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Colonisation and competitiveness of *Aspergillus* and *Penicillium* species on maize grain in the presence of *Fusarium moniliforme* and *Fusarium proliferatum*

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Abstract

The effects of different steady-state water activity levels (a_w, 0.93, 0.95 and 0.98) and temperature (15 and 25°C) on colonisation patterns of *Aspergillus* and *Penicillium* spp., when colonising irradiated maize grain in the presence of *Fusarium moniliforme* and *Fusarium proliferatum* were assayed in terms of populations (colony forming units, CFUs g grain^{-1}), seed infection and colonisation rates. The activity of *F. moniliforme* and *F. proliferatum* in grain reduced the presence of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus* to some extent, particularly at 15°C and higher water availabilities (0.95–0.98 a_w). In contrast, colonisation patterns of *Penicillium implicatum* on maize grain were unaffected by either *Fusarium* spp. in terms of CFUs or seed infection. Correlations were made between CFUs, seed infection, growth rates and niche overlap indices and hyphal interactions to try and link key indicators of competitiveness and dominance by an individual species. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: *Aspergillus* species; *Fusarium moniliforme*; *Fusarium proliferatum*; Maize grain; *Penicillium* species

1. Introduction

Maize grain is colonised by a mixture of spoilage fungi pre- and post-harvest. The dominant species depends on a number of abiotic and biotic factors. Of particular importance are the prevailing water availability and temperature conditions which determine the dominance of groups of fungi in the maize grain ecosystem. In recent years *Fusarium moniliforme* and *Fusarium proliferatum* have attracted much interest because of their competitive ability and the production of the fumonisin group of mycotoxins (Cawood et al., 1991). Detailed studies have previously shown that isolates of these two species germinate, grow and produce fumonisins over a wide range of environmental conditions (Marín et al., 1995a,b, 1996). Recently, Marín et al. (1998a) also demonstrated that these *Fusarium* species compete effectively and can exclude other species over a

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range of water activities and temperatures in vitro. However, few studies have examined the reasons why under certain conditions these Fusaria are competitive, while under others they may be dominated by other *Aspergillus, Eurotium* and *Penicillium* spp. The competing strategies of each species when co-cultured on maize grain have not been examined in detail previously and may help to understand the conditions under which these groups of species successfully dominate, particularly in the stored maize grain ecosystem.

Different approaches to fungal competition have been used before, such as development of an Index of dominance (I_D) values (Magan and Lacey, 1984, 1985; Cuero et al., 1987; Wheeler and Hocking, 1993; Marín et al., 1998b) and comparison of growth rates (Magan and Lacey, 1984, 1985; Cuero et al., 1987; Whipps and Magan, 1987; Ramakrishna et al., 1993, 1996a,b). More recently, Ramakrishna et al. (1996a,b) assessed the effects of fungal competition in terms of the population structures and levels of seed infection of barley grain to explain interactions between fungi in grain and their impact on mycotoxin production. Percentage of seed infection has also been frequently used as a measure of fungal interference (Cuero et al., 1987; Wicklow et al., 1988; Rheeder et al., 1990).

However, very little work has been done on the impact that the presence of *F. moniliforme* or *F. proliferatum* may have on the occurrence and development of other common maize fungi (Wicklow et al., 1980; Rheeder et al., 1990; Marín et al., 1998a,b). *F. moniliforme* has been demonstrated to act as an inhibitor of *A. flavus* and infection by other *Fusarium* spp. (Wicklow et al., 1988; Rheeder et al., 1990). Indeed, Yoshizawa (1996) reported a negative correlation between levels of fumonisins and aflatoxins in Thai corn. It has been suggested that the production of fumonisins by Fusaria of the section Liseola might give them an advantage in order to outcompete other fungal colonisers of maize grain (Marín et al., 1995a).

The objective of this study was to investigate the impact of *F. moniliforme* and *F. proliferatum* on the development of common co-colonisers such as *A. niger, A. flavus, A. ochraceus* and *P. implicatum* under different environmental conditions, by using four different criteria, (a) relative growth rates, (b) types of hyphal reactions, (c) population structure, and (d) percentage maize kernel infection.

2. Material and methods

2.1. Fungal isolates

Isolates of six different species were used in this study: *Fusarium moniliforme* Sheldon, *F. proliferatum* (Matsushima) Nirenberg, *A. niger* van Tieghem, *A. ochraceus* Wilhem, *A. flavus* Link, and *P. implicatum* Biourge. All species were isolated from maize and are common contaminants in Spain (Sala, 1993). All the isolates used are held in the Food Technology Department culture collection of the University of Lleida.

