ELSEVIER

International Journal of Food Microbiology 81 (2003) 185–193

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

SEM study of water activity and temperature effects on the initial growth of *Aspergillus ochraceus*, *Alternaria alternata* and *Fusarium verticillioides* on maize grain

M.R. Torres [a], A.J. Ramos [b,*], J. Soler [b], V. Sanchis [b], S. Marín [b]

[a] *Pathology Laboratory, CeRTA Post-Harvest Area, UdL-IRTA Center. Av. Rovira Roure, 191, 25198 Lleida, Spain*
[b] *Department of Food Technology, CeRTA-UTPV, University of Lleida. Av. Rovira Roure, 191, 25198 Lleida, Spain*

Received 28 September 2001; received in revised form 28 March 2002; accepted 31 May 2002

Abstract

A scanning electron microscopy (SEM) study of the effect of water activity (a_w), temperature and fungal interactions on the very early phases of *Aspergillus ochraceus*, *Alternaria alternata* and *Fusarium verticillioides* development on maize grains was carried out. Germination and growth of individual fungal strains were assayed at 0.92, 0.95 and 0.98 a_w, and 20 and 30 °C. Hyphal lengths were measured on micrographs taken by SEM at different periods of incubation. *A. alternata* had the highest linear growth at 0.98 a_w, and was the only species able to grow under all conditions tested, whereas *A. ochraceus* was not able to germinate at 0.92 a_w at any temperature assayed. *F. verticillioides* demonstrated a different behaviour depending on growth temperature. Fungal interactions were studied at 0.95a_w and 30 °C. *A. ochraceus* germination was inhibited when it was co-inoculated with one or two of the other species. *A. alternata* showed an increased growth rate when growing together with the other fungi, whereas growth of *F. verticillioides* was significantly inhibited when paired with *A. ochraceus*.

Keywords: Scanning electron microscopy (SEM); *Aspergillus ochraceus*; *Fusarium verticillioides*; *Alternaria alternata*; Water activity; Maize; Germination; Growth; Ecophysiology

1. Introduction

A wide variety of fungal spores naturally contaminate the surface of grains and, depending on environmental conditions, stored cereal grains may be colonised by a range of different species of fungi. Maize is particularly susceptible to colonisation and infection after silk emergence (Sutton, 1982; Miller, 1994). Biotic and abiotic parameters determine the extent of fungal colonisation and, among them, water activity (a_w), temperature and fungal interactions are the most important.

Germination and growth of fungi have frequently been studied on nutrient media, adjusted to different a_w values with glycerol or other solutes, and incubated at different temperatures, both when species are growing alone and when interacting with other species (Sung and Cook, 1981; Marín et al., 1998a; Abellana et al., 1999). However, results obtained on culture media cannot necessarily be extrapolated to natural

* Corresponding author. Tel.: +34-973-702811; fax: +34-973-702596.
E-mail address: ajramos@tecal.udl.es (A.J. Ramos).

0168-1605/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0168-1605(02)00226-X

systems, and complex interactions between more than one fungal species occurring at the first steps of fungal colonisation directly on maize grains have not been described.

Several studies carried out in our laboratory have demonstrated the effect of a_w and temperature on the microconidial germination and growth of *Fusarium verticillioides* and *Fusarium proliferatum* in maize-based media (Marín et al., 1995a, 1996), and on the germination of spores of *Aspergillus* and *Penicillium* (Marín et al., 1998d), based on microscopical observation of germ tubes.

The usual techniques to study fungal development on culture media or grains are based on the inoculation of fungal spores or mycelium on these substrates and on the observation of germination or growth directly by visual observation or by optical microscopy if the media is transparent enough. The behaviour of the spores in the initial hours of fungal colonisation on a natural substrate is difficult to observe. One of the most promising techniques is the utilisation of the scanning electron microscopy (SEM), a useful tool that allows visualisation of spores directly on a natural substrate and study of fungal development (Bacon et al., 1992). Only a few SEM studies on the influence of abiotic and biotic factors in the fungal colonisation of grains have been reported (Szekely et al., 1991; Ramakrishna et al., 1993, 1996).

In the present study, *Aspergillus ochraceus*, *Alternaria alternata* and *F. verticillioides* were selected as their spores could be easily distinguished by SEM. All are commonly isolated from maize grain and all are mycotoxin-producing species. The objectives of this study were: (i) to study the effect of a_w and temperature on the first steps of fungal development of *A. ochraceus*, *A. alternata* and *F. verticillioides* on maize grains, and (ii) to study their interactions.

