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### ► **To cite this version:**

Hend Bejaoui, Florence Mathieu, Patricia Taillandier, Ahmed Lebrihi. Fungal flora and ochratoxin A production during wine grape maturation in French vineyards during three years. *International Journal of Food Microbiology*, 2006, 111, sup. 1, pp.S46-S52. <10.1016/j.ijfoodmicro.2006.03.004>. <hal-03597551>

**HAL Id: hal-03597551**

**<https://hal.science/hal-03597551v1>**

Submitted on 4 Mar 2022

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# **Fungal flora and ochratoxin A production during wine grape maturation in French vineyards during three years**

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## **Abstract**

A survey was conducted in France over three years (2001-2003) in 10 vineyards from four winemaking regions with different geographical locations and climatic conditions. *Aspergillus* and *Penicillium* were isolated from the grapes. During 2001 and 2002, from setting to harvest, the total numbers of fungal isolates were respectively 721 and 711 increasing in 2003 to reach 1035. The *Aspergillus* genus was essentially represented by *Nigri* section (99%) and was predominant (80%  $\pm 4.6$ ) when compared to *Penicillium* (20%  $\pm 4.6$ ). Regardless of sampling year, 32.5% ( $\pm \sigma = 1.26$ ) of the fungal isolates were OTA producers and 93% ( $\pm \sigma = 2.65$ ) belonging to black aspergilli. The ochratoxigenic potential of the isolates and their occurrence on grapes revealed that *A. carbonarius* was the main OTA producer on

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22 French grapes. At harvest time, the fungal population was maximal and this was  
23 the most critical period influencing OTA contamination. Grapes from Languedoc-  
24 Roussillon region were most infested with ochratoxigenic fungi and had the highest  
25 concentrations of OTA. All these results could be of prime importance for further  
26 preventive action against ochratoxigenic species in vineyards to limit the  
27 contamination of grapes and grape products in France.

28 *Keywords:* Ochratoxin A; grapes; Black aspergilli; *A. carbonarius*; *A. niger*  
29 aggregate.

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## 30 **1. Introduction**

31 In the last few years, OTA has received increasing interest from both scientific  
32 communities and food committees because of its nephrotoxic (Krogh et al., 1974;  
33 Mortensen et al., 1983) teratogenic (Arora et al., 1981; Mayura et al., 1989),  
34 genotoxic (Dirheimer, 1998), immunosuppressive (Haubeck et al., 1981; Creppy et  
35 al., 1983) and carcinogenic (Boorman, 1989) properties. Its ingestion by humans,  
36 which occurs mainly through different plant-based foods and beverages, could lead  
37 to deterioration of liver or kidney function (Sweeney and Dobson, 1998).

38 Grapes and derived products such as dried vine fruit (McDonald et al., 1999), grape  
39 juices and wines (Scott and Kanhere, 1995; Zimmerli and Dick, 1996; Jorgensen,  
40 1998; Burdaspal and Legarda, 1999; Visconti et al., 1999; Otteneder and Majereus,  
41 2000) have been reported as potentially contaminated with OTA. Provisional  
42 estimates of the Codex Alimentarius Commission, based on limited European data,

43 suggested that red wine is the second major source of human exposure to OTA,  
44 following cereals and preceding coffee and beer (Walker, 1999). Swiss authors  
45 were the first to detect OTA in table wines collected from various European  
46 countries (Zimmerli and Dick, 1996) and red wines had higher concentrations than  
47 white ones (Ottender and Majerus, 2000). Two genera raised particular attention as  
48 the source of OTA, *Penicillium* and *Aspergillus* (Varga et al., 2001). Among the  
49 *Aspergillus*, the section *Nigri* was responsible for OTA production (Cabañes et al.,  
50 2002; Sage et al., 2002; Pechavy et al., 2003) and the species *A. niger* aggregate  
51 and *A. carbonarius* were considered to be particularly important (Abarca et al.,  
52 2001; Cabañes et al., 2002). According to different surveys grape products from the  
53 Mediterranean regions of south Europe (Burdaspal and Legarda, 1999; Battilani et  
54 al., 2003; Belli et al., 2004) and north Africa (Filali et al., 2001) were the most  
55 contaminated by OTA. In France, two preliminary studies revealed the presence of  
56 OTA in samples of grapes, musts and wines recovered from the South (Ospital et  
57 al., 1998; Sage et al., 2002).

58 This study was intended to assess the potential for ochratoxin A contamination of  
59 French grapes. It was done on a large sampling pattern of grapes recovered from  
60 different French vineyards in four winemaking regions with a variability of  
61 climatic conditions during three years. Screening the presence of OTA-producing  
62 species in *Penicillium* and *Aspergillus* section *Nigri*, following the dynamic of

63 these fungi during growing season and determining OTA contents in grapes were  
64 the principle objectives of this survey.

## 65 **2. Materials and Methods**

### 66 *2.1. French Study area*

67 The French mainland has almost a regular hexagonal form with 551,602 Km<sup>2</sup> area.  
68 From North to South, it extends between the parallels 51°5'27'' and 42°20'  
69 (Latitude North) and from West to East between the meridians 5°56' (Longitude  
70 West) and 7°9' (Longitude East). This large sweep shelters a big variety of climatic  
71 zones.

72 Ten vineyards located in 4 French winemaking regions with different climatic  
73 conditions were chosen in this study (see figure 1): Poitou-Charentes (PC)  
74 (vineyard 1), Languedoc-Roussillon (LR) (vineyards 2 to 8), Provence-Alpes Côtes  
75 d'Azur (PACA) (vineyard 9), and Alsace (Als) (vineyard 10). Eight varieties were  
76 analysed: Ugni Blanc (UB) from PC, Riesling (R) from Als, Cinsault (CN) from  
77 PACA and from LR, 5 varieties were considered: Sauvignon (SA), Muscat (Mu),  
78 Syrah (S), Carignan (CA) and Grenache (G). For the Syrah variety, three areas  
79 were considered (a, b and c). All the varieties analysed except Ugni Blanc, Riesling  
80 and Muscat were red vines.

