Available online at www.sciencedirect.com

SCIENCE DIRECT®

International Journal of Food Microbiology 96 (2004) 19–27

INTERNATIONAL JOURNAL OF Food Microbiology

www.elsevier.com/locate/ijfoodmicro

# Influence of water activity and temperature on growth of isolates of *Aspergillus* section *Nigri* obtained from grapes

N. Bellí, S. Marín, V. Sanchis, A.J. Ramos *

*Food Technology Department, CeRTA-UTPV, University of Lleida, Av. Alcalde Rovira Roure 191, 25198, Lleida, Spain*

Received 29 September 2003; received in revised form 18 February 2004; accepted 22 March 2004

**Abstract**

The effects of water activity ($a_w$) and temperature on growth of *Aspergillus* section *Nigri* isolated from wine grapes were investigated on an agar medium with composition similar to that of grapes. Temperatures in the range of 10–37 °C were tested. Optimum temperatures for growth were between 30 and 37 °C. Water activity levels ranging from 0.90 to 0.995 were tested. Optimum $a_w$ for growth was 0.98 in most cases. Statistical differences were found among the groups tested (*A. carbonarius*, *A. niger* aggregate and *A*. section *Nigri* uniseriates). Growth rates models for the factors assayed have been obtained.


*Keywords:* Water activity; Temperature; Solute; *Aspergillus* section *Nigri*; Growth; Grapes

## 1. Introduction

Ochratoxins are secondary metabolites produced by moulds belonging to several species of the genera *Aspergillus* and *Penicillium*. The most extensively studied compound of this group, ochratoxin A (OTA), has been shown to be a nephrotoxic, immunosuppressive, teratogenic and carcinogenic agent (JEFCA, 1991).

The presence of OTA has been reported in a number of plant products and occasionally in body fluids and kidneys of animals and humans (Xiao et al., 1996; Solti et al., 1997; Burdaspal and Legarda, 2000). First reported in wines in 1996 (Zimmerli and Dick, 1996), OTA has since been found in other grape beverages. Fungi belonging to *Aspergillus* section *Nigri* are the main OTA producers in these products. In addition, recent studies have shown that the three major black species, *A. carbonarius*, *A. niger* aggregate, and *A.* section *Nigri* uniseriates, are all very common in grapes at harvest (Battilani et al., 2003; Bellí et al., 2004).

The most used measure of the availability of water to microorganisms is water activity ($a_w$), the ratio of the vapour pressure of the water in the substrate to that of pure water at the same temperature and pressure. Other environmental factors such as temperature, pH, nutritional factors, etc. also affect mycelial growth and mycotoxin production by moulds. Temperature and $a_w$ are the main factors influencing germination, growth and sporulation of spoilage fungi (Magan and Lacey, 1984).

The objectives of this study were to determine in vitro the effect of $a_w$ and temperature on mycelial

* Corresponding author. Tel.: +34-973-702811; fax: +34-973-702596.

*E-mail address:* ajramos@tecal.udl.es (A.J. Ramos).

0168-1605/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2004.03.004

growth of several isolates of *Aspergillus* section *Nigri* isolated from grapes, on a synthetic nutrient medium similar to grape composition.

## 2. Materials and methods

### 2.1. Fungi

All the fungi used in this study were isolated from European wine grapes during the year 2001. Isolates were classified as *A. carbonarius* (W120, 93cr4, A0204, 36br4, 01UAs219, 01UAs294, A0933, CA332, Mu644), *A. niger* aggregate (93RJ4, 11V4, 01UAs203, 01UAs127, A1099, A1109, C432, Mu246), and *Aspergillus* section *Nigri* uniseriates (73r3, W118, 01UAs128, A0704, A0212).

Isolates were obtained from (1) the Faculty of Agriculture, Università Cattolica del Sacro Cuore, Piacenza, Italy; (2) the Institut National Polytechnique de Toulouse, Ecole Nationale Supérieure Agronomique de Toulouse, France; (3) the Departamento Engenharia Biologica, Universidade do Minho, Braga, Portugal; (4) Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Univ. Autònoma de Barcelona, Spain; (5) the Departament Tecnologia d'Aliments, Escola Tècnica Superior d'Enginyeria Agrària, Universitat de Lleida, Spain, where samples of each isolate are held in their culture collection.

### 2.2. Growth medium

A synthetic nutrient medium (SNM) with composition similar to that of grapes between veraison and ripeness (modified from Delfini, 1982) was used. It had the following composition: D(+) glucose, 70 g; D(−) fructose, 30 g; L(+) tartaric acid, 7 g; L(−) malic acid, 10 g; $(NH_4)_2SO_4$, 0.67 g; $(NH_4)_2HPO_4$, 0.67 g; $KH_2PO_4$, 1.5 g; $MgSO_4 \cdot 7H_2O$, 0.75 g; NaCl, 0.15 g; $CaCl_2$, 0.15 g; $CuCl_2$, 0.0015 g; $FeSO_4 \cdot 7H_2O$, 0.021 g; $ZnSO_4$, 0.0075 g; (+) catechin, 0.05 g, distilled water, 1 l; pH adjusted to 4.2 with KOH (2 N); agar, 20 g.

