Clarke) Pamp.
1060 essential oil as a mycotoxicant against postharvest mycobiota of table grapes. J Sci Food
1061 Agric 95(9):1932-1939.

1062 Sonker N, Pandey AK, Singh P and Tripathi NN. 2014. Assessment of *Cymbopogon citratus*
1063 (DC.) stapf essential oil as herbal preservatives based on antifungal, antiaflatoxin, and
1064 antiochratoxin activities and *in vivo* efficacy during storage. J Food Sci 79(4):1750-3841.

1065 Storari M, Broggini GAL, Bigler L, Cordano E, Eccel E, De Filippi R, Gessler C and Pertot I.
1066 2012. Risk assessment of the occurrence of black aspergilli on grapes grown in an alpine
1067 region under a climate change scenario. Eur J Plant Pathol 134:631-645.

1068 Storari M, Pertot I, Gessler C and Broggini GAL. 2010. Amplification of polyketide synthase
1069 gene fragments in ochratoxigenic and nonochratoxigenic black aspergilli in grapevine.
1070 Phytopathol Mediterr 49(3):393-405.

1071 Tancinova D, Felsoeciova S, Rybarik L, Maskova Z and Cisarova M. 2015. Colonization of
1072 grapes berries and cider by potential producers of patulin. Potravinarstvo Scientific Journal
1073 for Food Industry 9(1):138-142.

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

1074 Tancinova D, Maskova Z, Rybarik L, Felsoeciova S and Cisarova M. 2016. Colonization of
1075 grapes berries by *Alternaria* sp. and their ability to produce mycotoxins. Potravinarstvo
1076 Scientific Journal for Food Industry 10(1):7-13.

1077 Techarat S, Dachoupakan C and Cheewanun D. 2012. Growth and Ochratoxin A production of
1078 black aspergilli isolated from Thai wine grapes. As J Food Ag Ind 5(3):172-182.

1079 Terra MF, Lira NdA, Passamani FRF, Batista LR, Santiago WD and Cardoso MdG. 2016. Effect
1080 of Fungicides on Growth and Ochratoxin A Production by *Aspergillus carbonarius* from
1081 Brazilian Wine Grapes. J Food Protect 79(9):1508-1516.

1082 Tjamos SE, Antoniou PP, Kazantzidou A, Antonopoulos DF, Papageorgiou I and Tjamos EC.
1083 2004. *Aspergillus niger* and *Aspergillus carbonarius* in Corinth Raisin and Wine-Producing
1084 Vineyards in Greece: Population Composition, Ochratoxin A Production and Chemical
1085 Control. J Phytopathol 152(4):250-255.

1086 Trinidad AV, Ganoza FQ, Pinto VF and Patriarca A. 2015. Determination of mycotoxin profiles
1087 characteristic of *Alternaria* strains isolated from Malbec grapes. BIO Web of Conferences 5.

1088 Valero A, Marín S, Ramos AJ and Sanchis V. 2005. Ochratoxin A-producing species in grapes
1089 and sun-dried grapes and their relation to ecophysiological factors. Lett Appl Microbiol
1090 41(2):196-201.

1091 Var I, Erginkaya Z and Kabak B. 2011. Inhibition of ochratoxin A production of *Aspergillus*
1092 *carbonarius* by yeast species. Czech J Food Sci 29(3):291-297.

1093 Vezzulli S, Battilani P and Bavaresco L. 2007. Stilbene-Synthethase Gene expression after
1094 *Aspergillus carbonarius* infection in grapes. Am J Enol Viticult 58(1):132-134.

1095 Wang CW, Ai J, Liu YX, Lv HY, Fan ST and Yang YM. 2015. *Fusarium avenaceum*: A New
1096 Pathogen Causing Amur Grape (*Vitis amurensis*) Fruit Rot in Jilin Province, China. Plant Dis
1097 99(6):889-889.

1098 Zhu C, Shi J, Jiang C and Liu Y. 2015. Inhibition of the growth and ochratoxin A production by
1099 *Aspergillus carbonarius* and *Aspergillus ochraceus in vitro* and *in vivo* through antagonistic
1100 yeasts. Food Control 50:125-132.

1101

American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2019.19039
AJEV Papers in Press are peer-reviewed, accepted articles that have not yet been published in a print issue of the journal or edited or formatted, but may be cited by DOI. The final version may contain substantive or nonsubstantive changes.

Table 1 European Union Regulation on the amount of mycotoxins in grape products (consolidated European Commission (2006)).

