SHORT COMMUNICATION

A NEW SEMI-SELECTIVE MEDIUM FOR THE OCHRATOXIGENIC FUNGUS *ASPERGILLUS CARBONARIUS*

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SUMMARY

Aspergillus carbonarius (Bainier) Thom. and *Aspergillus niger* Van Tieghem are common fungal contaminants of several commodities, including grapes. *A. carbonarius* is the most important, if not the exclusive, responsible for wine contamination by ochratoxin A (OTA) in Mediterranean areas. Identification of *A. carbonarius* is made difficult by its high similarity with *A. niger*, the *Aspergillus* species most common on grapes, as well as with other species of the section *Nigri* of this genus. Hence, it requires deep knowledge of fungal taxonomy. A semi-selective medium based on Malt Extract Agar amended with appropriate antibiotics (chloramphenicol and chlortetracycline) and fungicides (dichloran and boscalid) was developed in order to speed up the quantitative detection of *A. carbonarius* in grapes and musts and improve risk assessment of OTA contamination in wine.

Key words: Ochratoxin A, toxigenic fungi, wine, grapevine.

Ochratoxin A (OTA) is a mycotoxin frequently found as contaminant in a great variety of agricultural commodities all over the world (Pittet, 1998; Logrieco *et al.*, 2002), including grapes, must and wine (Majerus and Otteneder, 1996; Zimmerli and Dick, 1996; Ospital *et al.*, 1998; Burdaspal and Legarda, 1999; MacDonald *et al.*, 1999; Visconti *et al.*, 1999; Otteneder and Majerus, 2000; Pietri *et al.*, 2001; Battilani and Pietri, 2002; Battilani *et al.*, 2002; Cabañes *et al.*, 2002; Sage *et al.*, 2002, 2004; Pollastro *et al.*, 2003, 2005; Bellí *et al.*, 2004; Serra *et al.*, 2004; Vercesi *et al.*, 2004; Bau *et al.*, 2005).

Originally isolated as secondary metabolite of *Aspergillus ochraceus* Wilhem, OTA is produced by several species of *Aspergillus* and *Penicillium* (IARC, 1993; Pitt and Hocking, 1997; Samson *et al.*, 2004; Medina *et al.*, 2005).

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OTA has nephrotoxic, hepatotoxic, teratogenic and immunotoxic effects on several animal species, and is responsible of kidney and liver tumours in mice and rat (IARC, 1993; WHO, 1996). It has been included in the group 2B, among substances with potential carcinogenic activity for humans (IARC, 1993).

Recently, maximum tolerable limits of OTA have been established for wine and other grape derivatives, in addition to numerous already regulated foods and feeds [Reg. (CE) N. 123/2005 of 26.1.2005 modifying the Reg. (CE) n. 466/2001].

Several species of *Penicillium* and *Aspergillus* are involved in secondary fungal rots of grape bunches in vineyards. Studies carried out in South Italy showed that *Aspergillus carbonarius* (Bainier) Thom is the main, if not exclusive, responsible for OTA contamination in wine (Battilani *et al.*, 2002; Pollastro *et al.*, 2003).

Detection and quantification of *A. carbonarius* present on grape bunches in the vineyard may be helpful in assessing the risk of OTA contamination in wine. But symptoms caused by *A. carbonarius* are not distinguishable by naked eyes from those caused by *Aspergillus niger* van Tieghem, the most common *Aspergillus* species in the field, as well as those caused by other species of the section *Nigri* of the genus.

Detection and quantification of toxigenic fungi is traditionally done using the serial dilution plating technique and selective or semi-selective media (Pitt and Hocking, 1997). Frequently, the lack of suitable selective media, as it occurs for *A. carbonarius*, makes the method very laborious, time consuming and costly, because personnel skilled in fungal taxonomy must identify single colonies under the microscope.

