Ecology of mycotoxin producing fungi present in wine grapes

Rita Serra, Luís Abrunhosa and Armando Venâncio*

Centro de Engenharia Biológica, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

*Correspondence to: avenan@deb.uminho.pt

Summary

In order to establish the potencial mycotoxin producer species hazardous for wine quality, surveys of filamentous fungi were made in Portuguese wine grapes. These studies were conducted from 1999 to 2002, and are still ongoing. Among the more relevant fungi detected in grapes are black aspergilli, namely *A. niger* aggregate and *A. carbonarius*, found to be responsible for the production of ochratoxin A in grapes. The results of these studies are discussed in an ecological point of view, and in terms of risk assessment for mycotoxins presence in the final product, wine.

Keywords: Ochratoxin A; Wine; Trichothecenes; Grapes; Mycotoxins

Introduction

Grapes are the start point for the winemaking process. A good wine starts its production at the vineyard, where aroma, colour and flavour are defined. However, as all natural products, grapes are subject to contamination with harmful substances. In the vineyard, grapes are constantly exposed to fungi, and given the appropriate conditions, infection of the vine or grapes can occur, and the production of mycotoxins may take place. The role of mycotoxins in agroecossystems is still greatly unknown, although several theories have been proposed for their functions (Bhatnagar *et al.*, 1992). It is known that in environments where several species compete among it self for the substrate, mycotoxins could be determinant to establish the mycoflora, and present an advantage for the fungus that produce it. Also, some mycotoxins have insecticide activity, such as ochratoxin A (Wicklow

et al., 1996), and it is believed that are a defence mechanism against predation by insects.

Over 300 mycotoxins (Betina, 1989) are known, but relatively few are considered important to human health (Pitt, 2000). In wine, it has been some concern about the presence of ochratoxin A, a mycotoxin with nephrotoxic and immunotoxic effects. In a recent report assessing the dietary OTA intake by European populations, wine was considered the second major source of intake, following cereals, contributing in 10 % (Miraglia and Brera, 2002). Less frequently, other mycotoxins have been reported in this commodity, such as trichothecenes (Schwenk *et al.*, 1989).

In surveys of Portuguese wine grapes that took place from 1999 to the current year, the main filamentous fungi present were evaluated through plating methods. Grapes were analysed along the maturation process. The most relevant mycotoxin producer species found are treated here from an ecological point of view, together with its relevance in terms of mycotoxin presence in wine and the ecology of the species.

Materials and Methods

In 1999, the filamentous fungi were detected in grapes according to Abrunhosa *et al.* (2001). In 2001 and 2002, sampling, detection and isolation of fungi, and the OTA determination are described in Serra *et al.* (in press).

Results and Discussion

Fungi in grapes

Table 1 shows the more frequent filamentous fungi detected in Portuguese grapes detected between June 2001 and September 2002.

Thirty eight genera were detected, being the more frequent ones *Alternaria*, *Cladosporium* and *Botrytis*. These genera are ubiquitous, present in air and soil, and they are common colonisers of the angiosperm phylloplane (Dix and Webster, 1995).

Table 1. Percentage of berries where filamentous fungi were detected (total of analysed berries: 3150)

Fungi	Colonised berries (%)
Alternaria spp.	53
Cladosporium spp.	51
Botrytis cinerea	35
Aspergillus niger aggregate	13
Epicoccum nigrum	10
Aureobasidium pullulans	8
Penicillium brevicompactum	5
Penicillium thomii	4
Rhizopus spp.	4
Stemphylium spp.	4
Ulocladium spp.	3
Penicillium spinulosum	2
Trichoderma spp.	2
Trichothecium roseum	2
Aspergillus carbonarius	1
Aspergillus flavus	1
Drechslera spp.	1
Fusarium spp.	1
Penicillium citrinum	1
Penicillium simplicissimum	1
Others	13

Mycotoxigenic saprophyte fungi were detected in grapes, being the more relevant ones, due to the importance of the mycotoxins produced to man, the genus *Aspergillus*, *Fusarium*, and *Penicillium*. *Trichothecium roseum*, was also detected, being its presence discussed due to its association with *Botrytis*.

Aspergillus

The main mycotoxin species detected were black *Aspergillus* (namely *A. carbonarius* and *A. niger* aggregate), and *A. flavus*. Recently it was found that ochratoxin A is produced in grapes by fungi from the black *Aspergillus* group, mainly by *A. carbonarius* species (Cabañes *et al.*, 2002; Sage *et al.*, 2002; Battilani *et al.*, 2003). However, some

ocratoxigenic strains in the *A. niger* aggregate were also detected (Serra *et al.*, 2003). From the 48 strains of *A. carbonarius* isolated in our laboratory, only one did not produce the mycotoxin, and its molecular profile suggests differences from the other *A. carbonarius* strains. On the other hand, from the 425 *A. niger* aggregate isolated strains, only 4 % were OTA producers.