Grape product		**Maximum levels (µg/kg)**
Dried vine fruits to be subjected to sorting, or other physical treatment	Aflatoxin B1	5
	Total aflatoxins	10
Dried vine fruits and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	Aflatoxin B1	2
	Total aflatoxins	4
Dried vine fruit (currants, raisins and sultanas)	Ochratoxin A	10
Wine (including sparkling wine, excluding liqueur wine and wine with an alcoholic strength of not less than 15 % vol), fruit wine, and grape juice	Ochratoxin A	2
Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	Patulin	50

Table 2 Biocontrol microorganisms with inhibitory potential on mycotoxin-producing fungi.

Biocontrol microorganism	**Target fungal species**	**Type of assay**	**Reference**
Aureobasidium pullulans	*A. carbonarius*	Grapes and at field	(Dimakopoulou et al. 2008)
Aureobasidium pullulans, *Candida sake* and *Cryptococcus magnus*	*A. tubingensis*	Grapes	(Pantelides et al. 2015)
Candida zemplinina M3, *Metschnikowia aff. fructicola* M179, *Pichia kluyveri* M117, *Saccharomyces cerevisiae* C297 and *S. cerevisiae* M114	*A. carbonarius* and *A. ochraceus*	*In vitro*; grapes	(Zhu et al. 2015)
Hanseniaspora uvarum	*A. tubingensis*	Grapes	(Apaliya et al. 2017)
Hanseniaspora uvarum, *Metschnikowia* (*pulcherrima* or spp.) and *Starmerella bacillaris*	*A. alternata*	Grapes	(Prendes et al. 2018)
Kloeckera apiculata and *Saccharomyces cerevisiae*	*A. carbonarius* and *A. ochraceus*	*In vitro*; grapes	(Cubaiu et al. 2012)
Kluyveromyces thermotolerans (*Lanchancea thermotolerans*)	*A. niger* aggregate and *A. carbonarius*	*In vitro*	(Ponsone et al. 2011)
	A. section *Nigri*	Greenhouse and field	(Ponsone et al. 2016)
Penicillium adametzoides	*A. carbonarius*	*In vitro*	(Ahmed et al. 2015)

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Table 3 Natural compounds with inhibitory potential on mycotoxin-producing fungi

Natural compound	Target fungal species	Type of assay	Reference(s)
Essential oils			
Ageratum conyzoides, *Cymbopogon citratus*, *Ocimum canum, Piper methysticum* and *Putranjeeva roxburghii*	*A. flavus*, *A. niger* and *A. ochraceus*	*In vitro*	(Sonker et al. 2014)
Artemisia nilagirica	*A. flavus*, *A. niger* and *A. ochraceus*	*In vitro* and on grapes	(Sonker et al. 2015)
Blumea membranacea, *Hyptis suaveolens* and *Ocimum gratissimum*	*A. flavus*, *A. niger* and *A. ochraceus*	*In vitro*	(Sonker et al. 2015)
Cinnanomum cassia, *Citrus reticulata*, *Cymbopogon citratus* and *Eygenia cariophyllus*	*A. carbonarius*	*In vitro*	(Lappa et al. 2017)
Cinnamomum verum, *Eruca sativ*, *Origanum vulgare* and *Thymus vulgaris*	*A. carbonnarius*	*In vitro* (synthetic grape medium)	(El Khour et al. 2016)
Cymbopogon citratus	*A. flavus*, *A. niger* and *A. ochraceus*	*In vitro* and on grapes	(Sonker et al. 2014)
Lavandula angustifolia MILLER., *Origanum vulgare* L., *Salvia officinalis* L. and *Thymus vulgaris* L.	*A. niger* and *A. tubingensis*	*In vitro*	(Cisarova et al. 2016)
Ocimum basilicum	*A. flavus*	*In vitro* and on grapes	(Kumar et al. 2011)
Others			
Carvacrol encapsulated with Halloysite nanotubes	*A. alternata*, *B. cinerea*, *P. digitatum*, *P. expansum* and *A. niger*	*In vitro* and on grapes	(Shemesh et al. 2016)
Chitosan	*F. oxysporum*	*In vitro* and on grapes	(Irkin and Guldas 2014)
Chitosan	*P. chrysogenum* and *A. parasiticus*	*In vitro*	(Irkin and Guldas 2014)
Chitosan and *Origanum vulgare* L. essential oil	*R. stolonifer* and *A. niger*	*In vitro* and on grapes	(dos Santos et al. 2012)
Garlic juice	*P. citrinum*, *P. expansum*, *P. puberulum* and *P. verrucosum*	*In vitro*	(El-Samawaty et al. 2013)
Isothiocyanates	*P. expansum* and *A. parasiticus*	*In vitro*	(Manyes et al. 2015)
Lecanicillium muscar enzymes	*A. carbonarius*	Grapes	(Barghini et al. 2013)
Metabolites of *Bacillus amyloliquefaciens*	*A. niger*	*In vitro*	(Raut et al. 2014)