Among the most used media in investigations on mycotoxigenic species of *Aspergillus* and *Penicillium* there are DYSG (Yeast extract Sucrose 18% Glycerol agar; Frisvad *et al.*, 1992), DG18 (Dichloran 18% Glycerol agar; Hocking and Pitt, 1980) and DRBC (Dichloran Rose Bengal Chloramphenicol agar; King *et al.*, 1979). In a preliminary work, however, these media showed a restricted germination of *A. carbonarius* conidia yielding an underestimated the number of colony forming units (CFU).

Recently, *in vitro* assays on the biological activity of

fungicides against *A. carbonarius* and *A. niger* showed some differential response of the two fungal species to few chemicals and, in particular, to the mitochondrial electron transport chain inhibitor boscalid (Cantus, BASF Agro), a new fungicide active against several pathogens, including *Botryotinia fuckeliana* (De Bary) Whetz. (Sauter *et al.*, 1999; Capriotti *et al.*, 2004), that is commercially available in many countries and will be shortly introduced in Italy. Germination of *A. niger* conidia was fully prevented on media amended with 1 µg ml^{-1} of active substance (a.s.) while germination of *A. carbonarius* conidia was not affected, even at concentration as high as 100 µg ml^{-1} a.s. (Pollastro *et al.*, 2005).

This paper deals with the development and validation of a new semi-selective medium for *A. carbonarius*.

Five isolates of each of the two species, *A. niger* and *A. carbonarius* obtained from rotting grapes were used. Conidia were scraped from the surface of 4 day-old colonies grown on Potato Dextrose Agar (PDA; per liter: infusion from 200 g peeled and sliced potatoes kept at 60°C for 1 h; glucose, 20 g; Oxoid agar N. 3, 20 g; adjusted at pH 6.5) and suspended in sterile water containing 0.05% Tween 20. Suspensions were titred with a haemocytometer. Aliquots (100 µl containing around 100 conidia) of each conidial suspension or their mixtures were plated on media in three-replicated Petri dishes (100 mm diam.) and maintained at 25±1°C in the darkness.

The following media were compared:

– DYSG (Yeast extract Sucrose Glycerol agar; Frisvad *et al.*, 1992): Difco yeast extract, 20 g l^{-1}; Oxoid agar N. 3, 20 g l^{-1}; sucrose, 150 g l^{-1}; K_2HPO_4, 1 g l^{-1}, $MgSO_4 \cdot 7H_2O$, 500 mg l^{-1}; $ZnSO_4 \cdot 7H_2O$, 10 mg l^{-1}; $CuSO_4 \cdot 5H_2O$, 5 mg l^{-1}; chloramphenicol, 50 mg l^{-1}; dichloran, 0.2% w/v in 1 ml of ethanol; glycerol, 220 ml l^{-1}; chlortetracycline, 50 mg l^{-1}.
– DYSG-M (DYSG modified): DYSG with half concentration of both sucrose and glycerol for reducing osmotic pressure of the medium.
– MESGA (Malt Extract Sucrose Glycerol Agar): as DYSG-M with no added salts and with yeast extract substituted by Oxoid malt extract, 20 g l^{-1}.
– MEA (Malt Extract Agar): Oxoid malt extract, 20 g l^{-1}; Oxoid agar N. 3, 20 g l^{-1}; chloramphenicol, 50 mg l^{-1}; dichloran 0,2% w/v in 1 ml of ethanol; chlortetracycline, 50 mg l^{-1}.

MESGA and MEA were either tested such as or added with 10 mg l^{-1} boscalid (Cantus, 50% a.s., BASF Agro) (MESGA-B and MEA-B, respectively). Chlortetracycline and boscalid were added to media after autoclaving.

Colonies were identified under the microscope and counted. Data on colony forming units (CFU) on each of the tested media are reported in Table 1.