2.2. Grain

Spanish maize grain was irradiated with 12 kGrays of gamma irradiation and stored at 4°C. The grain contained no fungal infection or contamination but had retained germinative capacity. The initial water content and water activity (a_w) of the grain were 13.9% and 0.71, respectively.

For the experiments, irradiated maize was weighed in sterile flasks and rehydrated to the desired treatment a_w levels (0.93, 0.95 and 0.98) by addition of sterile distilled water. The amount of water added was calculated from a moisture adsorption curve for the grain. The grain treatments were allowed to equilibrate at 4°C for 48 h, with periodic shaking. Finally, the a_w values were confirmed by using a Novasina Thermoconstanter TH200, Axair Ltd. Systems for Air Treatment, Pfäffikon, Switzerland.

2.3. Inoculation, incubation and growth assessment

Rehydrated maize was placed in 9-cm diameter sterile Petri plates (Bibby Sterilin Ltd., Stone, Staffs., UK) (20 g/plate, approximately) forming a single layer of grains. Then a 5-mm diameter agar disk was taken from the margin of a 5-day-old growing colony of each isolate on malt extract agar at 25°C and transferred to the centre of each plate. After that, plates containing grain at the same a_w were placed in sealed containers with beakers of glycerol–water solutions of the same a_w as the treatments in order to maintain the correct equilibrium relative humidity. Containers were incubated at 15 and 25°C. All treatments were repeated three times.

Every day during the incubation period growing

colonies were measured with the aid of a binocular magnifier (Leica, Z45E, Leica Inc., Buffalo, USA). Two diameters were obtained from each colony; then, growth rates (mm day^{-1}) were calculated by linear regression of colony radius versus time for each strain at each set of conditions tested. Then, relative growth rates were calculated as follows:

$$\text{relative growth rate} = \frac{\text{growth rate isolate}}{\text{growth rate } \textit{Fusarium} \text{ spp.}}$$

2.4. Interactions between species on maize grain

Under the same treatment conditions detailed above, pairs of species were inoculated as 5-mm diameter agar plugs of each species placed on the grain layer 4.4 cm apart. Treatments were incubated as described previously, and the experiment was repeated three times.

Periodically, growing colonies were observed macroscopically and the type of interaction assessed using a modified method of Magan and Lacey (1984). Their scores were based on intermingling (1), mutual inhibition on contact (2/2), mutual inhibition at a distance (3/3), dominance on contact (4/0), and dominance at a distance (5/0). The latter score was for the antagonized species. These scores were then added for each species individually to obtain an overall Index of Dominance (I_D). Instead of five categories, we only devised two types of categories as all interactions were either mutual antagonism on contact, or inhibition of one species and the inhibited species being overgrown. Numerical scores were given with both fungi being given 0 in the former category, and 1 or -1 for the dominant, and inhibited species in the latter category.

2.5. Fungal populations in mixed inoculation experiments and percentage infection of maize kernels

Flasks containing 75 g of rehydrated maize were inoculated with 1 ml of one of the spore suspensions described below; this volume was initially subtracted from the amount of water added to rehydrate the irradiated maize treatments. Spores were harvested from the surface of a 14-day-old colony of each isolate grown on malt extract agar at 25°C, and suspended in 0.01% Tween 80 sterile distilled water (1–2 drops in 1000 ml water). Spore suspensions consisted of a single inoculum of each strain (2×10^6 spores/ml) or a mixture (1+1) of a *Fusarium* species and one of the other isolates tested (4×10^6 spores/ml). Inoculum was homogeneously spread by vigorous shaking of the flasks and then maize was placed in sterile Petri plates (25 g/plate, approximately). After that, plates containing grain at the same a_w were placed in closed containers and incubated as described previously. Incubation periods were 2 and 4 weeks. All treatments were repeated three times.

After incubation, plates were destructively sampled and analysed for CFU g^{-1} by dilution plating using both Malt extract agar (MEA; 20 g malt extract; 20 g glucose; 1 g peptone; 1000 ml distilled water; pH=5.5) and low a_w Malt salt agar (MSA; 20 g malt extract; 20 g glucose; 10 g NaCl; 1 g peptone; 1000 ml distilled water; pH=5.5) as enumerating media. Peptone saline (8.5 g NaCl, 1 g peptone, 1000 ml distilled water, pH=5.5) was used as diluent, and homogenisation of samples was carried out by using an Stomacher Lab-Blender 400 (BA 6021, Seward Medical UAC House, London, UK). After incubation at 25°C, plates bearing between 5 and 150 colony forming units (CFUs) were enumerated for populations of individual *Aspergillus* and *Penicillium* species.