### 81 *2.2. Samples collection*

82 Samples were taken during three growth stages: green berry, early veraison and  
83 ripe berry (harvest time). For each vineyard and each stage, 10 bunches were

84 collected by following the two diagonals. After their rapid transport to the  
85 laboratory in cool boxes, mycological analyses were immediately done. The  
86 remaining samples were frozen at -20°C for subsequent analysis.

### 87 *2.3. Mycological analysis of grapes*

88 Five randomly choosed berries from each bunch were put onto the surface of the  
89 DRBC (Dichloran Rose Bengale Chloramphenicol) agar (Oxoid) (Cahgnier, 1998)  
90 and plates incubated at 25°C for 7 days. Samples were examined daily with a  
91 stereomicroscope and all *Aspergillus* and *Penicillium* species isolated on CZ  
92 (CZAPEK) (Cahgnier, 1998) medium and purified on CYA (CZAPEK Yeast  
93 extract Agar). All fungi isolated were identified by morphological characters  
94 according to the most accepted criteria of classification (Raper and Fennell, 1965;  
95 Klich and Pitt, 1988; Pitt and Hocking, 1997).

### 96 *2.4. Ochratoxigenic ability of the isolates*

97 OTA production ability of 2467 isolates was tested on CYA using the method of  
98 Bragulat et al. (1998). OTA was analysed by HPLC and ochratoxigenic potential  
99 was expressed as  $\mu\text{g g}^{-1}$  CYA. 86 isolates were deposited into ITEM and IMI  
100 collections.

### 101 *2.5. Ochratoxin A analysis in grapes*

102 Each year, at harvest time, the 10 samples recovered from each vineyard were  
103 weighed and crushed for 2 minutes at room temperature. The juice was recovered

104 after centrifugation and its exact volume was measured. 10 ml were adjusted to pH  
105 7.8 with KOH 2 M and diluted to make a final volume of 20 ml with PBS buffer (r-  
106 Biopharm, St Didier Au Mont D'Or, France). The diluted sample was loaded onto  
107 an OchraPrep (r-Biopharm, St Didier Au Mont D'Or, France) immunoaffinity  
108 column operating at a steady flow rate of 2 ml min<sup>-1</sup>. The immunoaffinity column  
109 was washed with 20 ml of sterile distilled water, and then dried with an air strain.  
110 OTA was eluted by applying successively 1.5 ml of methanol/acetic acid (98:2)  
111 and 1.5 ml of sterile distilled water. The eluted extract was further analysed by  
112 HPLC and OTA concentrations expressed in µg Kg<sup>-1</sup> grapes.

### 113 *2.6. High performance liquid chromatography (HPLC)*

114 OTA was detected and quantified by reversed-phase HPLC. The analysis was  
115 performed using a BIO-TEK HPLC System (Milan, Italy) equipped with a Solvent  
116 delivery system 525, column thermostat 582, autosampler 465, diode array detector  
117 545V, acquisition data Kroma System KS3000 and Spectra System FL3000  
118 fluorescence detector ( $\lambda_{\text{ex}}=332$  nm;  $\lambda_{\text{em}}=466$  nm). The analytical column was a 150  
119 x 4.6 mm, 5 µm, C18 reversed-phase ODB Uptisphere fitted with a guard column  
120 (10x4 mm) having the same stationary phase. During analysis, the column was  
121 maintained at 30°C, and OTA was eluted with a mobile phase consisting of a  
122 mixture of HPLC grade acetic acid in water 0.2 % (A) and acetonitrile (B) at a flow  
123 rate of 1 ml min<sup>-1</sup>. Analysis of OTA in grape juices was done over 45 min with a

124 linear gradient from 10 % to 50 % of B over the first 30 min followed by a linear  
125 gradient to 90 % of B from 30 to 35 min, then a steady flow of 90 % of B for 8 min  
126 finally reduced to 10 % for 2 min. Analysis of OTA produced by grape isolates was  
127 done using a run time of 20 min and an isocratic method [A (59%)-B (41%)].  
128 Ochratoxin A was identified by its retention time according to a standard (Sigma  
129 Aldrich, Steinheim, Germany) and quantified by measuring peak area according to  
130 a standard curve. The detection limit was  $0.025\mu\text{g l}^{-1}$ . All analysis were done in  
131 triplicate.

### 133 **3. Results**

#### 134 *3.1. Total fungi isolates*

135 During three years, genera of penicillia and aspergilli on wine French grapes were  
136 systematically isolated, counted and identified. In 2001 and 2002, the total fungal  
137 isolates were respectively 721 and 711 increasing in 2003 to reach 1035. Penicillia  
138 represented only 20% ( $\pm 4.6$ ) and the species included *P. expansum*, *P. spinulosum*,  
139 *P. glabrum*, *P. crustosum*, *P. brevicompactum*, *P. oxalicum*, *P. citrinum*, *P.*  
140 *glandicola* and *P. adametzoides*.

141 The *Aspergillus* genus was predominant (80%  $\pm 4.6$ ) and especially those belonging  
142 to the *Nigri* section (Black aspergilli) (99%). Among the remaining aspergilli (1%),  
143 isolates of *A. flavus*, *A. parasiticus* and *A. fumigatus* were sometimes found. Black  
144 aspergilli were represented by three populations: *A. carbonarius*, *A. japonicus* and

145 *A. niger* aggregate. Except for *A. japonicus*, all these species were biseriata. *A.*  
146 *carbonarius* was microscopically recognised and distinguished by conidial size and  
147 ornamentation. All the other black biseriata aspergilli isolates will be referred as *A.*  
148 *niger* aggregate. Of the total black aspergilli isolated, *A. japonicus* was in the  
149 minority, representing 16, 8 and 2% respectively in 2001, 2002 and 2003. From  
150 2001 to 2003, *A. niger* aggregate increased (36, 56 and 72%) whereas *A.*  
151 *carbonarius* decreased (48, 36 and 26%).