2. Materials and methods

2.1. Source of maize grain and gamma-irradiation treatment

Spanish dent maize grain, with an initial water content of 13.9% (0.71 a_w), was used for all experiments. Grain was sterilized with 12 kGy of gamma-irradiation. The grain contained no viable fungal infection or contamination but had retained germinative capacity. The gamma-irradiated maize was stored at 4 °C until use.

2.2. Water activity adjustment of gamma-irradiated maize

Water activity was adjusted to 0.98, 0.95 or 0.92 a_w by aseptically adding sterile distilled water to the grain in 1-l sterile flasks, using a moisture adsorption curve, and then equilibrating for at least 3 days at 7 °C with periodic shaking every 12 h. The amounts of water to be added were calculated experimentally and adjusted to the following equation: $x = 0.0689 \ln y + 1.0831$ ($r^2 = 0.970$); where x was desired a_w and y was the water volume to be added to the grain (ml). All a_w values were confirmed by using a Novasina Humidat IC I Thermoconstanter (Novasina, Zurich, Switzerland).

2.3. Source of isolates

A. ochraceus Wilhelm (NRRL 3174) and *A. alternata* (Fries:Fries) von Keissler A. (IMET 539) were collection strains, whereas *F. verticillioides* 25N was a fumonisin-producing strain isolated from maize grain in our laboratory. All strains were held in the Fungal Strain Collection of the Department of Food Technology of the University of Lleida (Spain).

2.4. Preparation of spore suspensions

Cultures of the three species were grown on potato-dextrose-agar Petri dishes at 25 °C, using for *A. alternata* a 12-h photoperiod system. Spore suspensions of each isolate were prepared by adding a glycerol–water solution, adjusted to the required a_w, to each 21 days old culture, and scraping the surface with a sterile spatula. Spore concentration was determined using a Thoma chamber, and adjusted to 2.5×10^5 spores ml^{-1} before mixing 100 μl of the spore suspension with an equal volume of either water activity adjusted sterile glycerol–water solution or a spore suspension of another species, to give a total of 250 spores of each species in 2 μl suspension. Suspensions were used immediately.

2.5. Inoculation of individual grains

Three gamma-irradiated maize grains, adjusted to 0.98, 0.95 or 0.92 a_w, were aseptically transferred, dent side down, to a one of the three divisions of a three-compartment Petri dish. Each grain was point inoculated with 2 μl of the spore suspension on the flat side. After inoculation, a glycerol–water solution adjusted to the same a_w as the grain was added in a second compartment of the Petri dish, and dishes with the same a_w were placed in sealed containers with beakers of glycerol–water solutions of the same