Thirty maize kernels were taken from each sample and assayed by direct plating on both MEA and MSA, after 2-min. surface disinfection with 2% sodium hypochlorite (2 ml NaClO in 100 ml solution). The percentage (%) of maize kernels infected by *Aspergillus* and *Penicillium* species was determined.

2.6. Statistical analyses of the data

Analyses of variance were made for colony radius after 8 days, CFUs g^{-1} and % of infection, by using SAS program version 6.11 (SAS Institute, Inc., Cary, NC, USA). CFU data were transformed prior analysis by $y=\log(CFU\ g^{-1})$, while % of infection was transformed by $y=\sin^{-1}\sqrt{(\%\ \text{infection}/100}$. The same software was used to obtain Pearson correlation coefficients and thus try to correlate the different approaches to fungal competition accumulated in this study and that obtained from a previous study (Niche Overlap Indices, NOI) (Marín et al., 1998b). Niche Overlap Indices were based on the common C-

sources assimilated by both interacting species paired/total C-sources assimilated by an interacting species.

3. Results

3.1. Differences in relative growth rates among species

All the species grew faster at 25°C than 15°C, while in general growth rates increased with a_w, except for *P. implicatum* which showed faster growth at 0.95 a_w, and even a higher growth rate at 0.93 than at 0.98 a_w (data not shown).

At 25°C *Fusarium* species were the slowest, with a maximum growth rate of 3.59 mm day^{-1} at 0.98 a_w, while *P. implicatum* was the fastest with a maximum of 6.32 mm day^{-1} at 0.95 a_w. *A. niger* had the best growth rate of the *Aspergillus* species on irradiated maize grain (data not shown).

Interestingly, *Fusarium* species grew faster than the other species at 15°C and 0.95–0.98 a_w, with a maximum growth rate of 1.78 mm day^{-1} at 0.98 a_w. *A. flavus* had less ability to grow at the lower temperature (data not shown).

Analysis of variance showed that there were statistically significant ($P<0.01$) differences in relation to a_w, temperature and species. In general, *P. implicatum* grew fastest, followed by *A. niger* and *A. ochraceus,* while there was little difference between the growth rates of A. *flavus* and the *Fusarium* species.

Table 1 shows the relative growth rates (RGR, growth rate of species/growth rate *Fusarium)* obtained by the four species tested under the different environmental conditions. Relative growth rates were in general <1 at 0.95–0.98 a_w at 15°C. *P. implicatum* had a RGR<1 only at 0.98 a_w and 15°C. Interestingly only *A. ochraceus* had a value <1 at 25°C and 0.98 a_w, showing poor ability of *Fusarium* species to grow quickly when compared to the others. Similar RGR values were obtained in relation to both *F. moniliforme* and *F. proliferatum*.

3.2. Hyphal interactions of Aspergillus and Penicillium species when paired with Fusarium species

Table 2 shows the scores obtained by *Aspergillus* and *Penicillium* species when paired with *F. moniliforme* and *F. proliferatum* under the different environmental treatments tested. In general, both *Aspergillus* spp. and *P. implicatum* were inhibited and often overgrown (−1) by both *Fusarium* species at 0.98 a_w. In contrast, at 0.93–0.95 a_w, they were either mutually inhibitory on contact (0) or dominant (1) against the *Fusarium* spp.

Both *Aspergillus* and *Penicillium* species behaved similarly, with *A. niger* and *A. ochraceus* least

Table 1
Effect of water activity and temperature on relative growth rates (growth rate species/growth rate *Fusarium)* of the species tested on irradiated maize grain

Temperature (°C)	Water activity	*A. niger*	*A. ochraceus*	*A. flavus*	*P. implicatum*
		with reference to *F. moniliforme*			
15	0.93 a_w	1.44	1.75	0.44	2.00
	0.95 a_w	0.80	0.88	0.39	1.25
	0.98 a_w	0.83	0.82	0.45	0.56
25	0.93 a_w	2.36	2.07	1.76	3.69
	0.95 a_w	2.05	1.57	1.40	2.92
	0.98 a_w	1.34	0.86	1.42	1.21
		with reference to *F. proliferatum*			
15	0.93 a_w	1.07	1.29	0.32	1.48
	0.95 a_w	0.70	0.77	0.34	1.10
	0.98 a_w	0.89	0.88	0.48	0.60
25	0.93 a_w	2.00	1.75	1.49	3.12
	0.95 a_w	1.75	1.34	1.19	2.49
	0.98 a_w	1.38	0.88	1.46	1.25

Table 2
Effect of water activity and temperature on type of interactions and scores given to *Aspergillus* and *Penicillium* species when growing paired with *F. moniliforme* and *F. proliferatum* [a]