### 152 3.2. Ochratoxigenic fungal isolates

153 For all isolates, the capacity for producing ochratoxin A was determined on a solid  
154 laboratory medium (CYA) after 7 days at 25°C. Whatever the sampling year, 32.5  
155 % ( $\pm 1.26$ ) of the total fungal isolates were ochratoxigenic. The OTA producing  
156 capacity of the isolates deposited in the ITEM and IMI collections are represented  
157 in table 1.

158 Over the three years a total number of 1974 black aspergilli were isolated. 745 of  
159 them were found to produce OTA (i.e. 37% regardless of the year). Whatever the  
160 year, among 148 *A. japonicus* tested, OTA producers never exceeded 20% with  
161 OTA production of between 0.001 and 0.01  $\mu\text{g g}^{-1}$  CYA. For 691 isolates of *A.*  
162 *carbonarius*, OTA producers represented respectively 72% and 80% in 2003 and  
163 2001 and reached 100% in 2002, with OTA production of between 0.1 and 7  $\mu\text{g g}^{-1}$   
164 CYA. Among 1102 of *A. niger* aggregate, ochratoxigenic isolates never exceeded  
165 20% and had OTA production of between 0.01 and 0.06  $\mu\text{g g}^{-1}$  CYA.

### 3.3. Fungi population evolution

#### 3.3.1. During the growing season

Temporal changes in total and ochratoxigenic black aspergilli during the growing season are shown in Fig. 2. At the green berry stage, no OTA producer isolates were found regardless of year. In early veraison, a few OTA producer fungi were recovered and predominantly *A. carbonarius*. At harvest time, in all three sampling years, fungal contamination highly increased. About 66% ( $\pm 11$ ) of the total isolates and 90% ( $\pm 3.1$ ) of the ochratoxigenic ones were isolated. At this time, 96% ( $\pm 0.5$ ) of these ochratoxigenic isolates were black aspergilli. *A. carbonarius* species were 98% ( $\pm 0.67$ ) in 2001 and 2002 and 48% in 2003. Ochratoxigenic *A. niger* aggregates were only found in 2003 and reached 50% of the total ochratoxigenic black aspergilli isolates.

#### 3-2- Regional variations and varieties

Eight grape varieties from four winemaking regions were analysed. The most fungal isolates were from the Languedoc-Roussillon region (ranged between 91 and 132), followed by CN from Provence-Alpes Côte d'Azur (between 17 and 56), R from Alsace (between 0 and 56) and finally UB from Poitou-Charentes (between 2 and 17) (Fig. 3).

No ochratoxigenic fungi were isolated on the UB variety and only three on Riesling one. On those two grapes varieties OTA was never detected.

186 On CN variety, no ochratoxigenic isolates were found in 2001, 3 *A. carbonarius* in  
187 2002 and 13 *A. niger* aggregates in 2003. OTA content in this variety was about  
188 0.18 ppb in 2002 and 0.11 ppb in 2003.

189 On Languedoc-Roussillon varieties, ochratoxigenic isolates were in majority  
190 represented by *A. carbonarius* (98% ( $\pm 2$ )) in 2001 and 2002 and by 52% *A.*  
191 *carbonarius* and 48% *A. niger* aggregate in 2003. The highest OTA concentrations  
192 in grapes from this region were observed in 2001 in CA (0.38 ppb), in 2002 in S  
193 (2.78 ppb) and in 2003 in SA (0.46 ppb).

194

## 195 **Discussion**

196 In our study, *Aspergillus* and *Penicillium* genera were isolated as they are potential  
197 OTA producers in various foodstuffs (Varga et al., 1996; Abarca et al., 1997;  
198 Sweeny and Dobson, 1998). In grapes, the predominance of *Aspergillus* compared  
199 to *Penicillium* was observed among total (80% versus 20%) and ochratoxigenic  
200 (93% versus 7%) isolates. It is known that *Penicillium* species appear more often in  
201 temperate and cold climates such as in northern Europe (Pitt and Hocking, 1997;  
202 Frisvad et al., 1999) whereas *Aspergillus* species are commonly associated with  
203 warmer and tropical regions (Pitt and Hocking, 1997; Pittet, 1998). Creppy et al.  
204 (1991) also outlined that ochratoxicosis in France was connected to aspergilli  
205 whereas in Germany or Scandinavia it could be linked to penicillia (Creppy et al.,  
206 1991). Although commonly reported in cereal and cereal products as principle

207 OTA producers (Pitt, 1987; Frisvad, 1989; Frisvad and Filtenborg, 1990),  
208 *Penicillium verrucosum* and *Aspergillus ochraceus* have never been isolated on  
209 French grapes. This observation was already demonstrated for grapes from south of  
210 France (Sage et al., 2002) and from Argentina (Da Rocha Rosa et al., 2002). *P.*  
211 *verrucosum* has never been mentioned as part of the normal microbiota of grapes  
212 (Cabañes et al., 2002; Sage et al., 2002; Serra et al., 2003; Da Rocha Rosa et al.,  
213 2002), whereas, *A. ochraceus* was already isolated among *Aspergillus* genus, on  
214 Brazilian (6%) (Da Rocha Rosa et al., 2002), Spanish (2.5%) (Belli et al., 2004),  
215 Portuguese (<1%) (Serra et al., 2003) and Italian (<0.6%) (Battilani et al., 2003)  
216 berries.