Fungi from the *A. niger* aggregate are the fourth more frequent fungi isolated in grapes. At harvest time they are dominant in some locations, as showed in Figure 1.

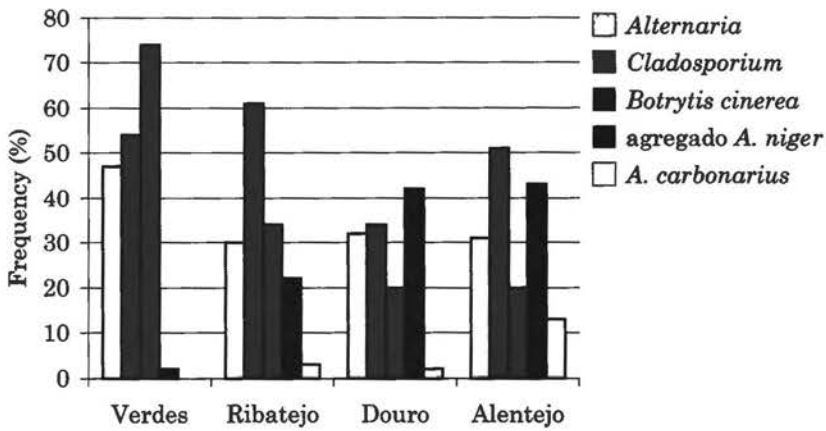

Figure 1. Presence of the 4 more frequent genera found at harvest time in 4 different winemaking Portuguese regions (Study made between June 2001 and September 2002).

In the Portuguese vineyards from Douro and Alentejo regions, the climatic conditions are hot and dry during summer time, and temperatures of 40 °C are frequently achieved. Black aspergilli possess high melanin content in their spores, which protect them from sunlight and UV radiation, and are xerophile. Therefore, these fungi are adapted to the environment of Mediterranean vineyards, with high temperatures and sun exposition and low humidity levels. The contamination of wines with ochratoxin A appears to be related with the region of its origin, being wines from Southern Europe and North Africa more contaminated. This is apparently due to the incidence of

A. carbonarius in wine grapes, which is higher in the Mediterranean basin area.

It is known that *A. flavus* competes with *A. niger*. We have found that *A. niger* is more frequent in wine grapes than *A. flavus* in all the studied locations. Therefore, we consider that aflatoxins might not present a problem for wine grapes, at least in the studied Portuguese conditions.

Fusarium

Fusarium species produce several mycotoxins. They are plant pathogens, identified as causal agents of mycotoxins in several food products. They were also detected in wine grapes, but infrequently, when compared to other mycotoxin producers such as *Aspergillus*. These strains were not identified to the species level, so it is not known if they are mycotoxigenic species.

Penicillium

The *Penicillium* species more frequently found were *P. brevicompactum*, *P. thomii* and *P. spinulosum*. Only *P. brevicompactum* produces mycotoxins, but their presence in foods is not considered relevant. *P. thomii* and *P. spinulosum* are not known mycotoxin producers. In 1999, however, *P. expansum* was the major *Penicillium* species isolated from grapes (Abrunhosa *et al.*, 2001). Since then, in the following years, its presence has been detected, but not very frequently. *Penicillium expansum* produces patulin, a carcinogenic mycotoxin. This mycotoxin is destroyed during the fermentation process; therefore it has not been detected in wines. However, it may be present in grapes and grape juice. Nevertheless, *Penicillium* does not look to be responsible for relevant mycotoxin production in grapes in our climatic conditions.

Trichothecium roseum

Trichothecium roseum is a know producer of trichothecene mycotoxins, such as trichothecin and trichothecolone, and it is a mycoparasite of *Botrytis* (Domsh and Gams, 1980). In cases where *Botrytis* rot attacked grapes, it was found growing over *Botrytis* (Figure 2).