Temperature (°C)	Water activity	*A. niger*	*A. ochraceus*	*A. flavus*	*P. implicatum*	Total
		paired with *F. moniliforme*				
15	0.93 a_w	0	1	0	0	1
	0.95 a_w	0	0	0	0	0
	0.98 a_w	0	−1	−1	−1	−3
25	0.93 a_w	1	0	1	1	3
	0.95 a_w	0	1	0	1	2
	0.98 a_w	0	0	0	−1	−1
	Total	1	1	0	0	2
		paired with *F. proliferatum*				
15	0.93 a_w	0	1	0	0	1
	0.95 a_w	0	0	0	0	0
	0.98 a_w	0	−1	−1	−1	−3
25	0.93 a_w	1	0	0	1	2
	0.95 a_w	0	1	1	1	3
	0.98 a_w	0	−1	0	−1	−2
	Total	1	0	0	0	**1**
	TOTAL	**2**	**1**	**0**	**0**	**3**

[a] 0: mutual antagonism; 1: dominance; −1: inhibition ('overgrown').

dominated at 15°C, while at 25°C the scores obtained by each species depended on water availability. There was no difference between the effect of *F. moniliforme* and that of *F. proliferatum* on the competing abilities of the species studied.

3.3. Effect of Fusarium species from section Liseola on populations of Aspergillus and Penicillium species

The effect of the different media (MEA, MSA) used for enumeration showed that in general there was not a significant difference (data not shown). Thus, results are only presented in this section based on MEA results. Table 3 shows how temperature (15 and 25°C), time (2 and 4 weeks), water activity (0.93, 0.95 and 0.98), and the presence of *Fusarium* species (control, *F. moniliforme* and *F. proliferatum*) had a significant influence on the results obtained. Analysis of variance revealed that most of the two-way interactions were also significant. The CFUs g^{-1} increased with temperature and a_w for all the species, except *A. ochraceus* which had a maximum at 0.93–0.95 a_w (4.2×10^7–9.8×10^8 CFU g^{-1}), and a minimum at 0.98 a_w (2.8×10^6–7.6×10^8 CFU g^{-1}).

Fig. 1 shows the effect of the presence of *Fusarium* species on colonisation of grain by *Aspergillus* species and *P. implicatum* at different a_w and temperature levels. Although populations of fungi were significantly higher after 4 weeks than after 2 weeks for all species, the trends observed for the level of inhibition were, in general, quite similar regardless of time of incubation (data not shown).

Colonisation of grain by *A. niger* based on CFUs g^{-1} grain was clearly inhibited by the presence of *Fusarium* species at 15°C at all a_w levels (5–40% reduction on a log basis), while the effect was less clear at 25°C. Similarly, *A. flavus* numbers were markedly inhibited at 15°C over the whole range of a_w (18–44% reduction), but not at 25°C.

In contrast, *F. moniliforme* significantly affected the development of *A. ochraceus* at 15 and 25°C, although the effect was more important at 15°C (24–48% of reduction). *F. proliferatum* also inhibited this species at both temperatures, but the effect was not as pronounced. At 25°C the inhibition was clearer at 0.98 a_w.

Table 3
Analysis of variance of the effect of temperature (T), time (t), water activity (a_w), and presence of *Fusarium* species (c), and their interactions on *Aspergillus* and *Penicillium* species populations on maize

Factor	*A. flavus*		*A. niger*		*A. ochraceus*		*P. implicatum*	
	MS	F	MS	F	MS	F	MS	F
c	18.83	266.08**	10.75	107.04**	43.85	699.69**	6.38	277.85**
T	207.11	2926.00**	90.81	903.98**	2.77	203.84**	2.21	96.24**
c×T	14.11	199.41**	15.34	152.74**	8.09	129.06**	0.02	0.89
t	2.44	34.53**	6.62	65.88**	8.11	129.38**	1.22	53.19**
c×t	0.82	11.66**	0.05	0.52	1.11	17.69**	1.38	60.26**
T×t	0.00	0.01	5.69	56.68**	3.77	60.15**	1.39	60.57**
c×T×t	0.16	2.28	0.98	9.81**	0.03	0.41	0.15	6.52**
a_w	2.19	30.89**	4.33	43.14**	3.89	62.10**	1.53	66.50**
c×a_w	3.08	43.48**	2.60	25.90**	4.93	78.69**	0.70	30.56**
T×a_w	0.26	3.68*	0.79	7.84**	9.60	153.18**	0.29	12.62**
c×T×a_w	0.19	2.68*	0.80	7.97**	1.02	16.30**	0.20	8.69**
t×a_w	0.00	0.07	2.68	26.67**	1.83	29.16**	4.45	193.74**
c×t×a_w	0.33	4.65**	0.34	3.36*	0.19	2.97*	0.69	30.18**
T×t×a_w	0.07	1.06	0.28	2.76	0.74	11.86**	0.33	14.60**
c×T×t×a_w	0.24	3.45*	0.32	3.16*	0.34	5.47**	0.06	2.40

* Significant $P<0.05$.
** Significant $P<0.01$.