217 In our study, among *Aspergillus* species, the black aspergilli were the most  
218 common (99%). This group was also predominant on Italian (97%) (Battilani et al.,  
219 2003), Spanish (97%) (Belli et al., 2004) and Portuguese (90%) (Serra et al., 2003)  
220 grapes. In 2003, the total fungal isolates highly increased (+44.5%) principally due  
221 to the increase in *A. niger* aggregate. Their highest growth rate and their tolerance  
222 to high temperatures (higher than 37°C) (Battilani et al., 2003), registered in  
223 weather stations in 2003, could explain their tendency to increase.

224 According to our study, the low number of *A. japonicus* species, the few  
225 ochratoxigenic isolates recovered and their low OTA-producing potential showed  
226 that *A. japonicus* was unimportant in OTA French grape contamination.

227 The number of *A. carbonarius* ( $230 \pm 26$  per year), the high frequency of  
228 ochratoxigenic isolates (between 70 and 100%) and their potential to produce OTA  
229 show that this species is the most OTA producer on grapes in France. Previous  
230 studies showed its invasive character to colonise and penetrate berries even without  
231 skin damage (Battilani and Pietri, 2002). *A. carbonarius* also reported as the main  
232 OTA producer in Spanish (Abarca et al., 2001; Cabañes et al., 2001), Portuguese  
233 (Serra et al., 2003) and Italian grapes (Battilani et al., 2003). Its ochratoxigenic  
234 potential was not only occasioned by its intrinsic toxigenic character but also due to  
235 its aggressiveness (Battilani et al., 2002).

236 The consistent presence of *A. niger* aggregate on grapes during the growing season  
237 and their important number compared to *A. carbonarius* could explain their  
238 importance. However, the lower frequency of ochratoxigenic *A. niger* aggregate  
239 (20%) and their lower ability to produce OTA compared to *A. carbonarius*, make  
240 them of second importance in OTA contamination. According to the literature 4%,  
241 16.6% and 30% of *A. niger* aggregate isolated respectively on Portuguese (Serra et  
242 al., 2003), Argentinean and Brazilian (Da Rocha Rosa et al., 2002) grapes were  
243 potential OTA producers.

244 During the growing season, the harvest time was considered as a critical period for  
245 fungal development. This was also reported on Portuguese grapes (Serra et al.,  
246 2003) and on Spanish berries (Belli et al., 2004). Acidity decrease, sugar  
247 accumulation and cuticule embrittlement in grapes may explain fungal abundance.

248 At this time, about 90% of ochratoxigenic isolates were from ripe berries and 92%  
249 were *A. carbonarius*. This has also been observed in Portuguese vineyards (Serra et  
250 al., 2003).

251 According to regions, in the western (Poitou-Charentes) or Eastern (Alsace)  
252 vineyards, limited fungal population was found. Over the three years, *A.*  
253 *carbonarius* was absent. Ochratoxigenic fungi isolated in those regions were also  
254 not relevant and only 3 *A. niger* aggregate from Alsace have been confirmed as  
255 OTA producers. Moreover, very low concentrations of OTA were measured in  
256 grapes from Poitou-Charentes (oceanic climate) and Alsace (continental climate).

257 French Mediterranean southern vineyards (LR and PACA) had a large fungal flora,  
258 especially of black aspergilli. These fungi were reported to be resistant to high sun  
259 exposure and to very hot (frequently achieving temperatures of 40°C during  
260 summer time) and dry environments with low rainfall levels characterising this  
261 climate (Serra et al., 2003). The presence of OTA on grapes from this area and the  
262 important ochratoxigenic isolates confirmed the high contamination in the  
263 Mediterranean region. High levels of OTA in wines were previously reported in  
264 South Europe (Battaglia et al., 1996; Zimmerli and Dick, 1996) and especially in  
265 Mediterranean areas (Zimmerli and Dick, 1996). However samples from PACA  
266 were less contaminated by ochratoxigenic fungi than those from LR and presented  
267 less OTA concentrations. This difference could not only due to species involved  
268 on grapes but also to variety, soil and growing area.

269 In conclusion, *A. carbonarius* followed by *A. niger* aggregate play a major role in  
270 OTA contamination of French grapes. Harvest time is the critical time where  
271 almost all ochratoxigenic fungi were detected and the highest amounts of OTA  
272 were measured in grapes. Southern Mediterranean regions and especially  
273 Languedoc-Roussillon were the most contaminated with OTA.

#### 274 **Acknowledgement**

275 This work was supported by grants from the European Union (QLK1-CT-2001-  
276 01761) and French “Ministère de la jeunesse de l’éducation et de la recherche”  
277 (AQS N°:02 PO571). The authors thank Patricia Nouvet for her technical  
278 assistance.

#### 279 **References**

280 Abarca, M.L., Bragulat, M.R., Castellà, G., Accensi, F., Cabanes, F.J., 1997. New  
281 ochratoxigenic species in the genus *Aspergillus*. *Journal of Food Protection* 60,  
282 1580-1582.

283 Abarca, M.L., Accensi, F., Bragulat, M.R., Cabañes, F.J., 2001. Ochratoxin A-  
284 producing black aspergilli in dried vine fruits. In: *Book of abstracts Bioactive*  
285 *fungus metabolites Impact and Exploitation*. Swansea, Wales, pp. 38.

286 Arora, R.G., Fröelén, H., 1981. Interference of mycotoxins with prenatal  
287 development of the mouse. II ochratoxin A induced teratogenic effects in relation  
288 to the dose and stage of ingestion. *Acta Agriculturae Scandinavica*

289 22, 535-552.

290 Battaglia, R., Hatzold, T., Kroes, R., 1996. Conclusion from the workshop on  
291 ochratoxin in food, organized by ILSI Europe in Aix-en-Provence, 10-12th  
292 January 1996. Food Additives and Contaminants 13, 1-3.