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Natural compound	Target fungal species	Type of assay	Reference(s)
Natamycin	*A. carbonarius*	*In vitro* (synthetic grape-juice medium)	(Kogkaki et al. 2016)
Pine-resin	*A. carbonarius*	*In vitro* (synthetic grape-juice medium)	(Kogkaki et al. 2016)

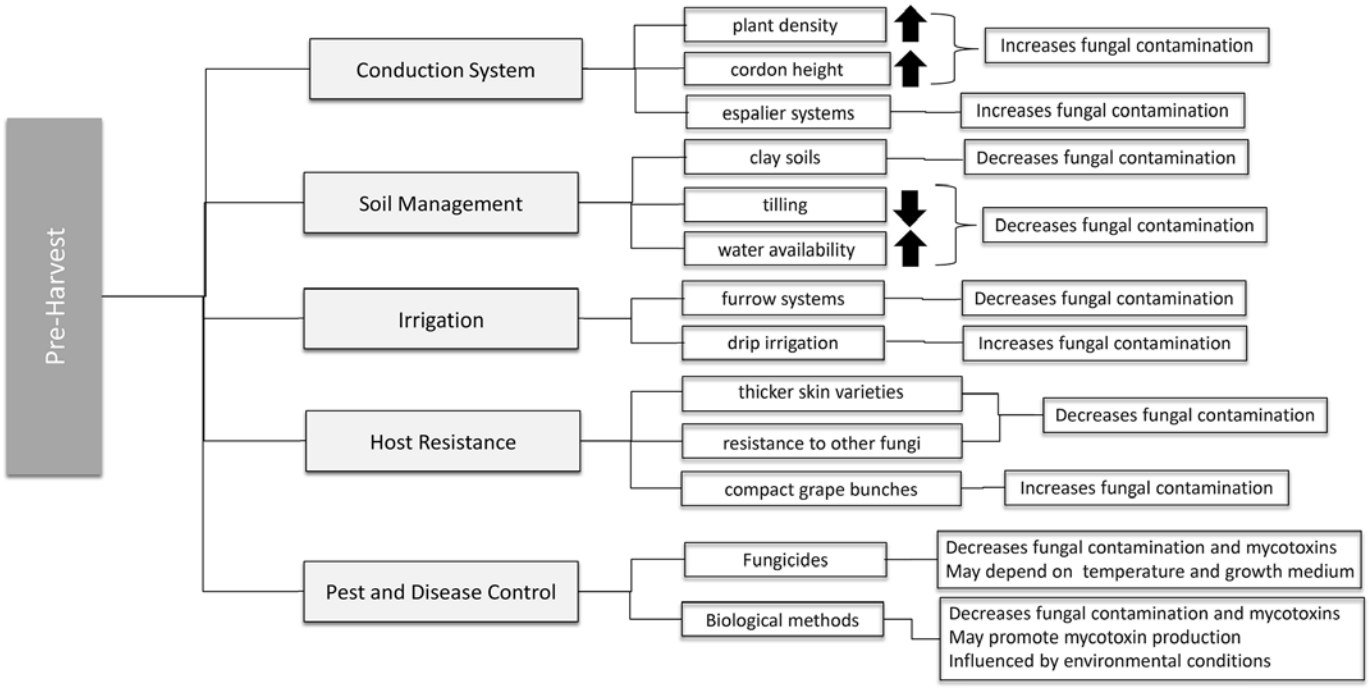

Figure 1 Parameters affecting mycotoxigenic fungi and their mycotoxins at the pre-harvest stage in the grape chain.