### 2.3. Water activity and solute type

Water activity was determined with a water activity meter (AquaLab, Decagon CX-2, Pullman, Washington, USA). Amounts of solute (glycerol or glucose) necessary to adjust the SNM growth medium to 0.90, 0.93, 0.95, 0.98 and 0.995 $a_w$ were calculated and added (Table 1). These amounts were determined by interpolation in two experimental curves, one for each solute, obtained by plotting different concentrations of solutes versus resulting $a_w$. Additional, control plates were prepared and measured at the end of each experiment in order to detect any significant deviation.

### 2.4. Inoculation and incubation

Fungi were grown on Czapek Yeast Autolysate Agar (CYA) for 7 days at 25 °C to obtain heavily sporulating cultures. Spore suspensions were obtained by harvesting spores of each isolate from these cultures and suspending them in sterile distilled water containing 0.005 % of a wetting agent (Tween 80, Probus, Barcelona, Spain). The final concentration of the spores was assessed by using a Thoma chamber, and was adjusted to $10^6$ spores $ml^{-1}$. SNM agar plates (20 ml) were needle inoculated centrally with each spore suspension. Plates with the same $a_w$ were sealed with parafilm, distributed in polyethylene bags (20 plates/bag) and finally incubated at the required temperature (10, 15, 20, 25, 30 and 37 °C).

### 2.5. Measurement of growth

Mycelial growth rates were determined by daily measurement of two right-angled diameters of the colonies. Measurements were carried out for a maximum of 60 days. Linear regression of colony radius against time (days) was used to obtain the growth rates (mm $day^{-1}$) under each set of growth conditions. Lag phase for growth was defined as the time (days) to reach 5 mm of diameter.

Table 1
Amounts (g) of solute (glycerol or glucose) necessary to make up 100 ml of medium (SNM) at the required $a_w$

| $a_w$ | Glycerol | Glucose |
|---|---|---|
| 0.90 | 29.6 | 50.0 |
| 0.93 | 21.8 | 38.1 |
| 0.95 | 16.3 | 27.8 |
| 0.98 | 7.7 | 8.9 |
| 0.995 | 3.2 | 0 |

### 2.6. Experiments

The study was divided into three parts as two preliminary trials were carried out before the main experiment.

#### 2.6.1. Effect of different solutes (glycerol and glucose) on the growth rates of six A. Section Nigri isolates, at 25 °C on SNM

A preliminary trial was carried out comparing growth rates of two isolates of *A. carbonarius* (W120, 93cr4), two isolates of *A. niger* aggregate (W119, 11V4) and two isolates of *A.* section *Nigri* uniseriates (73r3, W118), at different $a_w$ (0.90, 0.93, 0.95, 0.98 and 0.995), on SNM at 25 °C. The media were modified to achieve the correct $a_w$ by using either glycerol or glucose in order to study the effect of these solutes on the mould growth and to decide which would be more suitable for further studies.

A full-factorial design with three replicates was applied. The factors assayed were solute (glycerol and glucose) and isolates ($n=6$) as qualitative factors, and $a_w$ levels ($n=5$) as a quantitative factor. The response recorded was colony diameter.

#### 2.6.2. Effect of $a_w$ on growth rates of 20 A. Section Nigri isolates at 25 °C on SNM

Subsequent detailed experiments on the influence of $a_w$ on growth of a larger number of isolates (six *A. carbonarius*, eight *A. niger* aggregate and six uniseriates), were carried out at 25 °C on unmodified medium (0.995 $a_w$) and media modified with glycerol to 0.98, 0.95, 0.93 and 0.90 $a_w$. The objective was to verify the results of the first trial on $a_w$ influence on these species growth in order to establish interspecific differences.

A full-factorial design with three replicates was applied. The factors assayed were isolate ($n=20$) as a qualitative factor, and $a_w$ levels ($n=5$) as a quantitative factor. The response recorded was colony diameter.

#### 2.6.3. Temperature and $a_w$ influence on the growth of 10 black Aspergillus isolates on SNM

The combined effect of temperature and $a_w$ on the growth of 10 black Aspergilli isolates (four *A. carbonarius*, three *A. niger* aggregate and three uniseriates) on SNM was studied. This medium was modified in the range of $a_w$ 0.90–0.995 by the addition of glycerol. Plates were incubated at five different temperatures (10, 15, 20, 30, 37 °C).

A full factorial design with three replicates was applied. The factors assayed were isolate ($n=20$) as a qualitative factor, and $a_w$ levels ($n=5$) and temperature ($n=5$) as quantitative factors. The response recorded was colony diameter.

### 2.7. Statistical treatment of the results

Colony diameters (mm) were statistically analysed (SAS Institute, version 8.2, Cary, NC, USA), so that effects of single factors (isolate, solute, $a_w$ and temperature), and their interactions could be assessed at statistically significant differences ($p<0.05$). Growth rates were modelled by polynomial multiple lineal regression (MLR) with six coefficients ($b_0$, $b_1$, $b_2$, $b_{12}$, $b_{11}$ and $b_{22}$): growth rate $= b_0 + b_1\, a_w + b_2 T + b_{12} a_w T + b_{11} a_w^2 + b_{22} T^2$. The resulting response surface models (RSM) were obtained with the Unscrambler® software, version 7.6 (CAMO ASA, Oslo, Norway), including the significant factors, interactions and quadratic terms.