293 Battilani, P., Pietri, A., 2002. Ochratoxin A in grapes and wine. Plant pathology  
294 108, 639-643.

295 Battilani, P., Pietri, A., Bertuzzi, T., Languasco, L., Giorni, P., Kozakiewicz, Z.,  
296 2003. Occurrence of ochratoxin A producing fungi in grapes grown in Italy.  
297 Journal of food protection 66, 633-636.

298 Belli, N., Ramos, A.J., Sanchis, V., Marin, S., 2004. Incubation time and water  
299 activity effects on ochratoxin A production by *Aspergillus section Nigri* strains  
300 isolated from grapes. Letters in Applied Microbiology 38, 72-77.

301 Boorman, G., 1989. Ed. NTP Technical report on the toxicology and  
302 carcinogenesis studies of ochratoxin A (CAS No. 303-47-9) in: F344/N Rats  
303 (Gavage Studies) (NIH Publication No. 89-2813); National Toxicology  
304 Program, U.S. Department of health and human services: Research Triangle  
305 Park, NC.

306 Bragulat, M.R., Abarca, M.L., Accensi, F., Cabañes, F.J. New screening method  
307 for ochratoxigenic molds in pure cultures. Acta veterinaria Scandinavica. 1998,  
308 22, 515.

309 Burdaspal, P.A., Legarda, T.M., 1999. Ochratoxin A in wines and grape products  
310 originating from Spain and other European countries. *Alimentaria* 36, 107-113.

311 Cabañes, F.J., Accensi, F. Bragulat, M.R., Abarca, M.L., Minguez, S., Pons, A.,  
312 2001. *Aspergillus carbonarius* is a source of ochratoxin A contamination in  
313 wine. In: Book of abstracts “Bioactive Fungal Metabolites – Impact and  
314 exploitation” p 51 Swansea, wales.

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

318 Cahagnier, B., 1998. Moisissures des aliments peu hydratés. Lavoisier Tec et Doc,  
319 Paris, pp.85-88.

320 Creppy, E.E., Stormer, F.C., Rösenthaller, R, Dirheimer, G., 1983. Effects of two  
321 metabolites of ochratoxin A, (4R)-4-hydroxyochratoxin A and ochratoxin  
322 alpha on immune response in mice. *Infection Immunology* 39, 1015-1018.

323 Creppy, E.E., Betbeder, A.M., Gharbi, A., Counord, J., Castegnaro, M., Bartsch,  
324 H., Montcharmont, P., Fouillet, B., Chambon, P., Dirheimer, G., 1991. Human  
325 ochratoxicosis in France. IARC scientific publication N°115, International  
326 agency for research of cancer, Lyon, France, pp 145-151.

327 Da Rocha Rosa, C.A., Palacios, V., Combina, M., Fraga, M.E., De Oliveira  
328 Rekson, A., Mgnoli, C.E., Dalcerro, A.M., 2002. Food additives and  
329 contaminants 19, 4, 408-414.

330 Dirheimer, G., 1998. Recent advances in the genotoxicity of mycotoxins. Revue  
331 Méditerranéenne Vétérinaire (Toulouse) 149, 605-616.

332 Filali, A., Oumrani, L., Betbeder, A.M., Baudrimont, I., Soulaymani, R., Benyada,  
333 A., Creppy, E.E., 2001. Ochratoxin A in beverages from Morocco: a  
334 preliminary survey. Food additives and Contaminants 18, 565-568.

335 Frisvad J.C., 1989. The connexion between penicillia and aspergilli and  
336 mycotoxins with special emphasis on misidentified isolates. Archives of  
337 Environmental Contamination and Toxicology 18, 452-457.

338 Frisvad, J.C., Filtenborg, O., 1990. Secondary metabolites as consistent criteria in  
339 *Penicillium* taxonomy and a synoptic key to *Penicillium* subgenus *Penicillium*.  
340 In : Samson, R. A., Pitt, J. I. (eds.), Modern Concepts in *Penicillium* and  
341 *Aspergillus* classification. Plenum, New York, pp 373-384.

342 Frisvad, J.C., Filtenborg, O., Lund, F., Samson, R.A., 1999. The homogenous  
343 species and series in subgenus *Penicillium* are related to mammal nutrition and  
344 excretion, in integration of modern taxonomic methods for *Aspergillus* and  
345 *Penicillium* classification, ed. by Samson, R.A., Pitt, J.I., harwood academic,  
346 reading, UK, pp 259-277.

347 Haubeck, H.D., Lorkowski, G., Kölsh, E., Rösenthaler, R., 1981.  
348 Immunosuppression by ochratoxin A and its prevention by phenylalanine.  
349 Applied and Environmental Microbiology 41, 1040-1042.

350 Jørgensen, K., 1998. Survey of pork, poultry, coffee, beer and pulses for ochratoxin  
351 A. Food Additives and Contaminants 15, 550-554.

352 Klich, M. A., Pitt, J. I., 1988. A laboratory guide to common *Aspergillus* species  
353 and their teleomorphs. CSIRO Division of Food Processing, North Ryde.

354 Krogh, P., Hald, B., Giersten, P., Myken, F., 1974. Fate of ochratoxin A and  
355 citrinin during malting and brewing experiments. Applied Microbiology 28,  
356 31-34.

357 MacDonald, S.; Wilson, P.; Barnes, K.; Damant, A.; Massey, R.; Mortby, E.;  
358 Shepherd, M.J., 1999. Ochratoxin A in dried vine fruit: Method development  
359 and survey. Food additives and contaminants 16, 253-260.

360 Mayura, K., Edwards, J.F., Maull, E.A., Philips, T.D., 1989. The effects of  
361 ochratoxin A on postimplantation rat embryos in culture. Archives of  
362 Environmental Contamination and Toxicology 18, 411-415.

363 Mortensen, H.P., Hald, B., Madsen, A., 1983. Feeding experiments with ochratoxin  
364 A contaminated barley for bacon pigs. 5 Ochratoxin A in pig blood. Acta  
365 Agriculturae Scandinavica 33, 235-239.