Finally, *P. implicatum* populations were least affected at 15°C (0–19% reduction), with similar results at 25°C. The maximum inhibition of this fungus was caused by *F. moniliforme* after 4 weeks incubation.

Overall, the impact of *Fusarium* species on other competing species was often more important at high a_w levels. *F. proliferatum* exerted similar or no inhibition at 0.93–0.95 a_w, but inhibited all the species except for *A. flavus,* at 0.98 a_w In general, *F. moniliforme* inhibited the other competing species with increasing a_w.

3.4. Effect of Fusarium species from section Liseola on kernel infection by Aspergillus and Penicillium species

Table 4 shows how temperature (15 and 25°C), time (2 and 4 weeks), water activity (0.93, 0.95 and 0.98), and the presence of *Fusarium* species (control, *F. moniliforme* and *F. proliferatum)* had a significant influence on the percentage isolation of *Aspergillus* and *Penicillium* species from irradiated maize grain. Grain infection was higher at 25°C than at 15°C, and isolation of *Aspergillus* and the *Penicillium* species decreased with increasing water availability. The effect of time was significant for *A. niger* and *A. ochraceus,* where infection was lower initially but increased after 4 weeks incubation (data not shown).

A. niger was not inhibited when grown in combination with the *Fusarium* spp. at 25°C (Fig. 2), while it was inhibited at 15°C at 0.93–0.98 a_w, particularly at 0.98 a_w after 2 weeks (77% reduction) (data not shown). Similarly with *A. flavus,* it was not inhibited at 25°C, but at 15°C the *Fusarium* spp. significantly (58–100% reduction) inhibited infection over the whole a_w range tested.

In contrast, *A. ochraceus* was not competitive in the presence of the *Fusarium* spp. at 25°C and 0.98 a_w (76–100% reduction in infection), and at 15°C and 0.98 a_w. The effect of *F. moniliforme* was more marked than that of *F. proliferatum.* However, infection of grain by *P. implicatum,* was not affected by co-inoculation with the *Fusarium* species.

3.5. Correlation between different competing criteria for determining competitive strategies

There was good correlation between growth rates of *A. niger* and *A. flavus* with CFUs and seed infection (SI) (Table 5). Correlation was also found between niche overlap indices (NOI) and growth rate (GR) for the *Aspergillus* species. Moreover the RGR often correlated well with hyphal reactions (HR),

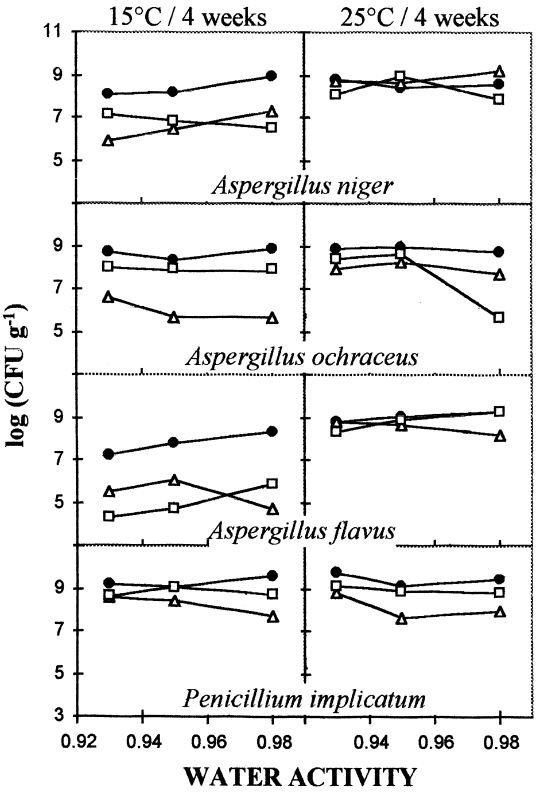

Fig. 1. Effect of water activity and temperature on *A. niger, A. ochraceus, A. flavus* and *P. implicatum* population (CFU g^{-1} irradiated maize) in pure culture (●), and in the presence of *F. moniliforme* (△) and *F. proliferatum* (□).

CFU and SI for *Aspergillus* species but not with the NOI. In general, no correlation was found between HR and CFU or SI; interestingly an inverse slight correlation was also found between HR and NOI. Moreover, good correlation was found between CFU and SI for the *Aspergillus* species. *P. implicatum* did not show much correlation between the parameters studied.