A. carbonarius and *A. niger* showed a restrict colony growth on DYSG, and 5-7 days were needed to obtain colonies visible by the naked eye; DYSG-M, MESGA and, especially, MEA allowed a faster growth and colonies appeared after 2-4 days. *A. carbonarius* and *A. niger* colonies were not distinguishable by naked eyes (Fig. 1). The recovery rate for *A. carbonarius* was 90% on MEA, 73-74% on DYSG-M and MESGA, and 51% on DYSG. The higher rate on MEA was likely due to a

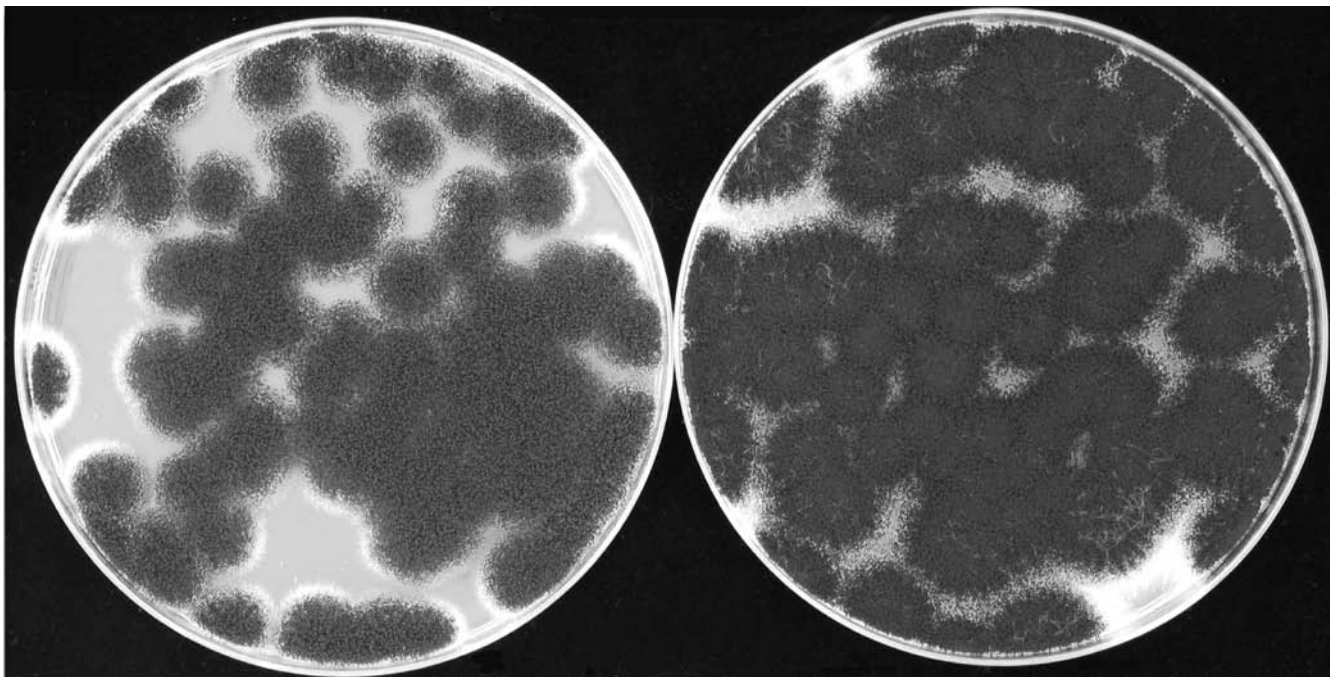

Fig. 1. *Aspergillus carbonarius* (on the right) and *Aspergillus niger* (on the left) on MEA six days after plating.

Table 1. Recovery of *Aspergillus niger* and *Aspergillus carbonarius* on different media [a].