## 3. Results and discussion

### 3.1. Effect of glycerol and glucose on the growth rates of six A. Section Nigri isolates at 25 °C on SNM

No significant differences between glycerol and glucose were found, although the growth was slightly faster and the lag phase for growth shorter with glucose, as sugars are the preferred carbon source for *A. niger* (Hatzinikolaou and Macris, 1995). However, glycerol was chosen for subsequent experiments as the dissolution of high amounts of glucose was more time-consuming than working with glycerol.

Significant differences were found between isolates, $a_w$ and their interaction. The differences in the isolates were due to differences among the different groups, as observed with further statistical analysis. Isolates belonging to the same group had similar growth rates and lag phases for growth under all the conditions tested, however, there were differences among the different groups: *A. carbonarius* growth rates were significantly lower than those of *A. niger*

aggregate and uniseriate isolates; moreover, lag phases for growth of *A. carbonarius* and uniseriates were higher than those of *A. niger* aggregate. Maximum growth rates and the shortest lag phases for growth were observed for all fungi at high $a_w$ (0.95–0.995) (Fig. 1).

### 3.2. Effect of $a_w$ on growth rates of 20 A. Section Nigri isolates at 25 °C on SNM

The isolates tested showed different growth rates and responses to $a_w$ and the significant differences found were mainly attributed to the group to which the isolate belonged (*A. carbonarius*, *A. niger* aggregate and uniseriates). Analysis of variance revealed that water relation profiles were similar for all the isolates of *A. niger* aggregate, being the ones which presented the fastest growth rates, higher than 5 mm day$^{-1}$ except at the lowest $a_w$ . All *A. carbonarius* isolates tested presented similar growth rates, below 5 mm day$^{-1}$, except for the isolate 01UAs294 which reached 7.8 mm day$^{-1}$ at 0.98 and 0.995 $a_w$. Uniseriate isolates also showed the same behaviour, with the maximum growth rates at 0.98 $a_w$, except for

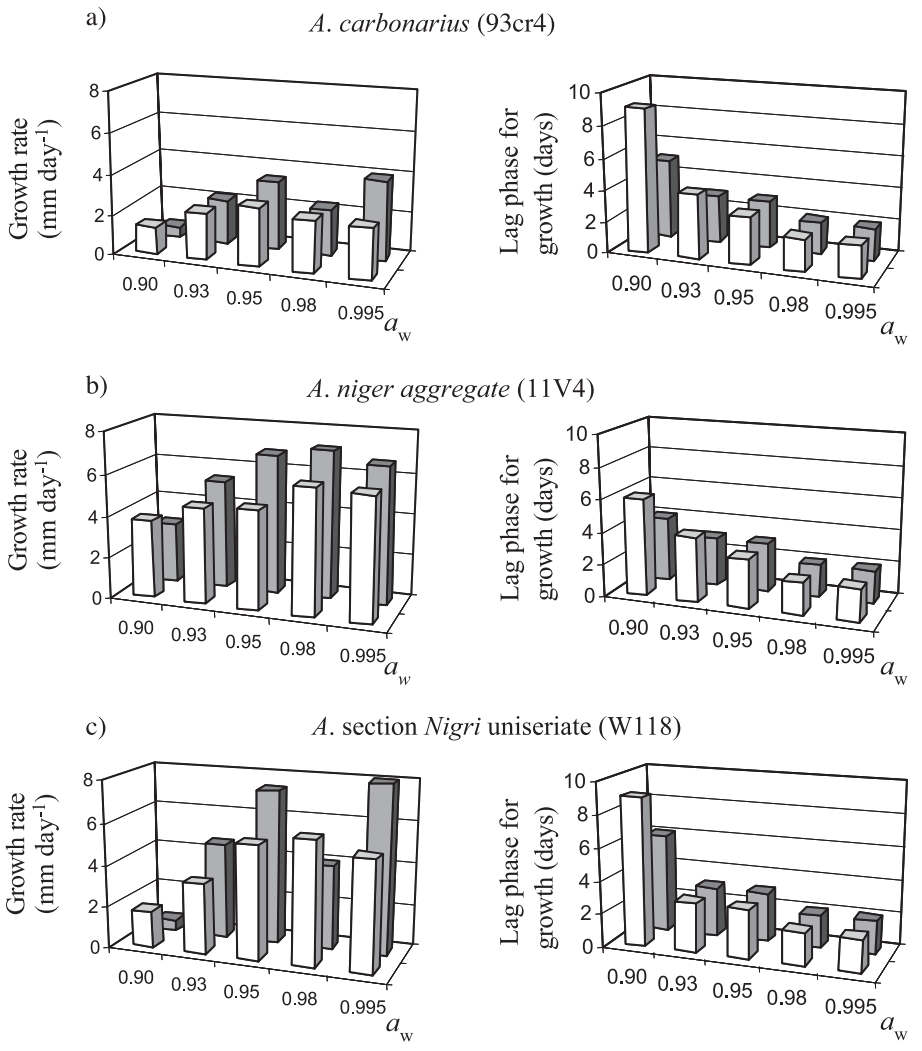


Fig. 1. Effect of $a_w$ on growth rate (mm day$^{-1}$) and lag phase (days until the colony diameter reached 5 mm) of three black *Aspergilli* isolates at 25 °C on SNM. Solutes were glycerol (□) and glucose (■).

isolate A0704, which presented the lowest growth rates values of this group.