4. Discussion

This study has shown how the outcome of interactions between fungi on maize grain depends on environmental conditions and the types of species present. This study has indicated that $a_w \times$ temperature interactions have a profound effect on the potential for fumonisin-producing *Fusarium* spp. to exclude other *Aspergillus* and *Penicillium* spp. from its niche. At 0.98 a_w the *Fusarium* spp. were particularly competitive. Previously, studies by Blaney et al. (1986) found a negative correlation between the isolation frequencies of *F. graminearum* and *F. moniliforme* and attributed this to competition for substrate, production of antagonistic substances, or environmental conditions that differentially influenced corn ear infection by these two fungi. Furthermore, Rheeder et al. (1990) found negative correlation between the presence of *F. moniliforme* and other *Fusarium* species and also concluded that environmental conditions accounted for these effects. However, few studies have examined the interaction of the *Fusaria* with other common *Aspergillus* colonisers of maize grain. The experiments were carried out with different *Aspergillus* species as indicator organisms. However, many Aspergillus species, in particular *A. niger* and *A. flavus*, have an optimum growth at 30–37°C (Marín et al., 1998c). The experiments were performed at 15 and 25°C. The possibility exists that the *Aspergillus* species are more competitive at their optimum temperature.

In our study, no correlation was found between hyphal reactions and CFUs, grain infection or growth rate. Previously, Magan and Lacey (1985) found little correlation between Indices of Dominance obtained on agar medium and populations on grain assessed by both direct and dilution plating. Magan and Lacey (1984), (1985); Whipps and Magan (1987); Wheeler and Hocking (1993) and Marín et al. (1998a) all failed to find a direct relationship between growth rate of the individual species and their competitiveness in terms of Index of Dominance (I_D), both on agar and cereal grain substrata. Thus, for example, Marín et al. (1998a) found that at lowered a_w conditions some *Penicillium* spp. were dominant (I_D) even though they grew more slowly; interestingly the *P. implicatum* isolate had the fastest growth rates on maize grain of those examined in the present study. Whipps and Magan (1987) reported that the interactions (I_D) changed markedly and were easily predicted from growth rates alone. All approaches, however, may be suitable for the determination of competitiveness. Two aspects of competition must be taken into account: primary resource capture and combat (Cooke and Whipps, 1993). Prolific production of spores, quick germination of these, possession of appropriate extracellular enzymes, and high growth rates allow species to

Table 4
Analysis of variance of the effect of temperature (T), time (t), water activity (a_w), and presence of *Fusarium* species (c), and their interactions on *Aspergillus* and *Penicillium* species infection of maize kernels

Factor	*A. flavus*		*A. niger*		*A. ochraceus*		*P. implicatum*	
	MS	F	MS	F	MS	F	MS	F
c	3.83	582.03**	1.34	153.71**	4.46	274.12**	0.02	49.89**
T	15.30	2324.87**	1.34	153.71**	0.29	17.90**	0.02	49.89**
c×T	3.83	582.03**	1.34	153.71**	1.86	114.27**	0.02	49.89**
t	0.01	2.02	0.11	12.44**	0.12	7.63**	0.00	3.73
c×t	0.01	1.73	0.11	12.44**	0.06	3.53*	0.00	3.73*
T×t	0.01	2.02	0.11	12.44**	0.00	0.26	0.00	3.73
c×T×t	0.01	1.73	0.11	12.44**	0.03	1.74	0.00	3.73*
a_w	0.09	13.37**	0.05	5.24**	2.90	178.57**	0.02	49.89**
c×a_w	0.03	4.48**	0.05	5.24**	0.87	53.78**	0.02	49.89**
T×a_w	0.09	13.37**	0.05	5.24**	0.87	53.39**	0.02	49.89**
c×T×a_w	0.03	4.48**	0.05	5.24**	0.33	20.16**	0.02	49.89**
t×a_w	0.02	2.65	0.01	1.54	0.04	2.46	0.00	3.73*
c×t×a_w	0.02	2.38	0.01	1.54	0.02	1.57	0.00	3.73**
T×t×a_w	0.02	2.65	0.01	1.54	0.08	5.19**	0.00	3.73*
c×T×t×a_w	0.02	2.38	0.01	1.54	0.04	2.56*	0.00	3.73**

* Significant $P<0.05$.
** Significant $P<0.01$.

succeed in primary resource capture. However, hyphal reactions reflect the ability of a species for combat and can be important when the density of the initial inoculum is high.