Fungal strain	N. plated conidia	N. recovered Colony Forming Units				Recovery rate (%)			
		DYSG	DYSG-M	MESGA	MEA	DYSG	DYSG-M	MESGA	MEA
				A. niger					
AN1	122.0	87.1	98.2	99.0	119.2	71.3	80.3	81.1	97.5
AN8	87.0	58.0	71.1	81.2	85.2	66.7	81.7	93.1	97.7
AN27	135.0	90.1	111.2	107.1	127.1	67.4	82.2	79.3	94.1
AN31	117.0	72.3	91.3	86.0	115.1	61.5	77.8	73.5	98.3
AN45	125.0	87.5	98.0	95.3	123.2	69.6	78.4	76.0	98.4
Average		79.0 c C	93.8 b B	93.6 b B	113.8 a A	67.4 c C	80.3 b B	79.8 b B	97.1 a A
				A. carbonarius					
AC12	92.0	47.5	67.0	71.2	82.1	51.1	72.8	77.2	89.1
AC13	131.0	67.3	89.2	90.1	118.2	51.2	67.9	68.7	90.8
AC25	119.0	59.1	90.1	89.3	107.2	49.6	76.5	74.8	89.9
AC32	125.0	62.1	93.2	90.3	113.2	49.6	74.4	72.8	90.4
AC48	111.0	60.2	85.5	89.1	99.3	55.0	76.6	80.2	89.2
Average		59.2 c C	85.0 b B	86.0 b B	104.0 a A	51.2 c C	73.5 b B	74.4 b B	90.0 a A

[a] Each figure is the average of three replicated Petri dishes inoculated with a 100-µl aliquot of suspension containing around 100 conidia. Mean values of each parameter, within each fungal species, followed by a same letter are not significantly different at the probability levels of P=0.05 (small letters) or P=0.01 (capital letters) according to the Duncan's Multiple Range Test.

Table 2. Recovery of *Aspergillus niger* and *Aspergillus carbonarius* on different media from artificially contaminated samples of washing suspensions from berries or musts [a].

Sample	Recovery rate %											
	A. niger				*A. carbonarius*				*A. niger*:*A. carbonarius* (1:1 mixture) [b]			
	MESGA	MEA	MESGA-B	MEA-B	MESGA	MEA	MESGA-B	MEA-B	MESGA	MEA	MESGA-B	MEA-B
					Washing suspension from berries							
Cabernet sauvignon	85.6	95.2	0.0	0.0	85.1	90.7	82.2	86.9	96.8:85.2	96.8:92.6	0.0:81.5	0.0:87.0
Montepulciano	87.2	99.0	0.0	0.0	79.4	88.8	77.6	94.4	92.1:81.5	92.1:99.9	0.0:85.2	0.0:90.7
Negroamaro	82.4	90.4	0.0	0.0	77.6	86.9	79.4	86.0	88.9:77.8	99.9:90.7	0.0:79.6	0.0:96.3
Primitivo	77.6	92.8	0.0	0.0	77.6	89.7	80.4	87.9	93.7:79.6	99.9:92.6	0.0:85.2	0.0:92.6
Average	83.2 b B	94.4 a A	0 c C	0 c C	79.9 b B	89.0 a A	79.9 b B	88.8 a A	81.0 b B	94.0 a A	82.9 b B	91.7 a A
					Must							
Cabernet sauvignon	84.0	98.4	0.0	0.0	79.4	86.9	80.4	88.8	90.5:88.9	84.1:90.7	0.0:81.5	0.0:96.3
Montepulciano	77.6	91.2	0.0	0.0	80.4	87.9	77.6	85.1	93.7:85.2	92.1:87.0	0.0:83.3	0.0:94.4
Negroamaro	79.2	94.4	0.0	0.0	82.3	88.8	81.3	87.9	93.7:96.3	98.4:98.1	0.0:87.0	0.0:90.7
Primitivo	80.8	96.8	0.0	0.0	85.1	86.9	83.2	91.6	95.2:96.3	96.8:90.7	0.0:83.3	0.0:94.4
Average	80.4 b B	95.2 a A	0 c C	0 c C	81.8 b B	87.6 a A	80.6 b B	88.4 a.A	91.7 a AB	91.6 a AB	83.8 b B	94.0 a A

[a] Each figure is the average of three replicated Petri dishes inoculated with a 100-µl aliquot of artificially-contaminated samples containing around 100 conidia. Mean values, within each fungal species and type of samples, followed by a same letter are not significantly different at the probability levels of P=0.05 (small letters) or P=0.01 (capital letters) according to the Duncan's Multiple Range Test.

[b] Samples were contaminated with a 1:1 mixture of conidia of the two fungal species. Mean values accompanied by statistical significance are referred to *A. carbonarius*.