Water activity effect was statistically significant for *A. carbonarius* and uniseriate isolates; *A. carbonarius* grew faster at higher $a_w$ (0.98–0.995), whereas similar growth rates were detected at 0.93 and 0.95 $a_w$, and the lowest growth rates were at 0.90 $a_w$ (Fig. 2). Growth of uniseriates was significantly lower at 0.90 $a_w$ but similar at the other $a_w$ levels tested. No differences on the growth rates of *A. niger* aggregate at the different $a_w$ levels were detected, although the optimum $a_w$ level for growth was between 0.98 and 0.995 $a_w$. As no statistical differences were found among the isolates tested into each group (except for the two cases mentioned above), Fig. 2 shows the influence of $a_w$ on the growth rates of two isolates of each group. The country of origin of each isolate did not have any significant influence on their growth rates.

This second trial confirmed that at 25 °C on SNM, all *A.* section *Nigri* grew better at high levels of $a_w$, with an optimum between 0.95 and 0.995 and also confirmed the differences on the growing patterns of the different groups in the section *Nigri*.

### 3.3. Temperature and $a_w$ influence on the growth of 10 black Aspergillus isolates on SNM

Lag phases for growth were noticeably influenced by both temperature and $a_w$. Generally, the time required to reach the linear phase increased with decreasing temperatures. Water activity influenced lag phases for growth in the same way as at 25 °C in the previous sections.

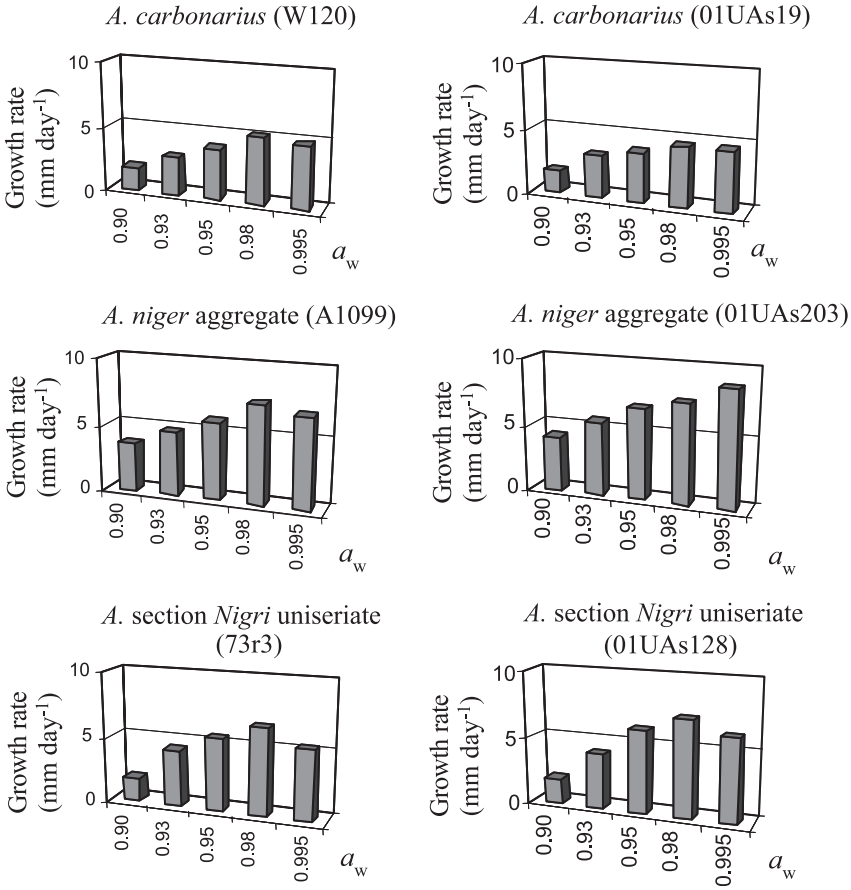


Fig. 2. Effect of $a_w$ on growth rate (mm day$^{-1}$) of six black *Aspergilli* isolates at 25 °C on SNM.

Table 2
Growth rates (mm day$^{-1}$) of 10 *A.* section *Nigri* isolates at different $a_w$ and temperatures on SNM

