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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% ± 4.6) when compared to *Penicillium* (20% ± 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.

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,

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

This study was intended to assess the potential for ochratoxin A contamination of French grapes. It was done on a large sampling pattern of grapes recovered from different French vineyards in four winemaking regions with a variability of climatic conditions during three years. Screening the presence of OTA-producing 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 Km² 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

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

2.3. *Mycological analysis of grapes*

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

2.4. *Ochratoxigenic ability of the isolates*

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

2.5. *Ochratoxin A analysis in grapes*

Each year, at harvest time, the 10 samples recovered from each vineyard were 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⁻¹. 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⁻¹ 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 (λ_{ex} =332 nm; λ_{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⁻¹. Analysis of OTA in grape juices was done over 45 min with a

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

3. Results

3.1. Total fungi isolates

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

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

145 *A. niger* aggregate. Except for *A. japonicus*, all these species were biseriate. *A.*
146 *carbonarius* was microscopically recognised and distinguished by conidial size and
147 ornamentation. All the other black biseriate 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 % (± 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% (± 11) of the total isolates and 90% (± 3.1) of the ochratoxigenic ones were isolated. At this time, 96% (± 0.5) of these ochratoxigenic isolates were black aspergilli. *A. carbonarius* species were 98% (± 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% (± 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

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

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

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

227 The number of *A. carbonarius* (230 ± 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 cuticle 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.

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(1)

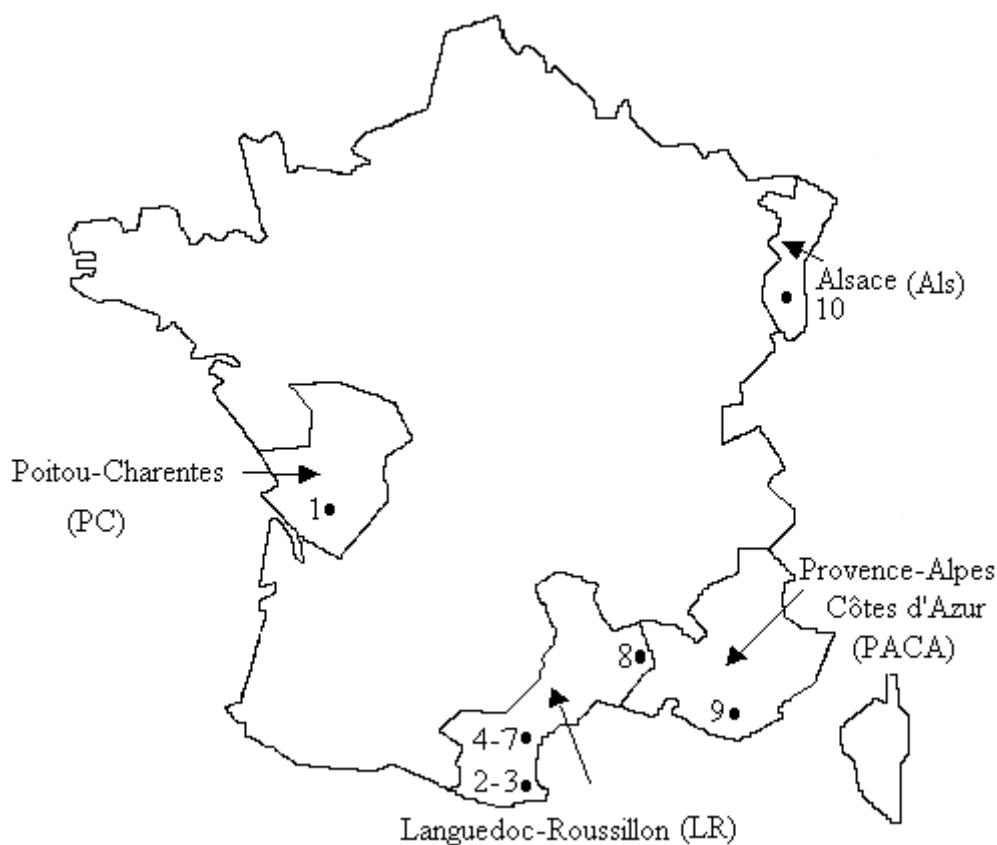


Fig 1: Location of the regions and vineyards (each number represented one vineyard) studied in France.

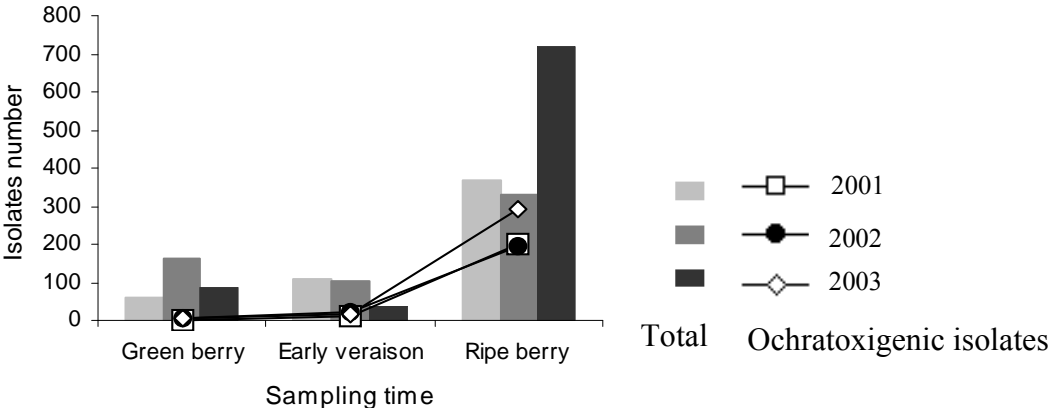


Fig. 2: Evolution of total and ochratoxigenic isolates of black aspergilli during growing season