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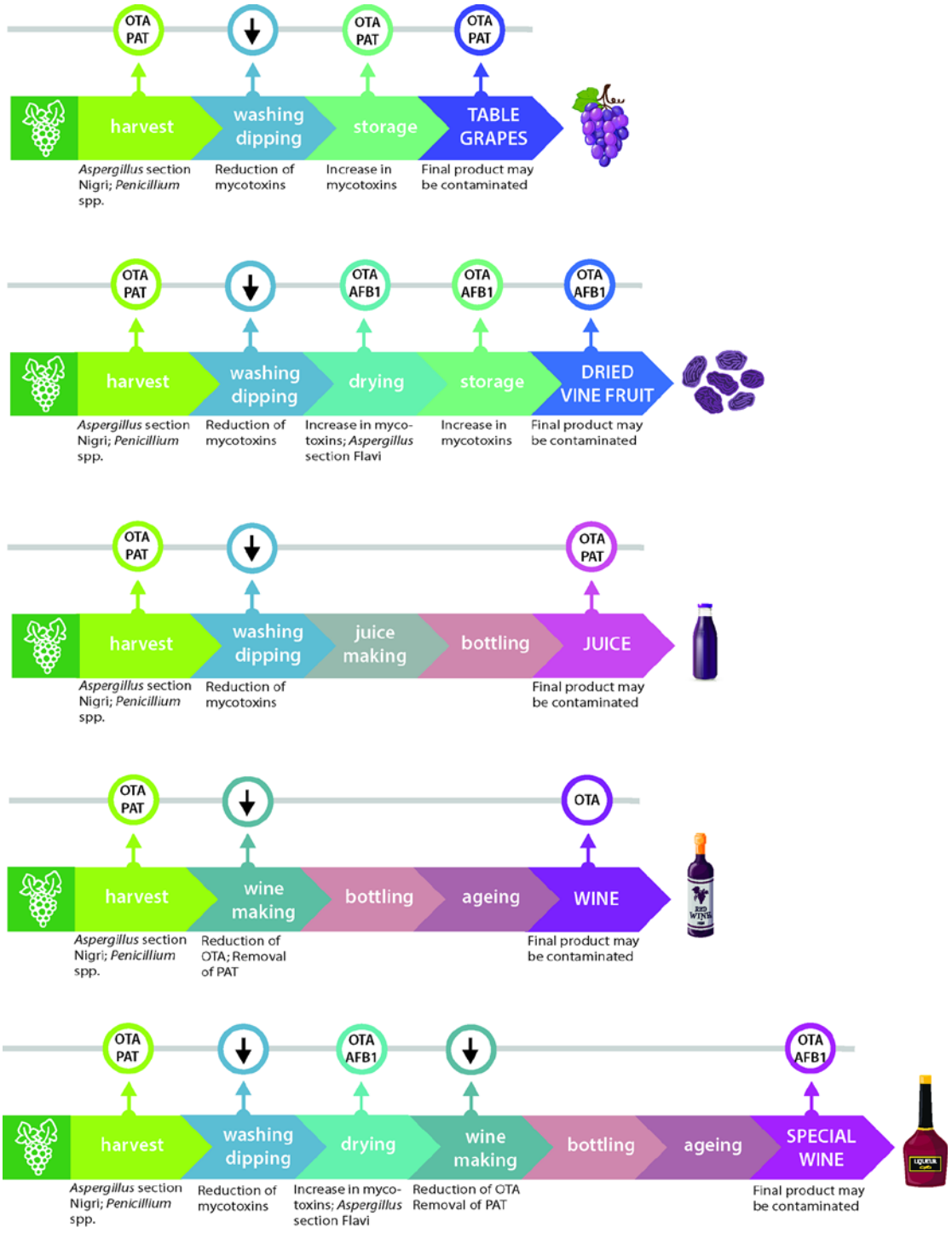

Figure 2 Simplified flowcharts for the main grape products: table grapes; dried vine fruits; grape juice; wine; and special wines (usually with ethanol content above 15%).

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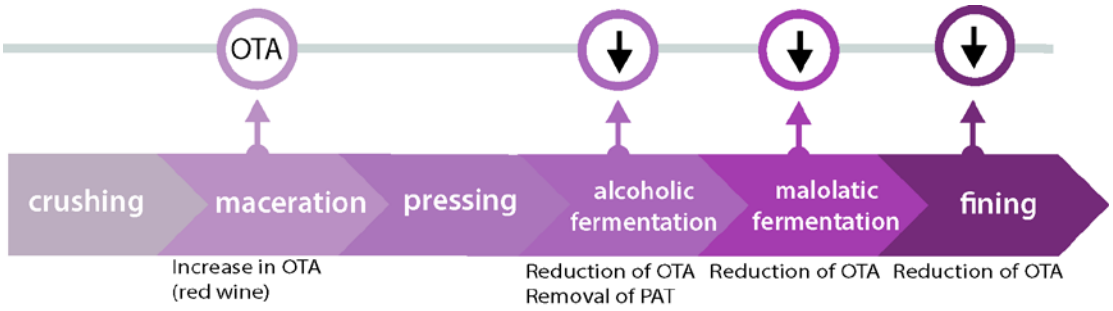

Figure 3 Simplified flowcharts for the main wine making steps.