| Isolate | $a_w$ | Growth rates (mm day$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10° C | 15° C | 20° C | 25 °C | 30° C | 37° C |
| *A. carbonarius* (36br4) | 0.90 | 0$^{a}$ | 0.13$^{a}$ | 0.83$^{a}$ | 1.77$^{a}$ | 1.94$^{a}$ | 0.54$^{a}$ |
| | 0.93 | 0$^{a}$ | 0.68$^{b}$ | 1.88$^{b}$ | 2.92$^{b}$ | 3.74$^{a}$ | 2.97$^{b}$ |
| | 0.95 | 0.28$^{b}$ | 1.14$^{c}$ | 2.35$^{c}$ | 3.92$^{b}$ | 4.22$^{b}$ | 3.32$^{c}$ |
| | 0.98 | 0.43$^{c}$ | 1.51$^{c}$ | 2.46$^{c}$ | 5.12$^{b}$ | 5.38$^{b}$ | 4.51$^{c}$ |
| | 0.995 | 0.31$^{b}$ | 1.05$^{c}$ | 1.3$^{bc}$ | 4.77$^{b}$ | 4.02$^{ab}$ | 3.2$^{c}$ |
| *A. carbonarius* (A0933) | 0.90 | 0$^{a}$ | 0.09$^{a}$ | 0.45$^{a}$ | 1.34$^{a}$ | 1.01$^{a}$ | 0.44$^{a}$ |
| | 0.93 | 0$^{a}$ | 0.69$^{b}$ | 1.61$^{b}$ | 2.06$^{ab}$ | 3.4$^{b}$ | 1.92$^{b}$ |
| | 0.95 | 0.21$^{b}$ | 1.01$^{c}$ | 2.17$^{c}$ | 2.94$^{b}$ | 3.65$^{b}$ | 2.04$^{b}$ |
| | 0.98 | 0.36$^{c}$ | 1.26$^{c}$ | 2.57$^{c}$ | 2.74$^{b}$ | 3.45$^{b}$ | 2.62$^{b}$ |
| | 0.995 | 0.16$^{a}$ | 0.92$^{c}$ | 2.07$^{c}$ | 3.36$^{b}$ | 3.76$^{b}$ | 2.24$^{b}$ |
| *A. carbonarius* (Mu644) | 0.90 | 0$^{a}$ | 0.51$^{a}$ | 1.46$^{a}$ | 1.55$^{a}$ | 3.47$^{a}$ | 4.06$^{a}$ |
| | 0.93 | 0$^{a}$ | 0.82$^{b}$ | 1.77$^{b}$ | 2.84$^{b}$ | 4.58$^{a}$ | 7.18$^{a}$ |
| | 0.95 | 0.39$^{b}$ | 0.99$^{c}$ | 2.31$^{c}$ | 3.05$^{b}$ | 5.15$^{a}$ | 7.3$^{a}$ |
| | 0.98 | 0.62$^{c}$ | 1.28$^{d}$ | 2.62$^{c}$ | 2.97$^{b}$ | 4.54$^{a}$ | 5.13$^{a}$ |
| | 0.995 | 0.4$^{d}$ | 0.89$^{d}$ | 1.85$^{c}$ | 2.90$^{b}$ | 4.51$^{a}$ | 4.68$^{a}$ |
| *A. carbonarius*(01UAs294) | 0.90 | 0$^{a}$ | 0.07$^{a}$ | 0.71$^{a}$ | 3.15$^{a}$ | 4$^{a}$ | 2.18$^{a}$ |
| | 0.93 | 0$^{a}$ | 0.33$^{a}$ | 2.14$^{b}$ | 5.18$^{a}$ | 5.85$^{a}$ | 5.15$^{b}$ |
| | 0.95 | 0$^{a}$ | 0.74$^{b}$ | 2.61$^{c}$ | 5.87$^{a}$ | 7.57$^{a}$ | 5.79$^{b}$ |
| | 0.98 | 0.14$^{b}$ | 1.43$^{c}$ | 2.73$^{bc}$ | 7.57$^{a}$ | 9.11$^{a}$ | 6.35$^{b}$ |
| | 0.995 | 0.09$^{ab}$ | 1.00$^{c}$ | 2.36$^{c}$ | 7.81$^{a}$ | 6.97$^{a}$ | 3.07$^{ab}$ |
| *A. niger* aggregate (11V4) | 0.90 | 0$^{a}$ | 0.64$^{a}$ | 1.62$^{a}$ | 4.16$^{a}$ | 4.62$^{a}$ | 4.89$^{a}$ |
| | 0.93 | 0$^{a}$ | 1.06$^{b}$ | 2.57$^{bc}$ | 6.34$^{a}$ | 7.04$^{a}$ | 7.23$^{a}$ |
| | 0.95 | 0.18$^{a}$ | 1.35$^{c}$ | 3.35$^{b}$ | 7.06$^{a}$ | 8.29$^{a}$ | 9.17$^{a}$ |
| | 0.98 | 0.39$^{b}$ | 1.38$^{c}$ | 3.16$^{b}$ | 8.64$^{a}$ | 9.34$^{a}$ | 8.9$^{a}$ |
