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Fungi and mycotoxins in vineyards and grape products

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Abstract

Many fungi may occur on grapes during growth in the vineyard, but the main concern from the viewpoint of mycotoxin contamination is the black Aspergilli, *Aspergillus carbonarius* and *A. niger.* These fungi are capable of producing ochratoxin A (OA) which may contaminate grapes and grape products such as wine, grape juice and dried vine fruit. Understanding the ecology and physiology of the black Aspergilli can provide tools for management of OA at all stages of grape production and processing. In the vineyard, careful management of cultivation, irrigation and pruning can assist in minimising the levels of black Aspergilli in the soil, which in turn, can minimise contamination of grapes by these fungi. Minimising damage to grapes on the vine by the use of open vine canopies, grape varieties with resistance to rain damage and by the management of insect pests and fungal diseases (*e.g.*, mildew, Botrytis bunch rot) can reduce the incidence of *Aspergillus* rot in mature berries. The risk of OA in table grapes can be minimised by careful visual inspection to avoid damaged and discoloured berries. In wine, harvesting grapes with minimal damage, rapid processing and good sanitation practices in the winery assist in minimising OA. During vinification, pressing of grapes, and clarification steps which remove grape solids, grape proteins and spent yeast can also remove a significant proportion of OA. For dried vine fruit production, avoiding berry damage, rapid drying, and final cleaning and sorting to remove dark berries can reduce overall OA levels in finished products.

Keywords: Ochratoxin; Grapes; Wine; Reduction; Vineyard management

1. Introduction

Several fungi are pathogenic to grapevines, infecting the roots, trunk, canes, leaves and berries (Pearson and Goheen, 1988). Fungi which commonly infect berries include the mildew pathogens *Erysiphe necator* (*Uncinula necator*) and *Plasmopara viticola*, as well as *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea*, *Cladosporium* spp ., *Penicillium* spp. and *Rhizopus* spp. (Hewitt, 1988; Emmett et al., 1992). Of these, strains of *Penicillium expansum* isolated from grapes may produce patulin (Abrunhosa et al., 2001). The aflatoxigenic species, *A. flavus* and *A. parasiticus*, have also occasionally been isolated from grapes (Da Rocha Rosa et al., 2002; Magnoli et al., 2003; Saez et al., 2004). *Trichothecium roseum* growing on grapes affected by 'grey rot' (*B. cinerea*) is the likely source of mycotoxins such as trichothecin (Serra et al., 2005). However, mycotoxins such as these are seldom detected in wine and other grape products and are currently of little concern for the grape and wine industries. The main mycotoxin of concern in grape products is ochratoxin A (OA). OA is produced primarily when *Aspergillus carbonarius* infects berries before harvest. The relatively few toxigenic strains of the related species, *A. niger*, may also contribute to OA contamination, as *A. niger* is by far the most common species of *Aspergillus* present on grapes (Chulze et al., 2006; Leong et al., 2006a; Varga and Kozakiewicz, 2006; Leong, 2007). Toxigenic isolates of *A. ochraceus* have only occasionally been isolated from grapes.

This paper reviews recent findings about strategies to minimise the potential for OA contamination of grapes in vineyards, and also deals with ways to reduce or remove OA from contaminated grape products during processing.

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2. Factors affecting *Aspergillus* in the vineyard environment

In a survey of six vineyards in Australia, soil and vine remnants on soil were the primary sources of *A. carbonarius* and *A. niger* (Leong et al., 2006a; Leong et al., 2006b). These fungi were also isolated occasionally from fallen dried berries, dead canes, vine bark, dried bunch stems and dead cover crop trash, but were seldom isolated from leaves (green and/or senescent), tendrils and green cover crop plants. In contrast, in Argentina. weeds are thought to represent an important inoculum source for *A. niger* in vineyards (Chulze et al., 2006).

In Australian studies, increased concentrations of *A. carbonarius* propagules occurred in surface soil (0–1 cm) compared with soil deeper within the profile, and in soil directly beneath vines compared with the inter-row area (Leong et al., 2006a,b). This association is likely to be due to damaged and dead berries falling onto the soil and providing a sugar-rich medium for the growth of indigenous saprophytic *Aspergillus* species. Thus, reducing berry drop and discarding rotten bunches away from vines may reduce the incidence of *A. carbonarius* in vineyard soil.