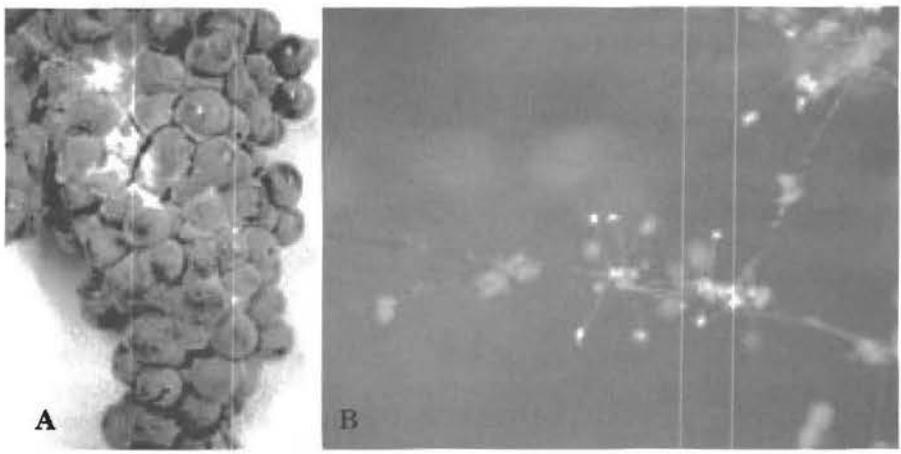

Figure 2. A) Rotten grape bunch with grey rot (*Botrytis*) with overgrowth of *Trichothecium roseum*; B) Detail of *T. roseum* conidiophores growing over *Botrytis* hypha (stereomicroscope image).

Occasionally, trichothecene mycotoxins were detected in wine (Schwenk *et al.*, 1989), and *T. roseum* was pointed out as the responsible for their production. *T. roseum* grows in grapes in conditions that favour *Botrytis* rot. So, according to our observations, the possible presence of thrichotecenes in wine depends on the sanitary conditions of grapes used for vinification.

Conclusions

Fungi are not just relevant in viticulture because they can produce rot, but also because they can produce mycotoxins. It is the ecology of fungi present in wine grapes that determines the potential mycotoxin producers. Climatic conditions, together with ecological relationships, such as competition or parasitism among species, are important to establish the risk of contamination in wines. Understanding the ecology of the vine brings us closer to control eventual problems.

Ochratoxin A is the mycotoxin most relevant in wines. However, little information on trichothecenes in wines is available, and its presence in this commodity is considered to be occasional.

The problematic of mycotoxins also highlights the need of good practices in winemaking, and making wine from grapes in good sanitary conditions. The vinification of rot grapes, is not just bad for sensorial wine parameters, but also has implications in terms of wine safety.

Safety

Ochratoxin A is a toxic compound that needs to be manipulated with care and with the appropriate safety precautions. Decontamination procedures for laboratory wastes have been reported by the International Agency on Research on Cancer (IARC) and were employed throughout this experimental work.

Acknowledgements

R. Serra and L. Abrunhosa were respectively supported by SFRH/BD/1436/2000 and SFRH/BD/11228/2002 grants from Fundação para a Ciência e Tecnologia.

References

Abrunhosa, L., Paterson, R. R. M., Kozakiewicz, Z., Lima, N. and Venâncio, A. (2001) Mycotoxin production from fungi isolated from grapes. *Letters in Applied Microbiology*, **32**, 240-242.

Battilani, P., Pietri, A., Bertuzzi, T., Languasco, L., Giorni, P. and Kozakiewicz, Z. (2003) Occurrence of ochratoxin A–Producing fungi in grapes grown in Italy. *Journal of Food Protection* **66**, 633-636.

Betina, V. (1989) Mycotoxins: Chemical, Biological and Environmental Aspects. Amsterdam: Elsevier.

Bhatnagar, D., Lillehoj, E. B. and Arora, D. K. (Eds) (1992) *Handbook of Applied Mycology* Vol. 5: Mycotoxins in ecological systems. New York: Marcel Dekker.

Cabañes, F. J., Accensi, F., Bragulat, M. R., Abarca, M. L., Castella, G., Minguez, S., and Pons, A. (2002) What is the source of ochratoxin A in wine? *International Journal of Food Microbiology*. **79**, 213-215.

Dix, N. J. and Webster, J. (1995) *Fungal Ecology* New York: Chapman & Hall.

Domsch, K. H., Gams, W. and Anderson, T. H. (1993) *Compendium of Soil Fungi* Volumes 1, 2. 2nd Edition. London: Academic Press.

Miraglia, M. and Brera, C. (2002) *Assessment of dietary intake of ochratoxin A by the population of EU member states.* Report on Tasks for Scientific Cooperation, Directorate-General Health and Consumer Protection.

Pitt, J. I. (2000) Toxigenic fungi: which are important? *Medical Mycology* **38**, 17-22.

Sage, L., Krivobok, S., Delbos, E., Seigle-Murandi, F. and Creppy, E. E. (2002) Fungal flora and ochratoxin A production in grapes and musts from France. *Journal of Agricultural and Food Chemistry* **50**, 1306-1311.

Serra, R., Abrunhosa, L., Kozakiewicz, Z. and Venâncio, A. (2003) Black *Aspergillus* species as ochratoxin A producers in Portuguese wine grapes. *International Journal of Food Microbiology* (in press).

Wicklow, D. T., Dowd, P. F., Alfatafta, A. A., and Gloer, J. B. (1996) Ochratoxin A: an antiinsectan metabolite from the sclerotia of *Aspergillus carbonarius* NRRL 369. *Canadian Journal of Microbiology* **42**, 1100-1103.