| | 0.995 | 0.47$^{b}$ | 1.14$^{c}$ | 2.49$^{c}$ | 9.53$^{a}$ | 5.24$^{a}$ | 7.54$^{a}$ |
| *A. niger* aggregate (A1109) | 0.90 | 0$^{a}$ | 0.69$^{a}$ | 1.77$^{a}$ | 3.08$^{a}$ | 4$^{a}$ | 3.81$^{a}$ |
| | 0.93 | 0.32$^{a}$ | 1.13$^{b}$ | 2.5$^{b}$ | 3.96$^{a}$ | 4.9$^{a}$ | 7.07$^{b}$ |
| | 0.95 | 0.45$^{b}$ | 1.41$^{c}$ | 2.79$^{c}$ | 4.27$^{a}$ | 5.1$^{a}$ | 7.83$^{b}$ |
| | 0.98 | 0.61$^{c}$ | 1.5$^{c}$ | 3.15$^{c}$ | 4.73$^{a}$ | 6.14$^{ab}$ | 6.29$^{b}$ |
| | 0.995 | 0.45$^{b}$ | 1.32$^{c}$ | 2.76$^{bc}$ | 4.63$^{a}$ | 5.56$^{b}$ | 5.09$^{b}$ |
| *A. niger* aggregate (01UAs127) | 0.90 | 0$^{a}$ | 0.52$^{a}$ | 1.81$^{a}$ | 4.00$^{a}$ | 4.26$^{a}$ | 3.92$^{a}$ |
| | 0.93 | 0$^{a}$ | 0.91$^{b}$ | 2.55$^{b}$ | 5.43$^{a}$ | 6.42$^{a}$ | 7.05$^{a}$ |
| | 0.95 | 0.22$^{a}$ | 1.49$^{c}$ | 3.16$^{b}$ | 6.28$^{a}$ | 7.27$^{a}$ | 7.16$^{a}$ |
| | 0.98 | 0.43$^{b}$ | 1.51$^{c}$ | 3.2$^{b}$ | 7.36$^{a}$ | 7.84$^{a}$ | 9.03$^{a}$ |
| | 0.995 | 0.12$^{a}$ | 1.23$^{c}$ | 2.51$^{ab}$ | 7.40$^{a}$ | 6.97$^{a}$ | 5.98$^{a}$ |
| *A.* section *Nigri* uniseriate (W118) | 0.90 | 0$^{a}$ | 0.44$^{a}$ | 1.59$^{a}$ | 1.77$^{a}$ | 2.64$^{a}$ | 3.74$^{a}$ |
| | 0.93 | 0$^{a}$ | 0.55$^{a}$ | 1.59$^{a}$ | 3.88$^{b}$ | 2.9$^{b}$ | 1.79$^{a}$ |
| | 0.95 | 0.46$^{b}$ | 0.92$^{b}$ | 2.22$^{b}$ | 6.01$^{b}$ | 5.87$^{b}$ | 2.31$^{a}$ |
| | 0.98 | 0.78$^{c}$ | 1.47$^{c}$ | 3.71$^{b}$ | 6.81$^{b}$ | 7.56$^{b}$ | 3.54$^{a}$ |
| | 0.995 | 0.06$^{ab}$ | 0.8$^{b}$ | 3.59$^{b}$ | 5.46$^{b}$ | 7.6$^{b}$ | 6$^{a}$ |
| *A.* section *Nigri* uniseriate (A0212) | 0.90 | 0$^{a}$ | 0.02$^{a}$ | 0.13$^{a}$ | 3.57$^{a}$ | 0.77$^{a}$ | 3.01$^{a}$ |
| | 0.93 | 0$^{a}$ | 0.23$^{b}$ | 0.94$^{b}$ | 3.46$^{b}$ | 3.98$^{b}$ | 0.51$^{ab}$ |
| | 0.95 | 0.06$^{b}$ | 0.29$^{b}$ | 1$^{b}$ | 6.37$^{b}$ | 6.99$^{b}$ | 0.89$^{ab}$ |
| | 0.98 | 0.23$^{c}$ | 0.8$^{c}$ | 2.72$^{c}$ | 8.67$^{b}$ | 5.52$^{b}$ | 5.45$^{c}$ |
| | 0.995 | 0.17$^{c}$ | 1.55$^{d}$ | 2.14$^{d}$ | 5.62$^{b}$ | 4.9$^{b}$ | 1.33$^{b}$ |
| *A.* section *Nigri* uniseriate (01UAs128) | 0.90 | 0$^{a}$ | 0$^{a}$ | 0.4$^{a}$ | 1.83$^{a}$ | 1.64$^{a}$ | 2.93$^{a}$ |
| | 0.93 | 0$^{a}$ | 0.35$^{b}$ | 1.64$^{b}$ | 4.10$^{b}$ | 2.93$^{b}$ | 2.38$^{a}$ |
| | 0.95 | 0$^{a}$ | 0.75$^{c}$ | 2.37$^{c}$ | 6.15$^{b}$ | 5.23$^{c}$ | 2.17$^{a}$ |
| | 0.98 | 0.14$^{b}$ | 1.37$^{d}$ | 3.25$^{c}$ | 7.19$^{b}$ | 7.01$^{bc}$ | 5.99$^{a}$ |
| | 0.995 | 0.11$^{b}$ | 1.36$^{d}$ | 1.72$^{c}$ | 6.17$^{b}$ | 6.99$^{c}$ | 3.6$^{a}$ |