366 Ospital, M., Cazabeil, M., Betbeder, A.M., Tricard, C., Creppy, E., and Medina, B.,  
367 1998. L'ochratoxine A dans les vins. *Revue Française d'œnologie* 169, 16-18.

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

371 Pechavy, M., Bejaoui, H., Mathieu, F., Nguyen, B. L., Roset, M., Lebrihi, A., 2003.  
372 Evolution de la population des champignons toxinogènes et suivi de la  
373 production d'OTA pendant la maturation des grappes de raisin. V<sup>IIème</sup>  
374 Symposium International d'œnologie, 19-21th june 2003. Bordeaux.

375 Pitt, J. I. 1987. *Penicillium viridicatum*, *Penicillium verrucosum* and production of  
376 ochratoxin A. *Applied and Environmental Microbiology* 53, 266-269.

377 Pitt, J.I., Hocking, A.D., 1997. *Fungi and Food Spoilage*, 2<sup>nd</sup> edn. Blackie  
378 Academic and Professional, London.

379 Pittet, A., 1998. Natural occurrence of mycotoxins in foods and feeds, an updated  
380 review. *Revue Méditerranéenne. Vétérinaire* 149, 479-492.

381 Raper, K.B., Fennell, D.I. 1965. *The genus Aspergillus*. Williams and Wilkins,  
382 Baltimore USA.

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

386 Serra, R., Abrunhosa, L., Kozakiewicz, Z., Venâncio, A., 2003. Black *Aspergillus*  
387 species as ochratoxin A producers in Portuguese wine grapes. *International*  
388 *Journal of Food Microbiology* 88, 63-68.

389 Scott, P.M., Kanhere, S.R., 1995. Determination of ochratoxin A in beer, *Food*  
390 *Additives and Contaminants* 12, 592-598.

391 Sweeny, M.J., Dobson, A.D., 1998. Review: Mycotoxin production by *Aspergillus*,  
392 *Fusarium* and *Penicillium* species. *International Journal of Food Microbiology*  
393 43, 141-158.

394 Visconti, A., Pascale, M., Centonze, G., 1999. Determination of ochratoxin A in  
395 wine by means of immunoaffimty column clean-up and high-perfonnance  
396 liquid chromatography. *Journal of Chromatography* 864, 89-101.

397 Varga, J., Kevei, E., Rinyu, E., Téren, J., Kozakiewicz, Z., 1996. Ochratoxin  
398 production by *Aspergillus* species. *Applied and Environmental Microbiology*  
399 62, 4461-4464.

400 Varga, J., Rigó, K., Téren, J. and Mesterházy, Á., 2001. Recent advances in  
401 ochratoxin research I. Production, detection and occurrence of ochratoxins.  
402 Cereal research communications 29, 85-92.

403 Walker, R., 1999. Mycotoxins of growing interest. In: Proceedings of the third  
404 Joint FAO/UNEP International Conference on Mycotoxins. Tunisia: Tunis.

405 Zimmerli, B., Dick, R., 1996. Ochratoxin A in table wine and grape-juice:  
406 occurrence and risk assessment. Food additives and contaminants 13, 655-668.

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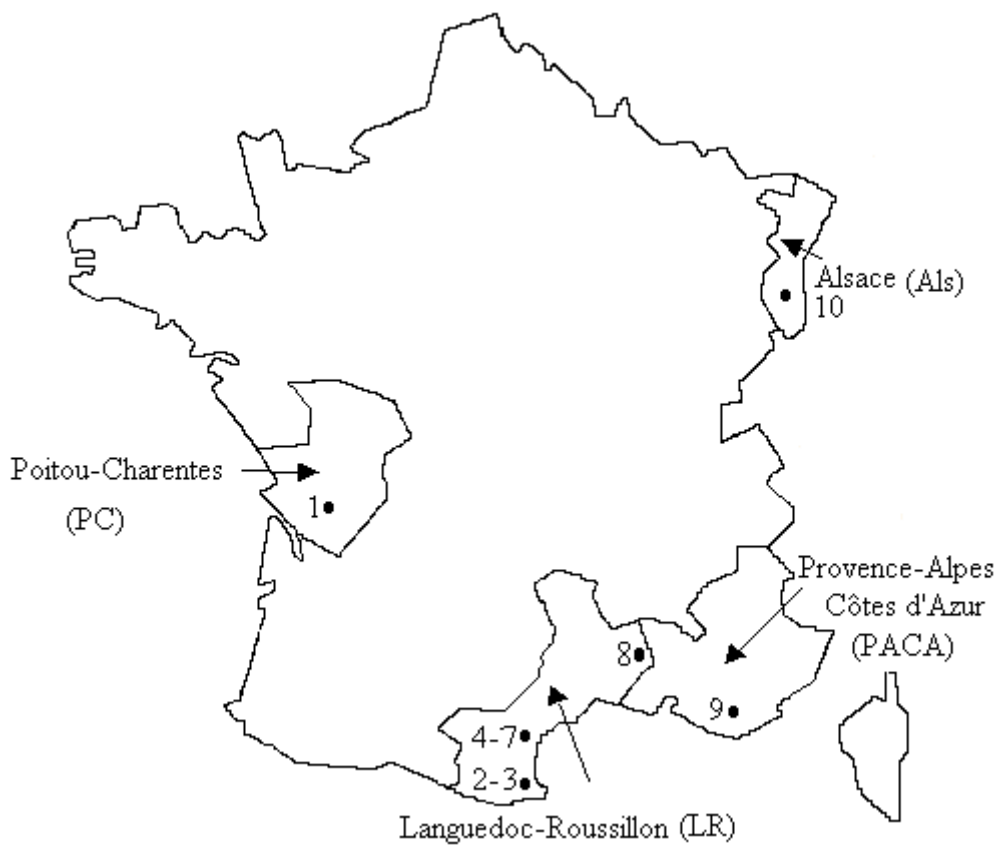
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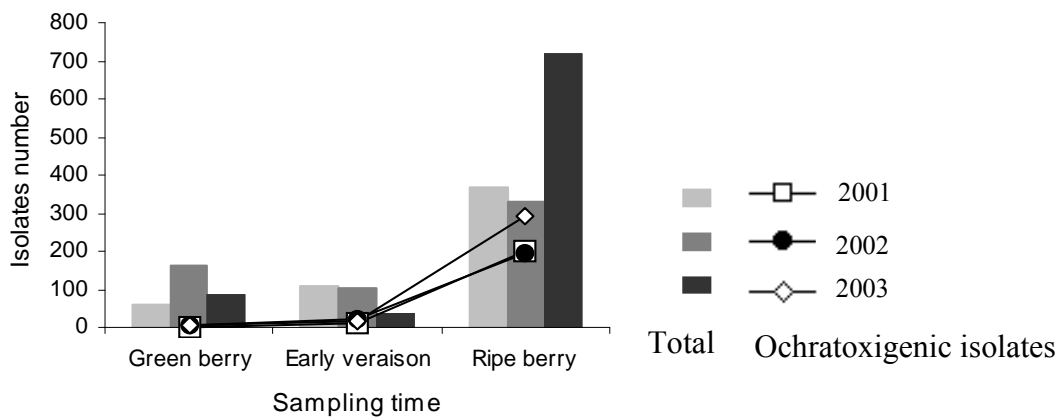
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422 Fig 1: Location of the regions and vineyards (each number represented one  
423 vineyard) studied in France.