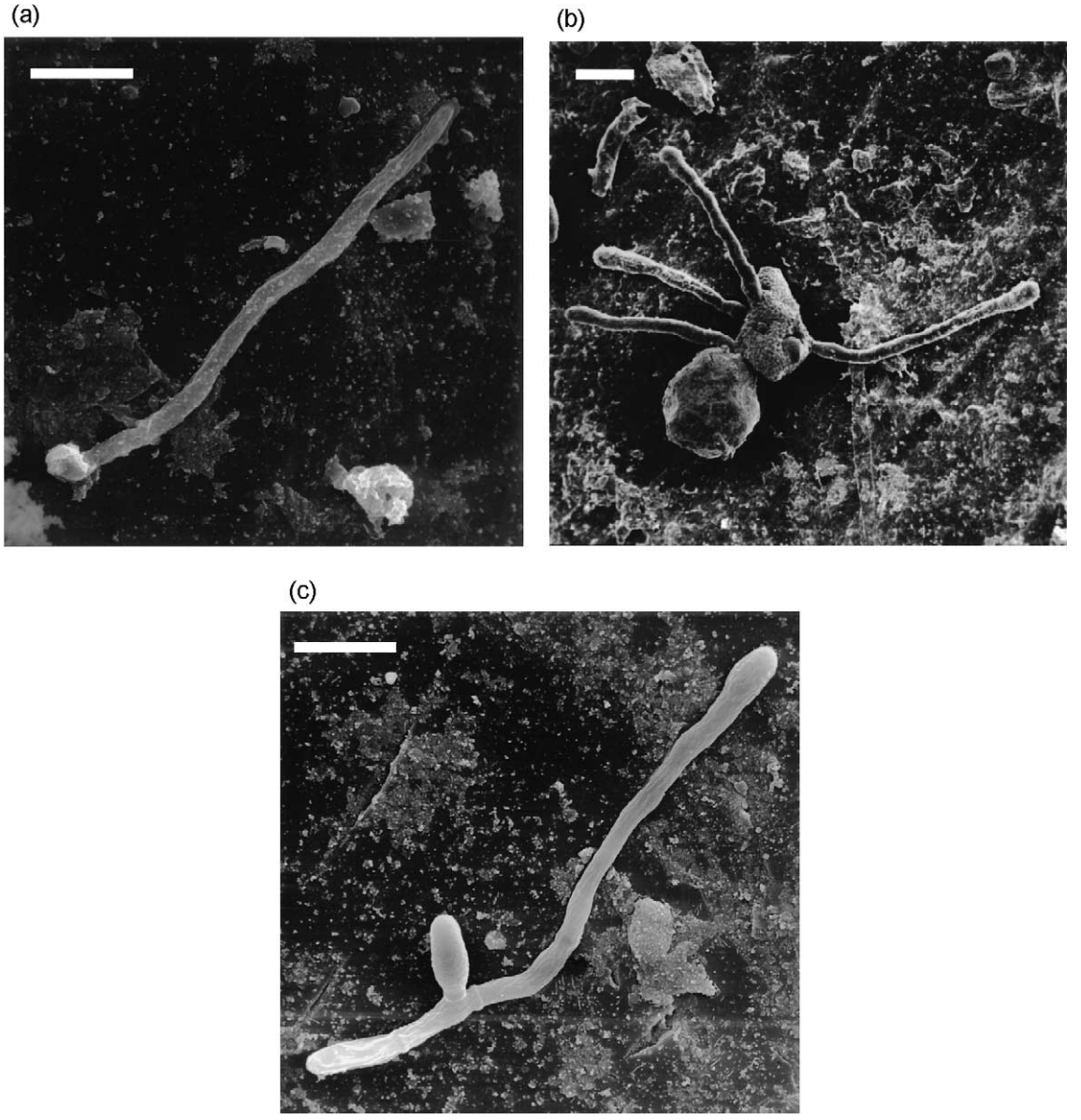

Fig. 1. Fungal development of (a) *A. ochraceus*, (b) *A. alternata* and (c) *F. verticillioides* on the surface of maize grain. Scale bar: 10 μm.

a_w as the treatments, in order to maintain the correct equilibrium relative humidity, and incubated at either 20 or 30 °C, for different incubation periods. The growth of germ tubes or hyphae of fungi on the maize surface was measured for up to 48 h at 20 °C and up to 40 h at 30 °C or until colonies became so enmeshed that they could not be distinguished from one another.

2.6. Observation of maize grain by SEM

2.6.1. Sample preparation

After different incubation periods, depending on the experimental conditions, grains from each treatment were fixed for 24 h by the vapours generated by a formaldehyde 40% (v/v) solution added to the third compartment of the Petri dish. Grains were dried at 42