For each isolate, data in the same column followed by different letters are significantly different in LSMEANS test.

There were statistical differences between the growth of the different isolates tested, due again to the group to which they belonged (*A. carbonarius*, *A. niger* aggregate and uniseriate). Growth was influenced by both factors assayed ($a_w$ and temperature) and their interaction. Table 2 shows growth rates and statistical significance of each isolate under all $a_w$ at the different temperature levels tested. The growth of *A. niger* aggregate was higher than the growth of the other two groups, except at low temperatures (10–20 °C), when the growth rates were very similar for the isolates of different groups. At 30 and 37 °C, *A. carbonarius* growth rates were below 5 mm day$^{-1}$. Again, the isolate 01UAs294 grew faster than the other isolates of *A. carbonarius* tested, but this isolate has been shown to present some molecular differences in comparison with the others (Dr. J. Cabañes, Animal Health and Anatomy Department, Autonomous University of Barcelona, Spain, personal communication). Furthermore, there were differences on the growth of the isolates belonging to the other two groups of black Aspergilli, although these differences were not attributable to the country of origin.

In all cases, the minimum growth rates were at 10 °C, increasing slightly at 15 °C and again at 20 °C. Similar results for *A. carbonarius* were found by Mitchell et al. (2003), who reported no isolate growing at 10 °C, whereas at 15 °C growth was only possible at higher $a_w$ levels. In our study, at the highest temperatures (30 and 37 °C), all the isolates showed higher growth rates than at the remaining temperatures with 30 °C being the optimum for *A. carbonarius* and uniseriate isolates, although the latter had highest growth rates at 37 °C than at 30 °C when $a_w$ level was the lowest (0.90). Similarly, other authors found optimum temperature for *A. carbonarius* between 25 and 35 °C (Leong et al., 1999; Mitchell et al., 2003). In the present study, all *A. niger* aggregate isolates growth rates at 30 and 37 °C were not significantly different regardless of $a_w$. The effect of environmental factors on the growth of one isolate of *A. niger* was studied by Vats and Banerjee (2002) and found that the isolate grow best at 30 °C but contrary to us, found a sharp decline at 37 °C and no growth at 45 °C and above.

For all the isolates and temperatures assayed, growth rates increased with $a_w$ reaching the optimum at 0.98 $a_w$ and decreasing slightly at 0.995 $a_w$. At 10 °C, the growth of all the isolates was negligible when $a_w$ levels were low (0.90 and 0.93 $a_w$), increasing slightly at 0.95, 0.98 and 0.995 $a_w$. All the isolates were more tolerant of low $a_w$ at temperatures close to the optimum.

Models of growth rates versus $a_w$ and temperatures were obtained (Fig. 3). Negative values must be interpreted as null values. Table 3 presents the model coefficients estimated by multiple linear regression for main effects, as well as interaction and quadratic terms.

Results obtained on culture media cannot easily be extrapolated to natural systems as the reactions in the

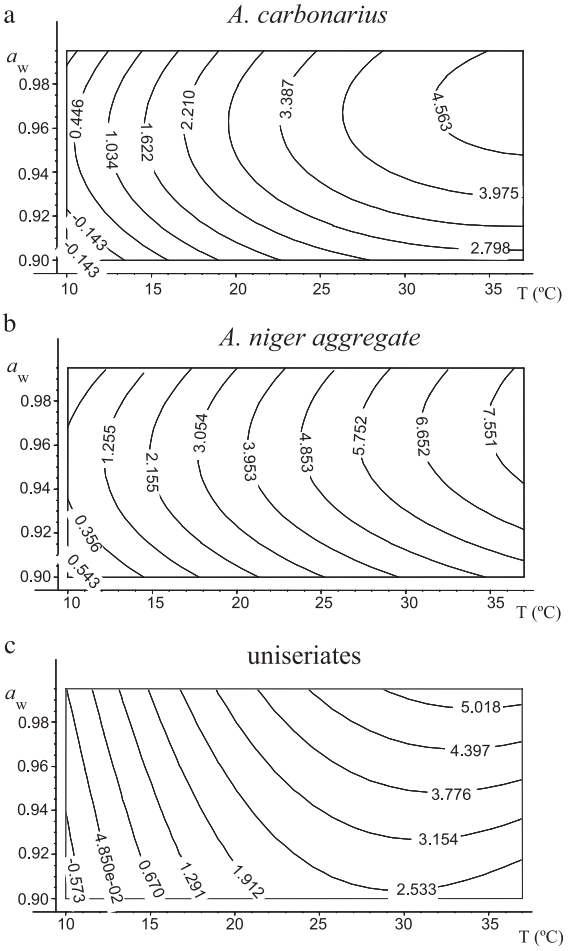


Fig. 3. Response surface contour plots showing the effect of $a_w$ and temperature on growth rates (mm day$^{-1}$) of *Aspergillus* section *Nigri*: (a) *A. carbonarius*, (b) *A. niger* aggregate and (c) uniseriates, on SNM.

Table 3
Model coefficients obtained by MLR for effects of $a_w$ and temperature on growth rates (mm day$^{-1}$) on SNM

| Factors | Regression coefficients | | |
|---|---|---|---|
| | *A. carbonarius*[a] | *A. niger* aggregate[b] | Uniseriates[c] |
| Intercept ($b_0$) | − 11.316** | − 11.926** | − 21.258** |
| $a_w$ ($b_1$) | 11.290** | 10.674** | 21.465** |
| Temperature ($b_2$) | 0.172** | 0.276** | 0.172** |
| $a_w \times T$ ($b_{12}$) | 0.195$^{ns}$ | 0.268* | 0.321* |
| $a_w^2$ ($b_{11}$) | − 0.505** | − 0.656** | $3.587 \cdot 10^{-2}$ ns |
| $T^2$ ($b_{22}$) | − 0.535$^{ns}$ | − 0.310* | − 0.859$^{ns}$ |
| $R^2$ | 0.693 | 0.907 | 10.659 |