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428 Fig. 2: Evolution of total and ochratoxigenic isolates of black aspergilli during  
429 growing season

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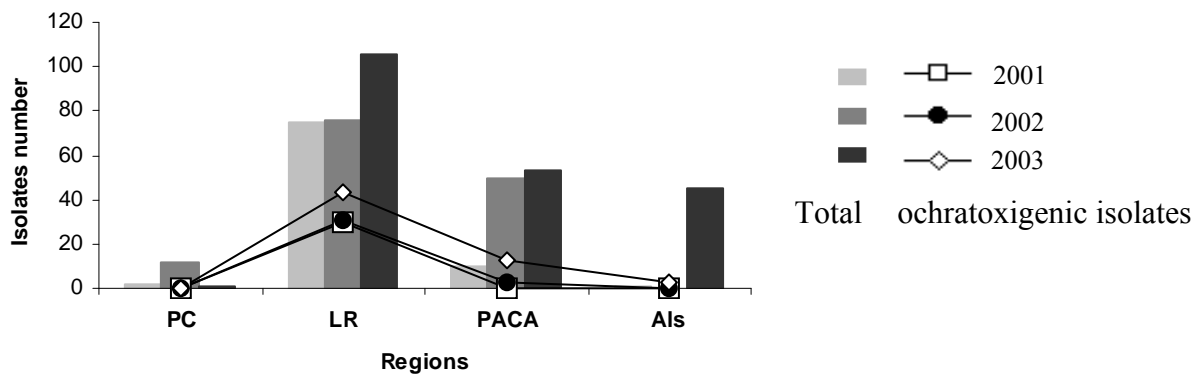
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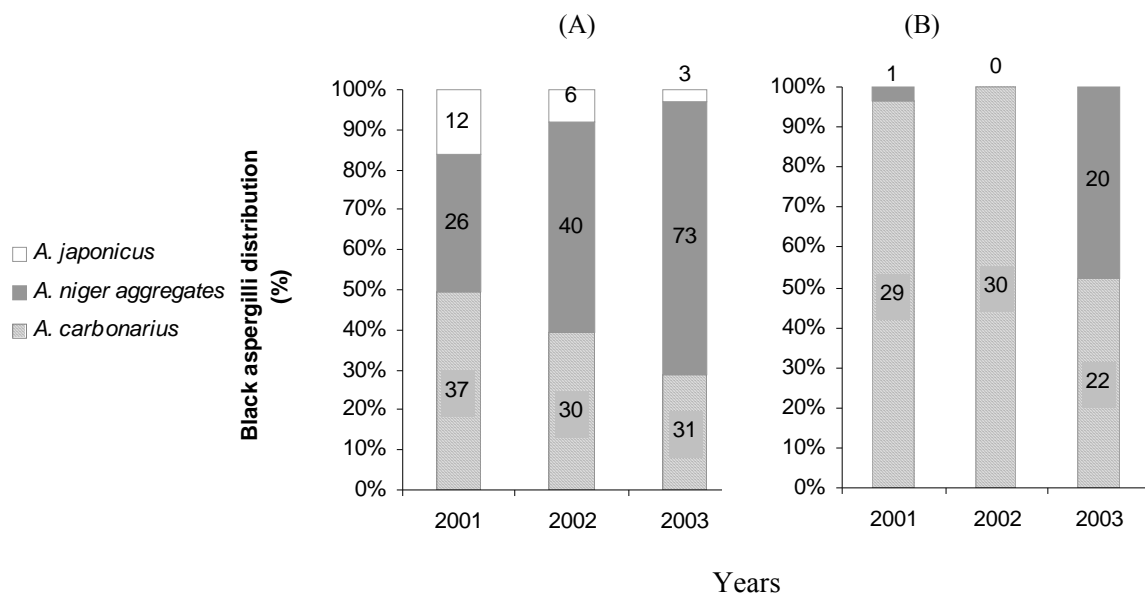


455 Fig. 3 Distribution of total and ochratoxigenic black aspergilli isolates in different  
456 regions during 2001, 2002 and 2003 (fungi isolates number represented in LR are  
457 mean values calculated on 7 vineyards)