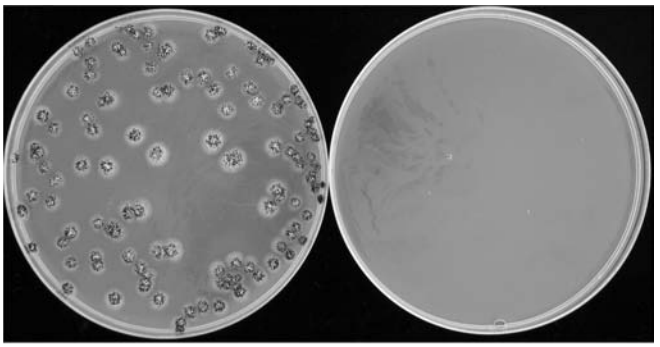

Fig. 2. *Aspergillus carbonarius* (on the right) and *Aspergillus niger* (on the left) on MEA-B three days after plating.

marked sensitivity of *A. carbonarius* to high osmotic pressure of the other media. *A. niger* proved less sensitive to high osmotic pressure than *A. carbonarius*, yielding a recovery rate of 67.4% on DYSG as compared to 80.3% on DYSG-M, 79.8% on MESGA, and 97.1% on MEA.

A limited *A. carbonarius* development was generally recognised in Petri dishes in which *A. niger* and *A. carbonarius* conidia were plated in a 1:1 ratio. *A. niger* and *A. carbonarius* were recovered in a ratio of 1:0.5 on DYSG, 1:0.7 on DYSG-M and MESGA and 1:0.9 on MEA.

MESGA-B and MEA-B were also tested. *A. niger* did not grow on either medium even after prolonged incubation (10-15 days), whereas colonies of *A. carbonarius* appeared within 8-10 days on MSGA-B and 3-5 days on MEA-B (Fig. 2), recovery rates being about 80% and 95%, respectively (data not shown) .

For further evaluation on the selectivity of MESGA and MEA, as such or supplemented with boscalid, the above described procedure was applied to samples of washing water from berries and must obtained at véraison to which known numbers of *A. niger* and/or *A. carbonarius* conidia were added. The results confirmed the selectivity of MEA-B and MESGA-B that allowed the growth and counting of *A. carbonarius* colonies but impaired the appearance of *A. niger* colonies. The mean value of recovery rate of *A. carbonarius* was 80% on MESGA-B and 89% on MEA-B (Table 2).

MEA and MEA-B were also compared using samples of washing suspension from berries and must obtained at vintage time from 22 vineyards of 7 grapevine cultivars. Very similar results in the detection of *A. carbonarius* were obtained with the two media (r^2=0.97; Table 3).

It must be emphazised, however, that MEA-B allowed direct counting of *A. carbonarius* colonies, whereas MEA required discrimination of the two fungal species through observations under the microscope. In these experiments, neither MEA nor MEA-B, prevented completely the growth of contaminant micro-organisms, mostly yeasts, especially in Petri dishes, where less diluted suspensions were plated.

In conclusion, serial dilution plating on MEA-B or MESGA-B and colony counting is a simple and inexpensive technique that in a relatively short time allows

Table 3. Detection of *Aspergillus niger* and *Aspergillus carbonarius* in samples of must obtained at vintage time from different vineyards using MEA such as or added with boscalid (MEA-B) [a].