Moreover, in our study good correlation was found for some of the species between relative growth rate and hyphal reactions; this might mean that if one species is able to grow comparatively faster than another, then it will be able to overgrow the competitor when their hyphae meet. Thus the ability to grow faster might not, per se, be a guarantee of dominance, except where a wide difference in growth rates between species occurs. Interestingly, relative growth rates in general correlated well for *Aspergillus* species with CFU and SI which suggests that a species with a fast growth rate under certain environmental conditions is able to colonise a greater proportion of the grain ecosystem and, if competitive, exclude other species with lower growth rates. Consequently RGR could in some circumstances be an easy measure to predict which species, e.g. *Aspergillus* vs *Fusarium* spp., might dominate the ecosystem under different environmental conditions.

In freshly harvested grain, contaminant spores of individual species are likely to vary in concentration and spatially. In our studies this was not taken account of because of the complexity of the matrix of treatments required. However, low concentrations of spores of one species may, under conducive environmental conditions, be outcompeted by more stress-tolerant and competitive species. Metabolites, perhaps including mycotoxins, produced in grain by either species may then either inhibit a competing species or they could be degraded and utilised. Overall, only two reaction types were observed in this study: Inhibition on contact (combative interaction) and overgrow (combative interaction with secondary resource capture). Growth rates of pure cultures on maize grain are probably a function of primary resource capture prior to any competitive interactions occurring. Thus, fungi in stored grain may form discrete 'mutually inhibited' colonies (Ramakrishna et al., 1993).

Interestingly, in the present study the *Aspergillus* spp. were inhibited by the *Fusarium* species under environmental conditions to which they were better adapted, e.g. 15°C and high a_w, in terms of growth rate, but not in terms of CFUs. This implies that the relative growth rate may be an important measure of the competing ability of a species. Heavy-sporulating species *(Aspergillus* and *Penicillium* spp.) are selected for in the dilution plating method (Magan and Lacey, 1988) at the expense of *Fusarium* species

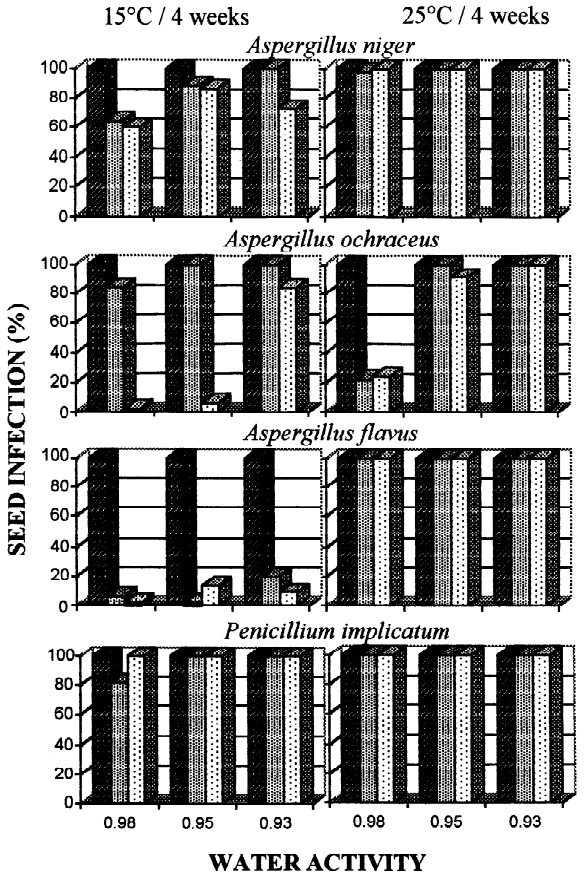

Fig. 2. Effect of water activity and temperature on *A. niger, A. ochraceus, A. flavus* and *P. implicatum* maize grain infection *(%)* in pure culture (■), and in the presence of *F. moniliforme* (▩) and *F. proliferatum* (▩).

(especially *F. proliferatum*) producing wet spore droplets in a matrix. However, in our study the heavily sporulating *P. implicatum* was quite competitive not only in terms of CFU, but in infection percentage and growth rate.

Recent work has also examined the use of niche overlap index (NOI) as a measure of fungal competitiveness and niche exclusion under different environmental parameters (Marín et al., 1998b). The total and common C-sources utilised by each fungus was found to be markedly influenced by both a_w and temperature. This implies that the amount of niche overlap changes with environmental conditions. The strain of *F. proliferatum* used in that study used more C-sources than *F. moniliforme,* but had a narrower overlap with other species, suggesting that *F. proliferatum* might be more competitive. Again, there was no general correlation between ID and NOI. *A. niger, A. flavus, A. ochraceus, P. aurantiogriseum,* and *F. graminearum* were among the species which shared their niches with fumonisin-producing *Fusarium* species, and thus competed for the same sources, and consequently are likely to inhibit each other. In the present study only correlations between growth rate and NOI for some species were significant. An inverse correlation between hyphal reactions and NOI was obtained and could be explained by the fact that the more an individual species shares its niche with others, the more likely it will be dominated by them.