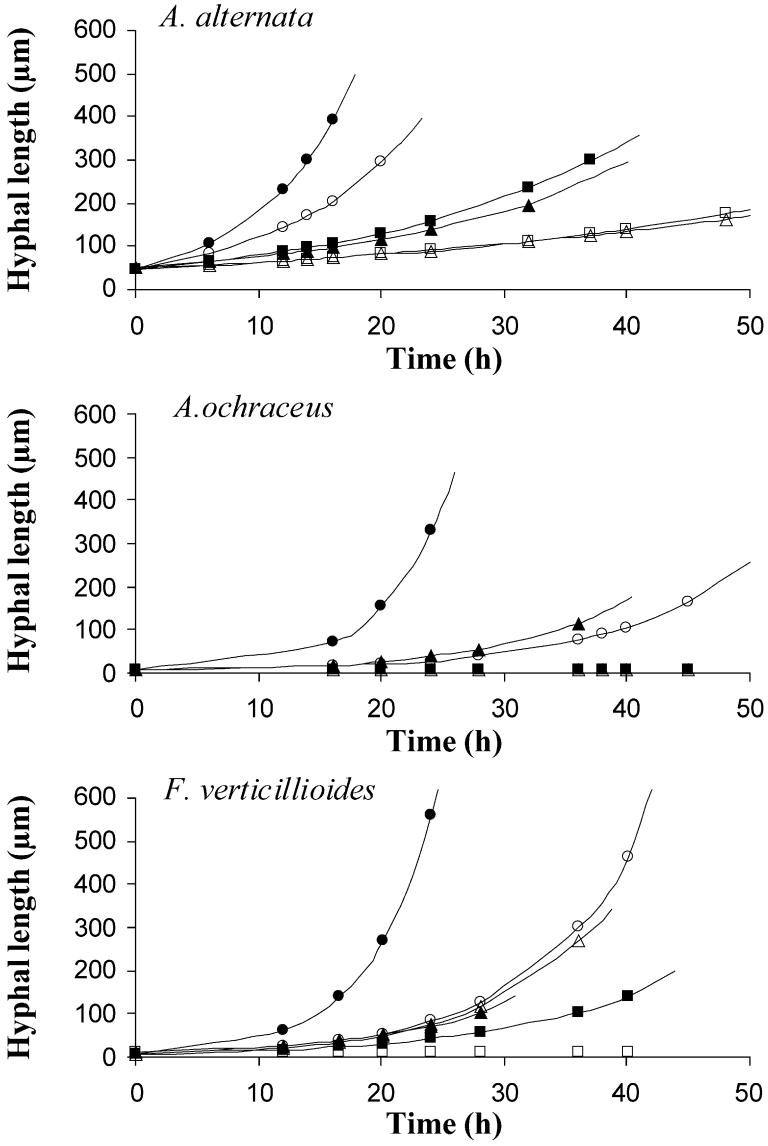

Fig. 2. Hyphal length of *A. alternata*, *A. ochraceus* and *F. verticillioides* growing alone on the surface of maize grain vs. time (□, 0.92 a_w/20 °C; △, 0.95 a_w/20°C; ○, 0.98 a_w/20°C; ■, 0.92 a_w/30°C; ▲, 0.95 a_w/30°C; ●, 0.98 a_w/30°C).

°C for at least 48 h and were then mounted on aluminium stubs, and coated with gold using a Balzers SCD 050 (Balzers Instruments, Fürstentum, Liechtenstein) sputter coater.

2.6.2. Measurement of hyphae

The lengths of the longest hyphae from 20 germinating spores for each of the three fungal species growing alone, or 15 spores in the presence of another species on the grain surface were measured directly using a Scanning Electron Microscope Zeiss DSM940A (Carl Zeiss, Oberkochen, Germany) on photographs taken with the SEM at magnifications from 2000 to 10,000. Graphs of hyphal growth versus time were constructed and data fitted to an exponential curve from which growth rate at each time of incubation could be obtained.

2.7. Statistics

Statistical analysis for the different sets of results were carried out using SAS package (version 6.11, SAS Institute).

3. Results

3.1. Effect of temperature and water activity on spore germination on the surface of maize grains

SEM micrographs of maize kernels showing the development of fungal spores on the surface of the grain are shown in Fig. 1.

Fig. 2 shows the hyphal growth of *A. alternata*, *A. ochraceus* and *F. verticillioides* growing alone on the

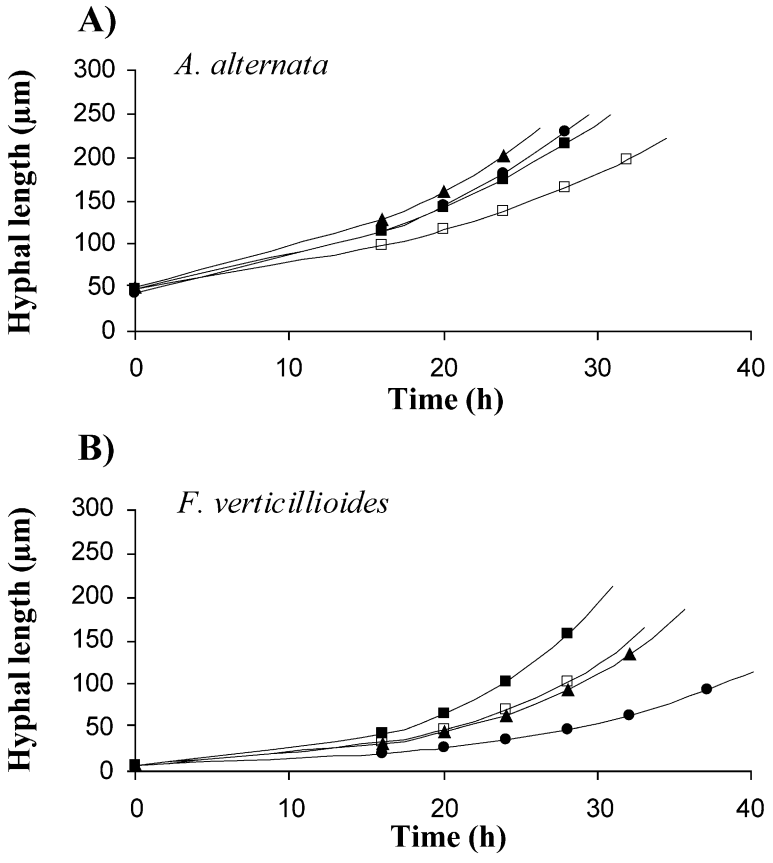

Fig. 3. Effect of fungal interactions on spore germination and hyphal growth at 0.95 a_w and 30°C. (A) □, *A. alternata* (Aa); ●, Aa vs. *F. verticillioides* (Fv); ■, Aa vs. A. ochraceus (Ao); ▲, Aa vs. Fv-Ao. (B) □, *F. verticillioides* (Fv); ●, Fv vs. Ao; ■, Fv vs. Aa; ▲, Fv vs. Aa-Ao.