Vineyard N.	Cultivar	Colony forming units ($\cdot 10^4$ ml^{-1})			
		MEA		MEA-B	
		A. niger	*A. carbonarius*	*A. niger*	*A. carbonarius*
1	Cabernet sauvignon	475.7	76.3	0.0	96.7
2	Cabernet sauvignon	34.3	0.7	0.0	1.7
3	Cabernet sauvignon	537.5	3.3	0.0	1.3
4	Lambrusco	1,092.3	11.7	0.0	16.0
5	Lambrusco	927.7	91.7	0.0	101.3
6	Lambrusco	119.3	0.1	0.0	0.3
7	Merlot	345.7	2.7	0.0	12.3
8	Merlot	92.7	0.7	0.0	6.7
9	Merlot	897.7	0.3	0.0	1.3
10	Montepulciano	729.7	0.1	0.0	0.3
11	Montepulciano	1,125.7	87.3	0.0	93.3
12	Negroamaro	1,103.3	127.7	0.0	180.7
13	Negroamaro	1,307.7	417.7	0.0	327.0
14	Negroamaro	112.7	0.3	0.0	1.3
15	Negroamaro	110.3	2.7	0.0	12.3
16	Negroamaro	430.7	31.7	0.0	28.7
17	Primitivo	1,906.3	75.7	0.0	55.3
18	Primitivo	295.7	20.3	0.0	20.3
19	Primitivo	775.3	31.3	0.0	31.3
20	Primitivo	445.3	1.3	0.0	2.7
21	Sangiovese	925.7	138.5	0.0	138.5
22	Sangiovese	692.3	9.7	0.0	8.5

[a] Each figure is the average of three replicated Petri dishes. Correlation index for data concerning *A. carbonarius* obtained with the two tested media was r^2= 0.97.

detection and quantification of *A. carbonarius* in samples of grape bunches or musts. MEA-B is the best medium as it required in most cases only 3-5 days for the detection of *A. carbonarius*. MESGA-B can be of help just when sour rot is very common in vineyards. Its osmotic pressure, higher than that of MEA-B, prevents partially yeast growth, although it delays the detection of *A. carbonarius* by 8-10 days. The new media will be helpful for estimating the abundance of *A. carbonarius* population in vineyards during grape ripening, which is a critical control point for assessing the risk of OTA contamination in wine and establishing appropriate preventive actions.

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REFERENCES

Battilani P., Pietri A., 2002. Ochratoxin A in grapes and wine. *European Journal of Plant Pathology* **108**: 639-643.

Battilani P., Pietri A., Pascale M., 2002. Funghi tossigeni e micotossine nella filiera viti-vinicola. *Informatore fitopatologico* **52**: 23-27.

Bau M., Bragulat M.R., Abarca M.L., Minguez S., Cabañes F.J., 2005. Ochratoxigenic species from Spanish wine grapes. *International Journal of Food Microbiology* **98**: 125-130.

Bellí N., Pardo E., Marín S., Farré G., Ramos A.J., Sanchis V., 2004. Occurence of ochratoxin A and toxigenic potential of fungal from Spanish grapes. *Journal of the Science of Food and Agriculture* **84**: 541-546.

Burdaspal P.A., Legarda T.M., 1999. Ocratoxina A en vinos, mostos y zumos de uva elaborados en Espãna y en otros paises europeos. *Alimentaria* **299**: 107-113.

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

Capriotti M., Del Vecchio A., Fagnani A., Gentili E., Bellettini L., Balzaretti G., Coatti M., Manaresi M., 2004. BAS 510 F (boscalid): nuovo fungicida multifunzionale. In: Brunelli A. and Canova A. (eds.). *Atti Giornate Fitopatologiche, Vol. 2, Montesilvano* 2004, 55-60.

Frisvad J.C., Filtenborg O., Lund F., Thrane U., 1992. New selective media for the detection of toxigenic fungi in cereal products, meat and cheese. In: Samson R.A., Hocking A.D., Pitt J.I., King A.D. (eds). Modern Methods in Food Mycology, pp. 255-261. Elsevier, Amsterdam, The Netherlands.

Hocking A.D., Pitt J.I., 1980. Dichloran-glycerol agar medium for enumeration of xerophilic fungi from low moisture foods. *Applied and Environmental Microbiology* **39**: 488-492.

IARC, 1993. Some naturally occurring substances: Food Items and Constituents, Heterocyclic Aromatic Amines, and Mycotoxins. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 56. International Agency for Research on Cancer, Lyon, France.

King A.D., Hoking A.D., Pitt J.I., 1979. Dichloran- rose Bengal medium for enumeration and isolation of molds from foods. *Applied and Environmental Microbiology* **37**: 959-964.