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473 Fig. 4: Black aspergilli species distribution among total (A) and ochratoxigenic (B)  
474 isolates in Languedoc-Roussillon region and during 2001, 2002 and 2003.  
475 (values mentioned on the graphics are average isolate numbers calculated on 7  
476 vineyards of LR region)

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Table 1 : Deposited isolates in ITEM and IMI collections

Isolate N°	Identification	IMI N°	ITEM N°	OTA production level ( $\mu\text{g g}^{-1}$ CYA)
A2215	<i>A.japonicus</i>	389196	5322	0
A411	<i>A.japonicus</i>	389195	5321	0
AX35	<i>A.japonicus</i>	389197	5323	0
B111	<i>A.japonicus</i>	389198	5325	0
B712	<i>A.japonicus</i>	389199	5326	0
C133	<i>A.carbonarius</i>	388568	4781	13,1
C134	<i>A.carbonarius</i>	388490	4796	10,6
C135	<i>A.carbonarius</i>	388569	4782	17
C232	<i>A.carbonarius</i>	388478	4791	3,46
C234	<i>A.carbonarius</i>	388488	4797	8,2
C235	<i>A.carbonarius</i>	388458	4810	1,23
C336	<i>A.carbonarius</i>	388479	4792	3,48
C338	<i>A.carbonarius</i>	388459	4811	0
C432	<i>A.niger</i>	388498	4823	0,25
C513	<i>A.japonicus</i>	389200	5327	0
C518	<i>A.japonicus</i>	389201	5328	0
C535	<i>A.niger</i>	388560	4815	0,11
C536	<i>A.carbonarius</i>	388570	4783	10,86
C633	<i>A.carbonarius</i>	388486	4809	0
C637	<i>A.carbonarius</i>	388480	4793	6,76
C933	<i>A.carbonarius</i>	388484	4798	4
CA122	<i>A.japonicus</i>	389206	5333	0
CA223	<i>A.japonicus</i>	389207	5334	0
CA332	<i>A.carbonarius</i>	388572	4789	25,3
CA532	<i>A.carbonarius</i>	388496	4805	26
CA924	<i>A.japonicus</i>	389208	5335	0
CA932	<i>A.niger</i>	388483	4830	0
CaX34	<i>A.niger</i>	388505	4828	0
CAX38	<i>A.niger</i>	388566	4821	0
CN311	<i>A.japonicus</i>	389205	5332	0
CX33	<i>A.niger</i>	388499	4824	0,09
G132	<i>A.japonicus</i>	389213	5339	0
G221	<i>A.japonicus</i>	389210	5336	0
G231	<i>A.japonicus</i>	388506	4814	0
G431	<i>A.niger</i>	388507	4829	0
G522	<i>A.japonicus</i>	389211	5337	0
G731	<i>A.carbonarius</i>	388573	4790	2,82
G732	<i>A.carbonarius</i>	388494	4806	2,63
G738	<i>A.carbonarius</i>	388495	4807	37,5
G921	<i>A.japonicus</i>	389212	5338	0
G936	<i>A.japonicus</i>	389214	5340	0

GX312	<i>A.niger</i>	388567	4822	0
Mu141	<i>A.carbonarius</i>	388574	4785	4,05
Mu143	<i>A.niger</i>	388561	4816	0
Mu148	<i>A.niger</i>	388502	4826	0
Mu212	<i>A.japonicus</i>	389202	5329	0
Mu245	<i>A.carbonarius</i>	388489		0
Mu246	<i>A.niger</i>	388487	4817	0
Mu247	<i>A.carbonarius</i>	388481	4794	8,78
Mu249	<i>A.niger</i>	38562	4818	0
Mu343	<i>A.niger</i>	388563	4819	0
Mu412	<i>A.japonicus</i>	389203	5330	0
Mu441	<i>A.carbonarius</i>	388575	4786	15
Mu541	<i>A.japonicus</i>	389204	5331	0
Mu543	<i>A.carbonarius</i>	388491	4801	6
Mu642	<i>A.carbonarius</i>	388576	4787	13,16
Mu644	<i>A.carbonarius</i>	388492	4802	14
Mu649	<i>A.carbonarius</i>	388577	4788	9,45
Mu7412	<i>A.carbonarius</i>	388485	4804	7,43
Mu746	<i>A.carbonarius</i>	388493	4803	10,84
Mu747	<i>A.niger</i>	388564	4831	0
Mu848	<i>A.niger</i>	388503	4832	0
Mu943	<i>A.niger</i>	388504	4827	0
MuX42	<i>A.carbonarius</i>	388482	4795	6,5
MuX44	<i>A.niger</i>	388565	4820	0
SA332	<i>A.carbonarius</i>	388497	4799	25
SA411	<i>A.japonicus</i>	389209	5324	0
SA436	<i>A.carbonarius</i>	388500	4808	0
SA636	<i>A.carbonarius</i>	388571	4784	1,64
SA731	<i>A.niger</i>	388501	4825	0
2A138	<i>A.niger</i>	390084		0
2A215	<i>A.japonicus</i>	390080		0
2A235	<i>A.carbonarius</i>	390090		14,96
2B437	<i>A.carbonarius</i>	390093		3,62
2C236	<i>A.carbonarius</i>	390094		1,82
2CA331	<i>A.niger</i>	390089		0
2CA332	<i>A.carbonarius</i>	390092		4,94
2CA432	<i>A.niger</i>	390087		0
2CN331	<i>A.niger</i>	390088		0
2G221	<i>A.japonicus</i>	390083		0
2G621	<i>A.japonicus</i>	390082		0
2MU135	<i>A.niger</i>	390086		0
2MU531	<i>A.carbonarius</i>	390091		2
2SA 511	<i>A.japonicus</i>	390081		0
2SA331	<i>A.niger</i>	390085		0

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