ns = Not significant. $R^2$ = percentage of variation explained by the model.
Values of the interaction and square effects for:
[a] *A. carbonarius*: $T^*a_w = 0.1010(T - 22.4) \times 29.1636(a_w - 0.951)$; $(T)^2 = 0.1010(T - 22.4))^2$; $(a_w)^2 = (29.1636(a_w - 0.951))^2$.
[b] *A. niger* aggregate: $T^*a_w = 0.1008(T - 22.4) \times 29.1145(a_w - 0.951)$; $(T)^2 = (0.1008(T - 22.4))^2$; $(a_w)^2 = (29.1145(a_w - 0.951))^2$.
[c] Uniseriates: $T^*a_w = 0.1008(T - 22.4) \times 29.1145(a_w - 0.951)$; $(T)^2 = (0.1008(T - 22.4))^2$; $(a_w)^2 = (29.1145(a_w - 0.951))^2$.
* Significant $p < 0.05$.
** Significant $p < 0.01$.

field can be modified by the ecosystem (Magan and Lacey, 1984). However, these preliminary results can provide an indication of the growing patterns of these species as affected by environmental conditions, and may be a first step for further experimental design at field conditions. Furthermore, studies directly on grapes are in progress.

## 4. Conclusions

*A*. section *Nigri* may grow at all the temperatures tested in this study, ranging from 10 to 37 °C, with an optimum between 30 and 37 °C, with the latter conditions being frequent in the field close to harvest time. However, at 10 °C the growth of all the isolates was negligible when $a_w$ levels were low (0.90 and 0.93 $a_w$). The optimum $a_w$ for black Aspergilli to grow, seems to be at 0.98, similar to the $a_w$ of grapes in the field. Thus, field conditions are likely to be conducive to optimum growth of these species. Furthermore, there were statistical differences between the growth of the different isolates tested, mainly due to the group to which the isolate belonged (*A. carbonarius*, *A. niger* aggregate and uniseriate), with the *A. niger* aggregate presenting the fastest growth rates in the three trials and *A. carbonarius* the lowest.

A better knowledge of the growth condition for OTA-producing fungi is critical for an understanding of the ecological niche occupied by these black *Aspergillus* spp. Moreover, further studies should be conducted to improve understanding of the ecology and to study the effect of the variables that most likely would influence toxin production by the main ochratoxigenic moulds in grapes. Based on these studies, remedial measures to prevent their establishment could be implemented.

## Acknowledgements

The authors are grateful to the Catalonian Government (Direcció General de Recerca, Generalitat de Catalunya), the Spanish Government (CICYT, Comisión Interministerial de Ciencia y Tecnología, project AGL 2001 2974-C05-02), and the EC, Quality of Life Programme (QoL), Key Action 1 (KA1) on Food, Nutrition and Health (QLRT-2000-01761) for their financial support. We also thank the participants of this European project for providing us with the isolates used in this study.

## References

Battilani, P., Pietri, A., Bertuzzi, T., Languasco, L., Giorni, P., Kozaakiewicz, Z., 2003. Occurrence of ochratoxin A-producing fungi in grapes grown in Italy. Journal of Food Protection 66, 633–636.

Bellí, N., Pardo, E., Marín, S., Farré, G., Ramos, A.J., Sanchis, V., 2004. Occurrence of ochratoxin A and potential producers on grapes from Spain. Journal of the Science of Food and Agriculture (in press).

Burdaspal, P.A., Legarda, T.M., 2000. Datos sobre la presencia de ocratoxina A en plasma humano en España. Alimentaria Mayo, 98–103.

Delfini, C., 1982. In: Scialpi, L. (Ed.), Tecnica di Microbiologia Enologica. Rome.

Hatzinikolaou, D.G., Macris, B.J., 1995. Factors regulating production of glucose-oxidase by *Aspergillus niger*. Enzyme and Microbial Technology 17, 530–534.

JEFCA, 1991. Evaluation of certain food additives and contaminants. 37th report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 806, Geneva, pp. 29–31.

Leong, S.L., Pitt, J.I, Hocking, A.D., 1999. Black Aspergilli on

dried vine fruits. IXth Int. Cong. of Mycology, Int. Union of Microbiol Soc., Sydney, NSW, Australia, 16–20 August, 1999. Abstract MP15.12.

Magan, N., Lacey, J., 1984. Effect of temperature and pH on water relations of field and storage fungi. Transactions of the British Mycological Society 82, 71–81.

Mitchell, D., Aldred, D., Magan, N., 2003. Impact of ecological factors on the growth and ochratoxin A production by *Aspergillus* carbonarius from different regions of Europe. Aspects of Applied Biology, 68.

Solti, L., Salamon, F., Barna-Vetró, I., Gyöngyösi, A., Szabó, E., Wolfing, A., 1997. Ochratoxin content of human sera determined by a sensitive ELISA. Journal Analytical Toxicology 21, 44–48.

Vats, P., Banerjee, U.C., 2002. Studies on the production of phytase by a newly isolated strain of *Aspergillus niger* var teigham obtained from rotten wood-logs. Process Biochemistry 38, 211–217.

Xiao, H., Marquardt, R.R., Abramson, D., Frohlich, A.A., 1996. Metabolites of ochratoxins in rat urine and in culture of *Aspergillus ochraceus*. Applied Environmental Microbiology 62, 648–655.

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