(3)

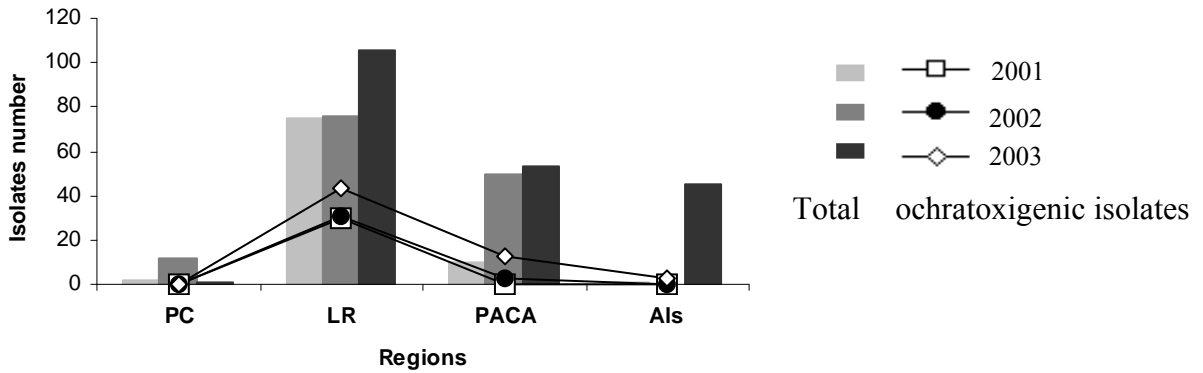


Fig. 3 Distribution of total and ochratoxigenic black aspergilli isolates in different regions during 2001, 2002 and 2003 (fungi isolates number represented in LR are mean values calculated on 7 vineyards)

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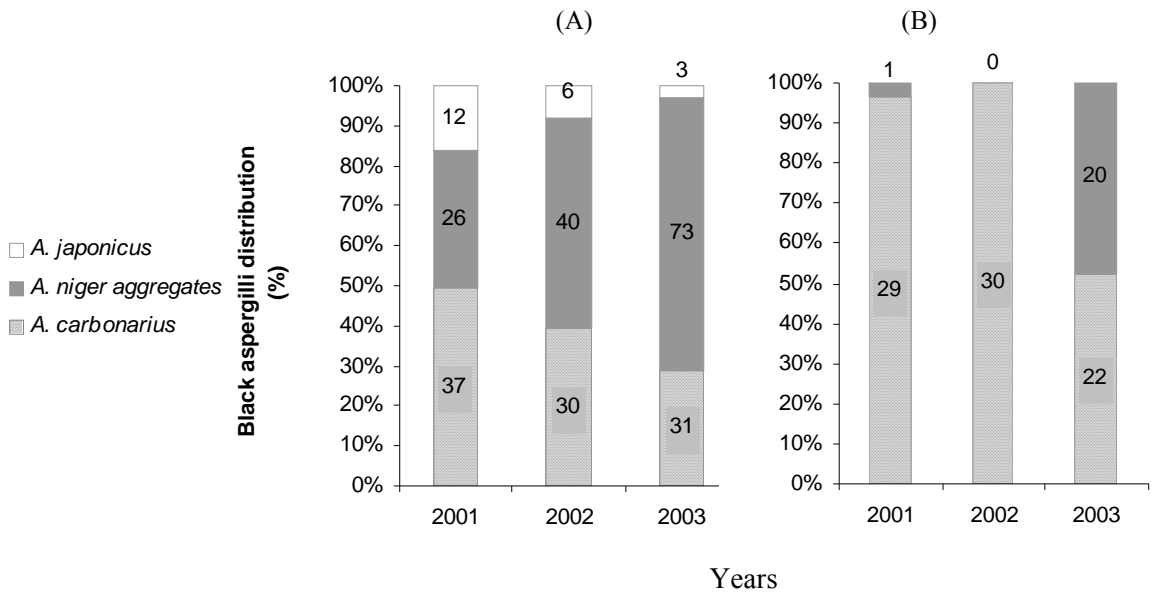


Fig. 4: Black aspergilli species distribution among total (A) and ochratoxigenic (B) isolates in Languedoc-Roussillon region and during 2001, 2002 and 2003. (values mentioned on the graphics are average isolate numbers calculated on vineyards of LR region)

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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 |

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|---------|----------------------|--------|------|-------|
| 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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