surface of maize at 20 and 30 °C versus time. As it can be observed, *A. alternata* was able to germinate under all conditions tested, exhibiting a very similar response at 0.92 and 0.95 a_w at both temperatures assayed. At 0.98 a_w, germination was very fast, resulting in growth rates of 18.6 and 51.1 μm h^{-1} at 20 and 30 °C, respectively, after 16 h.

At 20 °C and 0.92–0.95 a_w, there was no germination of *A. ochraceus* over the period of time studied. At 0.98 a_w, exponential growth started after 15 h, reaching a growth rate of 15 μm h^{-1} after 45 h. At 30 °C, growth was observed at 0.95–0.98 a_w, attaining a growth rate higher than 60 μm h^{-1} after 24 h at 0.98 a_w.

No germination of *F. verticillioides* was observed at 0.92 a_w and 20 °C. At 20 °C, hyphal growth was very similar at 0.95 and 0.98 a_w during the first 24 h of incubation. At 30 °C, germination was observed at the three a_w assayed. The maximum growth rate was obtained at 0.98 a_w and 30 °C, with more than 100 μm h^{-1} at 24 h.

3.2. Effect of fungal interactions on spore germination on the surface of maize grains

Fig. 3 shows the effect of fungal interactions on spore germination and hyphal growth when fungi where assayed together at 0.95 a_w and 30 °C. Growth rates calculated from exponential curves obtained at 16 and 24 h incubation are shown in Table 1.

When *A. ochraceus* was incubated with *F. verticillioides* and/or *A. alternata*, no germination was observed after 60 h incubation. At 0.95 a_w and 30 °C, co-inoculated strains and time of incubation both had a significant effect on the growth of *A. alternata* and *F. verticillioides*.

Duncan's test (results not shown) showed significant differences in means between individual growth of *A. alternata* and co-inoculation of this species with the other two, together or separately. *A. alternata* growing alone showed the lowest growth rate (6 μm h^{-1} at 24 h), and when the three species were growing together, almost a two-fold increase of growth rate was observed (11.7 μm h^{-1} at 24 h).

Duncan's test (results not shown) showed a significant effect due to fungal interactions for *F. verticillioides*, except when it grew with *A. alternata* plus *A. ochraceus*. When *F. verticillioides* was co-inoculated with only one of these fungi, the result was quite different. Germination and growth were promoted when *F. verticillioides* was paired with *A. alternata*, with a growth rate of 11 μm h^{-1} at 24 h, almost double the growth rate that was observed when it was growing alone. By contrast, when *F. verticillioides* was paired with *A. ochraceus*, growth rate was drastically reduced to only 2.6 μm h^{-1} at 24 h.

Table 1
Growth rates (μm h^{-1}) at 16 and 24 h of incubation of *A. ochraceus*, *A. alternata* and *F. verticillioides* alone or in the presence of other species on the surface of maize grain at 0.95 a_w and 30 °C

	Growing in the presence of	Growth rate (μm h^{-1})	
		16 h	24 h
A. ochraceus		1.56	3.41
alone	*A. alternata*	n.g.[a]	n.g.
	F. verticillioides	n.g.	n.g.
	A. alternata + *F. verticillioides*	n.g.	n.g.
A. alternata		4.25	6.03
alone	*A. ochraceus*	6.05	9.23
	F. verticillioides	6.69	10.71
	A. ochraceus + *F. verticillioides*	7.38	11.73
F. verticillioides		3.13	6.67
alone	*A. ochraceus*	1.46	2.61
	A. alternata	4.65	11.05
	A. ochraceus + *A. alternata*	2.83	5.88

[a] n.g.: no germination.

4. Discussion

When grains are harvested, their surfaces are contaminated with a complex mixture of fungi. Fungal propagules compete for space and substrate, although the interaction between species depends on the initial separation of the spores and colonies within the grain mass. Knowledge of influence of a_w and temperature on fungal germination and growth is of prime importance in understanding the ecology of different fungal species growing alone or with other fungal strains (Magan and Lacey, 1988).