Logrieco A., Bailey J.A., Corazza L., Cooke B.M., 2002. Mycotoxins in plant disease. *European Journal of Plant Pathology* **108**: 597-734.

MacDonald B.A., Wilson S., Barnes P., Damant K., Massey A., Mortby R., Shepherd M.J., 1999. Ochratoxin A in dried vine fruit: method development and survey. *Food Additives and Contaminants* **16**: 253-260.

Majerus P., Otteneder H., 1996. Detection and occurrence of ochratoxin A in wine and grape juice. *Deutsche Lebensmittel-Rundschau* **92**: 338-390.

Medina A., Mateo R., López-Ocaña L., Valle-Algarra F.M., Jiménez M., 2005. Study of Spanish grape mycobiota and ocratoxin A production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section *Nigri*. *Applied and Environmental Microbiology* **71**: 4696-4702.

Ospital M., Cazabeil J.M., Betbeder A.M., Tricard C., Creppy E., Médina B., 1998. L'ochratoxine A dans les vins. Office International de la Vigne et du Vine. Feuillet Blue N° 45 S.A. 2519/290198.

Otteneder H., Majerus P., 2000. Occurrence of ochratoxin A in wines: influence of the type of wine and its geographical origin. *Food Additives and Contaminants* **17**: 793-798.

Pietri A., Bertuzzi T., Pallaroni L., Piva G., 2001. Occurrence of ochratoxin A in Italian wines. *Food Additives and Contaminants* **18**: 647-654.

Pitt J.I., Hocking A.D., 1997. Fungi and food spoliage. 2nd Ed. Blackie Academic and Professional, London, UK.

Pittet A., 1998. Natural occurrence of mycotoxins in foods and feeds. An updated review. *Revue de Médicine Vétérinaire*, 149: 479-492.

Pollastro S., Dongiovanni C., Abbatecola A., Tauro G., Natale P., Pascale M., Visconti A., Faretra F., 2003. Wine contamination by ochratoxin A in south Italy: causes and preventive actions. *Journal of Plant Pathology* **85**: 281 (abstract).

Pollastro S., Dongiovanni C., De Miccolis Angelini R.M., Abbatecola A., Natale P., De Guido M.A., Faretra F., 2005. Marciumi dell'uva e contaminazione del vino da ocratossina A. *Informatore fitopatologico* **55**: 15-21.

Sage L., Krivobok S., Delbos E., Seigle-Murandi F., 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.

Sage L., Garon D., Seigle-Murandi F., 2004. Fungal microflora and ochratoxin A risk in French vineyards. *Journal of Agricultural and Food Chemistry* **52**: 5764-5768.

Samson R.A., Houbraken J.A.M.P., Kuijpers A.F.A., Frank J.M., Frisvad J.C., 2004. New ochratoxin A or sclerotium producing species in *Aspergillus* section *Nigri*. *Studies in Mycology* **50**: 45-61

Sauter H., Steglich W., Anke T., 1999. Strobilurins: evolution of a new class of active substances. *Angewandte Chemie International Edition* **38:** 1328-1349.

Serra S., Maddau L., Franceschini A., 2004. Ochratoxin A and producing fungi in Sardinian vineyards. *Journal of Plant Pathology*, **86**: 304 (abstract).

Vercesi A., Mogicato M., Toffolatti S., Masi G., Savino M., Borgo M., 2004. Effetto di diverse strategie antibotritiche sulla presenza di *Aspergillus* spp. e *Penicillium* spp. su uve in Puglia. In: Brunelli A. and Canova A. (eds.). *Atti Giornate Fitopatologiche, Vol. 2, Montesilvano* 2004, 235-242.

Visconti A., Pascale M., Centonze G., 1999. Determination of ochratoxin A in wine by means of immunoaffinity column clean-up and high performance liquid chromatography. *Journal of Chromatography* **864**: 89-101.

WHO, 1996. Ochratoxin A, toxicological evaluation of certain food additives and contaminants. Food Additives Series 35. World Health Organization, Geneva, Switzerland.

Zimmerli B., Dick R., 1996. Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. *Food Additives and Contaminants* **13**: 655-668.

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