This is the first time that impact of *F. proliferatum* on infection of irradiated maize grain by other fungal species has been shown and demonstrated to be similar to that of *F. moniliforme* depending on interacting *Aspergillus* or *Penicillium* spp. The impact of *F. moniliforme* sometimes resulted in interesting interactions, especially when paired with *A. ochraceus* in terms of CFU and grain infection, while there was no difference in hyphal reactions and RGR values. The study of Marín et al. (1998b) showed that *F. moniliforme* and *F. proliferatum* were able to dominate several other common maize-contaminating fungi over a wide range of temperature and water availability conditions on maize extract agar. However, based on I_D values, this isolate of *F. proliferatum* was more dominant than that of *F. moniliforme,* particularly at 0.994 and 0.98 a_w.

Previously, Rheeder et al. (1990) pointed out that *F. moniliforme* may serve as a deterrent to kernel invasion by other seed-infecting fungi, with potential for use as a biocontrol agent. However, stable non-mycotoxigenic strains would have to be examined to try and control infection and fumonisin production by these Fusaria both pre- and post-harvest in maize, as has been demonstrated for *A. flavus* and other species (Cole and Cotty, 1990).

Our results suggest that although *A. flavus* and *A. ochraceus* strains produce aflatoxins and ochratoxins, respectively, they were inhibited consistently by *F. moniliforme.* Previous work by Wicklow et al. (1988) indicated that common fungal colonists of maize kernels interfere with the ability of *A. flavus* to infect preharvest maize; *F. moniliforme* was particularly effective in inhibiting kernel infection by *A.*

Table 5
Pearson correlation coefficients among growth rate (GR), relative growth rate (RGR), hyphal reaction scores (HR), CFU, seed infection (SI) and niche overlap index (NOI) for *Aspergillus* and *Penicillium* species

	A. niger	*A. ochraceus*	*A. flavus*	*P.implicatum*
GR vs NOI	0.464	0.646*	0.533*	–
GR vs HR	0.195	0.008	0.115	0.574*
GR vs CFU	0.911*	0.395	0.910*	0.252
GR vs SI	0.851*	0.145	0.953*	0.294
RGR vs NOI	0.004	−0.295	0.468	–
RGR vs HR	0.689*	0.539*	0.129	0.892*
RGR vs CFU	0.498	0.371	0.930*	0.026
RGR vs SI	0.752*	0.531*	0.966*	0.364
CFU vs NOI	0.619*	0.144	0.494	–
CFU vs HR	0.345	0.337	0.100	0.015
CFU vs SI	0.830*	0.861*	0.873*	−0.100
SI vs NOI	0.266	−0.059	0.282	–
SI vs HR	0.416	0.484	0.321	0.363
NOI vs HR	0.272	−0.589*	−0.496	–

* Significant correlation with a 95% confidence level.

flavus. It has been suggested that kernels initially infected with *F. moniliforme* may be resistant to later infection by *A. flavus* if the *Fusarium* hyphae induce metabolic host resistance (e.g., papillae, cell wall thickening, phytoalexins). The presence of competing fungi may explain why some kernels with high levels of aflatoxin may be often located next to kernels that were toxin-free (Wicklow et al., 1988). *F. moniliforme* is capable of rapidly colonising wounded kernel tissues, thus reducing the resource nutrient pool available to fungi such as *A. flavus,* or may interfere in some other way with its ability to produce aflatoxin (Ullstrup, 1970).

P. implicatum remained unaffected by competition in terms of both seed infection and number of CFU, while for *Aspergillus* species both CFU and percentage seed infection decreased, demonstrating a clear parallel in the decrease in colonisation by both *A. ochraceus* and *A. flavus*. Ramakrishna et al. (1996b) in a similar study with *P. verrucosum* identified four different patterns which depended upon the competing species involved: (1) the percent seed infection and CFU were unaffected by competition, as for *P. implicatum*; (2) the percent seed infection and number of CFU increased initially more slowly during competition than in pure culture; (3) seed infection and CFU were markedly decreased by competition, as shown for *Aspergillus* species in this study; and (4) seed infection was markedly decreased but its sporulation was unaffected during competition. We found significant correlation between CFU and grain infection in *Aspergillus* species while not for *P. implicatum*. They found significant correlation between CFU and seed infection for 20 and 30°C, and 0.90–0.95 a_w, but not at 0.97 a_w (Ramakrishna et al., 1996b).

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