Different methods have been used to determine fungal growth on different substrates and conditions, including measurements of fungal biomass, ergosterol, rate of germinated spores or linear growth (Seitz et al., 1979; Cahagnier et al., 1983; Matcham et al., 1985; Marfleet et al., 1991; Tothill et al., 1992; Marín et al., 1998d). None of them is appropriate when the objective is to understand the germination and growth in the very early phases of fungal colonisation directly on grain samples. In our study, SEM was used to observe fungal germination and growth.

Although the relationship between factors such as a_w or temperature has been extensively studied for several fungi growing on grains (Marín et al., 1995b, 1998b; Ramos et al., 1998), few studies on the very early stages of fungal infection of grains have been published (Ramakrishna et al., 1993, 1996; Swart et al., 1995; Ramos et al., 1997). SEM is a useful tool for evaluation of the fungal growth before mould growth is visible. The method for sample preparation described here is a fast and easy way to prepare samples for SEM observation. It differs from conventional methods in that there is no dehydration with ethyl alcohol series and no fixation with toxic osmium tetroxide. Although turgidity of hyphae was diminished by the method applied, the quality of images was sufficient to enable measurement of hyphal growth on micrographs, and time required for sample preparation was considerably less.

Our results have shown the effects of temperature, a_w and fungal competition on germination and initial growth on maize surface. In general, the lag time for spore germination increased with decreasing a_w and temperature. Germination usually occurred within 8 h and rarely took longer than 16 h at 0.92–0.98 a_w and 20–30 °C.

All three species tested germinated by germ tubes, which grew on the surface of maize grain without appearing to penetrate the grain tissue. Fungal spores contain nutrient reserves that can support growth for a limited period (Gottlieb, 1978). Soluble nutrients at the grain surface are required until the hyphae reach cracks or wounds where they can penetrate. If such nutrients are absent, the fungal growth may be able to extend only to some limited distance, and with reduced branching (Ramakrishna et al., 1993).

According to Ramakrishna et al. (1993), by inoculating only at one point of the grain surface, the physical and nutritional environment of all spores was similar. However, this is not what occurs in nature where spores are likely to be unevenly dispersed, and growth rates may differ on different parts of the grain depending on nutrient availability.

A. alternata had the fastest linear growth at 0.98 a_w and was the only species able to grow under all the conditions tested. Similar results have been observed when infection of table grape bunches by *A. alternata* was investigated using SEM. Conidia in 20-μl drops of spore suspension germinated readily on the fruit surface, and within 16 h grew extensively on berries, pedicels and rachises of immature and mature bunches (Swart et al., 1995).

By contrast, *A. ochraceus* was not able to germinate at 0.92 a_w at either temperature used and at 0.95 a_w and 20 °C in the time of incubation assayed. However, according to Ramos et al. (1998), who used the same strain as employed in this study, growth of this fungus on a barley meal extract agar showed a minimum a_w for visible growth of 0.808 at 25 °C. It is possible that longer periods of incubation enabled germination of *A. ochraceus* on maize grains.

The response of *F. verticillioides* depended on temperature assayed. At 20 °C, no germination was detected at 0.92 a_w, and growth was very similar at 0.95 and 0.98 a_w, at least during the first 24 h. However, at 30 °C, there was growth at the three a_w assayed, the strongest growth being observed at 0.98 a_w. Our studies agree with those published by Marín et al. (1998c) using the same strain that showed that optimum conditions for growth of *F. verticillioides* on maize were 0.98 a_w and 25 °C.

Woods and Duniway (1986) found that optimum and minimum a_w values for growth of *F. verticillioides* were 0.98 and 0.87, respectively. Similarly, Marín et al. (1995a) demonstrated the influence of temperature and a_w on *F. verticillioides*, concluding that the minimum a_w for growth of this species was at 0.89–0.90 a_w at 25–30 °C.

Studies on maize extract agar at 3% (w/v) have demonstrated that spores of *F. verticillioides* and *F. proliferatum* have a lag phase that varies from 50 to 100 h at 0.88 a_w to a few hours at 0.98 a_w and 25 °C (Marín et al., 1996). Our study, on whole maize grains, showed slightly higher lag times, possibly due to a poorer accessibility to nutrients in the grain compared with an agar-based medium. According to

Ramakrishna et al. (1993), due to high concentrations of soluble nutrients and the free movement of metabolites in agar media, the visible effects of interaction between species could be intense, and therefore, extrapolation of results from in vitro studies to natural substrates may not be appropriate.