Moderate temperatures (*ca* 25 °C), regular cultivation, and drying of soils increased the incidence of *A. carbonarius* in soils (Leong et al., 2006a; Kazi et al., 2006). Of these, it is possible to manage cultivation and irrigation to minimise *A. carbonarius*, *viz*. by adopting minimal tillage practices and maintaining fairly constant soil moisture. The latter effect was particularly marked in clay loam soils compared with sandy soils. Addition of certain mulches, such as hay or sawdust, to moist soil, further reduces the population of *A. carbonarius*. In the presence of moisture, mulches are thought to release compounds that stimulate the growth of saprophytic competitors, and that may be fungistatic or fungicidal to *A. carbonarius*.

OA has been detected in agricultural soils in Denmark (Mortensen et al., 2003) and may be translocated through coffee plants (Mantle, 2000). However, due to the miniscule concentrations of OA that may occasionally be present in soil, it is unlikely that such translocation contributes to OA contamination of grapes. The significance of ochratoxigenic fungi in soils lies in the movement of spores by air from the soil onto berry surfaces. Indeed, the incidence of *A. carbonarius* spores in air samples increased closer to the soil, and a severe dust storm likewise, resulted in increased *A. carbonarius* on bunches (Leong et al., 2006a).

3. Factors affecting *Aspergillus* and ochratoxin A production in grape berries

In Europe, increased incidence of black *Aspergillus* spp. on grapes in particular regions or during certain seasons was generally associated with hot, dry weather in southern latitudes (Battilani et al., 2006a; Varga and Kozakiewicz, 2006; Leong, 2007), although hot weather coupled with increased humidity and rainfall was noted to increase *Aspergillus* incidence and OA contamination in certain studies (Battilani et al., 2003; Roset, 2003; Bellí et al., 2005). Such findings may partly explain the increased incidence of OA in wines from the warmer regions of southern Europe (Varga and Kozakiewicz, 2006); however, it should be noted that this trend was not observed in wines from warm climate regions in Australia (Hocking et al., 2003). In Australia, no clear effects of climate of viticultural regions on the incidence of black *Aspergillus* spp. have been reported (Leong et al., 2007), although incidence appeared to decrease in cool climate regions such as Tasmania.

On grapes, black *Aspergillus* spp. typically increase from berry set until harvest (Leong, 2007), perhaps because the surface of immature green berries and exposure to UV light represent a hostile environment for *A. carbonarius* spores (Leong et al., 2006d). At veraison, the berry skin softens and sugar content increases; from this stage until harvest, berries are most susceptible to infection by *A. carbonarius* and also are capable of supporting OA production, particularly when damaged (Battilani et al., 2006d; Leong et al., 2006d, 2007; Leong, 2007). Delayed harvest of mature berries also increases the risk of OA contamination (Roset, 2003; Gambuti et al., 2005).

Temperature and water activity are likely to affect the rate of growth and OA production by black *Aspergillus* species in berries. These effects have been studied by growing strains on a synthetic grape juice medium (SGM) designed to simulate the berry composition at early veraison. On this medium, the optimum temperature for growth of *A. carbonarius* (30 °C) was lower than that for *A. niger* (approximately 35 °C) (Battilani et al., 2006d; Leong, 2007). For both species, the optimum water activity for growth was approximately 0.97–0.99, however the optimum for *A. niger* was closer to the upper limit of this range and this species was also more tolerant of water activities below 0.95 (Leong et al., 2006c). Reports of optimum water activity for toxin production differed among authors quoted above but were within the range 0.95–0.995, which also supported good growth. In contrast, the optimum temperature for toxin production for both species (15–20 °C) was consistently lower than the optimum for growth.

The relative importance of temperature and water activity on fungal growth rate and OA production in berries is difficult to assess. As the sugar content of berries increases from veraison until harvest, the water activity decreases and comes within the optimal range for supporting OA production. Regarding temperature, the data obtained above, with incubation at constant temperatures, suggested that OA production by *A. carbonarius* would be favoured at 20 °C. Yet, subjecting isolates to diurnal temperature fluctuations (28 °C/20 °C) did not increase OA yield on SGM over that obtained at 28 °C. However, alternating photoperiods increased fungal growth rate (Bellí et al., 2006). Some trends established on SGM were also observed in berries inoculated with *A. carbonarius* and incubated at constant temperatures – OA production was greatest at temperatures below 25 °C (Battilani et al., 2004); however, growth was also enhanced at 20 °C rather than 25 °C, a finding contrary to the reports of maximum growth on SGM at approximately 30 °C discussed above.

Black *Aspergillus* spp. can degrade OA after it has been produced in culture media by cleavage of the molecule into the isocoumarin portion, ochratoxin α, and phenylalanine and thence to other undetermined products (Valero et al., 2006; Leong, 2007). It is thought that degradation of OA could be beneficial to the fungus as a source of organic nitrogen to support further growth, but it is not known if this process occurs in naturally infected grapes.