The structure of the maize grain and the wax deposits on the surface affect fungal colonisation of kernels. It has been shown by SEM that an *A. flavus*-resistant maize genotype (GT-MAS:gk genotype) showed a rougher surface and with abundant wax deposits compared with susceptible kernels as Deltaphine G-4666, Asgrow RX 947 and Pioneer 3154 (Russin et al., 1997).

A. ochraceus was unable to germinate within 60 h under the conditions assayed (0.98 a_w/30 °C) when co-inoculated with *F. verticillioides* or *A. alternata*. The origin of this inhibition is unknown, but it could be due to production of inhibitory fungal metabolites by the other two species.

F. verticillioides responded differently when grown with one of the other two species, growing more strongly with *A. alternata*. As *A. alternata* showed the fastest growth, this increment in the growth rate of *F. verticillioides* could be a strategy to avoid being surpassed by *A. alternata* in the colonisation of the maize grain. This result agrees with Magan and Lynch (1986), who demonstrated that *Fusarium culmorum* competed successfully with *Alternaria* spp. when both were growing on grains.

By contrast, when *F. verticillioides* grew together with *A. ochraceus*, its growth rate was drastically reduced by more than 48.9% at 24 h. The mechanism of this inhibition by spores of *A. ochraceus* is still unknown.

When *A. alternata* was co-inoculated with one of the other fungal strains assayed, it always showed an increase in the growth rate, being the aggressive competitor when the three strains were growing together.

Similar studies of fungal competition, observed by SEM on grain surfaces, have been published. Ramakrishna et al. (1993) studied germination, growth and interactions of *A. flavus*, *P. verrucosum*, *F. poae* and *H. burtonii*, either alone or in the presence of other species, on barley grains at 0.97–0.90 a_w and 20–30 °C. Results showed that spore germination was unaffected by the presence of another species. A second study with longer incubation periods showed that *P. verrucosum* spores on barley grains were unaffected by the presence of *A. flavus*, *H. burtonii* and *F. sporotrichioides* spores (Ramakrishna et al., 1996).

Our results have shown the effect of ecophysiological conditions on early phases of spore development on maize grains, and how initial fungal interactions influence the final colonisation.

Acknowledgements

This work was supported by the European Union (QLRT 1999 00996), the Spanish Goverment (Comisión Interministerial de Ciencia y Tecnología, CICYT ALI98-0509-C04-01) and the Council of Lleida.

References

Abellana, M., Benedí, J., Sanchis, V., Ramos, A.J., 1999. Water activity and temperature effects on germination and growth of *Eurotium amstelodami*, *E. chevalieri* and *E. herbariorum* isolates from bakery products. Journal of Applied Microbiology 87, 371–380.

Bacon, C.W., Bennett, R.M., Hinton, D.M., Voss, K.A., 1992. Scanning electron microscopy of *Fusarium moniliforme* within asymptomatic maize kernels and kernels associated with equine leukoencephalomalacia. Plant Disease 76, 144–148.

Cahagnier, B., Richard-Molard, D., Poisson, J., 1983. Evolution of the ergosterol content of cereal grains during storage—a possibility for a rapid test of fungal development in grains. Science de l'Alimentation 3, 219–244.

Gottlieb, D., 1978. The Germination of Fungus Spores. Meadowfield Press, Durham, UK.

Magan, N., Lacey, J., 1988. Ecological determinants of mould growth in stored grain. International Journal of Food Microbiology 7, 245–256.

Magan, N., Lynch, J.M., 1986. Water potential, growth and cellulolysis of fungi involved in decomposition of cereal residues. Journal of General Microbiology 132, 1181–1187.

Marfleet, I., Magan, N., Lacey, J., 1991. The relationship between fungal biomass, ergosterol and grain spoilage. In: Fleurat-Lessard, F., Ducom, P. (Eds.), Proceedings of the 5th International Working Conference on Stored Product Protection. INRA, Bordeaux, France, pp. 405–412.

Marín, S., Sanchis, V., Magan, N., 1995a. Water activity, temperature and pH effects on growth of *Fusarium moniliforme* and *F. proliferatum* isolates from maize. Canadian Journal of Microbiology 41, 1063–1070.

Marín, S., Sanchis, V., Vinas, I., Canela, R., Magan, N., 1995b. Effect of water activity and temperature on growth and fumonisin B1 and B2 production by *F. proliferatum* and *F. monili-*

forme on maize grain. Letters in Applied Microbiology 21, 298–301.

Marín, S., Sanchis, V., Teixidó, A., Sáenz, R., Ramos, A.J., Viñas, I., Magan, N., 1996. Water and temperature relations and microconidial germination of *Fusarium moniliforme* and *F. proliferatum* from maize. Canadian Journal of Microbiology 42, 1045–1050.

Marín, S., Companys, E., Sanchis, V., Ramos, A.J., Magan, N., 1998a. Effect of water activity and temperature on competing abilities of common maize fungi. Mycological Research 120, 959–964.

Marín, S., Sanchis, V., Arnau, F., Ramos, A.J., Magan, N., 1998b. Colonisation and competitiveness of *Aspergillus* and *Penicillium* species on maize grain in the presence of *Fusarium moniliforme* and *Fusarium proliferatum*. International Journal of Food Microbiology 45, 107–117.

Marín, S., Sanchis, V., Rull, F., Ramos, A.J., Magan, N., 1998c. Colonization of maize grain by *Fusarium moniliforme* and *Fusarium proliferatum* in the presence of competing fungi and their impact on fumonisin production. Journal of Food Protection 61, 1489–1496.

Marín, S., Sanchis, V., Sáenz, R., Ramos, A.J., Viñas, I., Magan, N., 1998d. Ecological determinants for germination and growth of some *Aspergillus* and *Penicilllium* spp. from maize grain. Journal of Applied Microbiology 84, 25–36.

Matcham, S.E., Jordan, B.R., Wood, D.A., 1985. Estimation of fungal biomass in a solid substrate by three independent methods. Applied Microbiology and Biotechnology 21, 108–112.

Miller, J.D., 1994. Epidemiology of *Fusarium* ear diseases of cereals. In: Miller, J.D., Trenholm, H.L. (Eds.), Mycotoxins in Grain, Compounds Other Than Aflatoxin. Eagan Press, St. Paul, MN, pp. 19–36.

Ramakrishna, N., Lacey, J., Smith, J.E., 1993. Effects of water activity and temperature on the growth of fungi interacting on barley grain. Mycological Research 97, 1393–1402.

Ramakrishna, N., Lacey, J., Smith, J.E., 1996. Colonization of barley grain by *Penicillium verrucosum* and ochratoxin A formation in the presence of competing fungi. Journal of Food Protection 59, 1311–1317.

Ramos, A.J., Soler, J., Torres, M.R., Marín, S., Magan, N., 1997. Scanning electron microscopy study of germination and growth of *Fusarium moniliforme* on the surface of maize grain. Cereal Research Communications 25, 809–810.

Ramos, A.J., Labernia, N., Marín, S., Sanchis, V., Magan, N., 1998. Effect of water activity and temperature on growth and ochratoxin production by three strains of *Aspergillus ochraceus* on a barley extract medium and on barley grains. International Journal of Food Microbiology 44, 133–140.

Russin, J.S., Guo, B.Z., Tubajika, K.M., Brown, R.L., Cleveland, T.E., Widstrom, N.W., 1997. Comparison of kernel wax from maize genotypes resistant or susceptible to *Aspergillus flavus*. Phytopathology 87, 529–533.

Seitz, L.M., Sauer, D.B., Burroughs, R., Mohr, H.E., Hubbard, J.D., 1979. Ergosterol as a measure of fungal growth. Phytopathology 69, 1202–1203.

Sung, J.M., Cook, R.J., 1981. Effect of water potential on reproduction and spore germination by *Fusarium roseum* "*graminearum*", "*culmorum*", and "*avenaceum*". Phytopathology 71, 499–504.

Sutton, J.C., 1982. Epidemiology of wheat head blight and maize ear rot caused by *F. graminearum*. Canadian Journal of Plant Pathology 4, 195–209.

Swart, A.E., Lennox, C.L., Holz, G., 1995. Infection of table grape bunches by *Alternaria alternata*. South African Journal of Enology and Viticulture 16, 3–6.

Szekely, J.G., Chelack, W.S., Delaney, S., Marquardt, R.R., Frohlich, A.A., 1991. Scanning electron microscope observations of growth and ochratoxin-A production of *Aspergillus alutaceus* variety *alutaceus* (formerly *A. ochraceus*) on gamma-irradiated barley. Food Structure 10, 295–302.

Tothill, I.E., Harris, D., Magan, N., 1992. The relationship between fungal growth and ergosterol content of wheat grain. Mycological Research 96, 965–970.

Woods, D.M., Duniway, J.M., 1986. Some effects of water potential on growth, turgor, and respiration of *Phytophthora cryptogea* and *Fusarium moniliforme*. Phytopathology 76, 1248–1253.