Phytopathologia Mediterranea (2014) 53, 3, 459–469
DOI: 10.14601/Phytopathol_Mediterr-13932

RESEARCH PAPER

Influence of water activity and anti-fungal compounds on development and competitiveness of *Fusarium verticillioides*

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Summary. This investigated the roles of water activity (a_w) and fungicides on the competitiveness of two *Fusarium verticillioides* strains against other spoilage fungi commonly present in maize (*F. proliferatum, Aspergillus niger, A. flavus, A. ochraceus* and *Penicillium verrucosum*). Fungal strains were inoculated on artificial media containing maize flour. The effects were determined of three a_w levels (0.99, 0.98 and 0.95) and three fungicides (tebuconazole, procloraz and prothioconazole) on fungal interactions, the Index of Dominance (I_D) of isolates and fumonisin B_1+B_2 (FBs) production. The two strains of *F. verticillioides* showed similar behaviour in conditions where water was freely available (0.99 a_w); at 0.98 and 0.95 a_w both *F. verticilliodes* strains had the lowest total I_D scores (8–6 and 10–12, respectively). They showed the same ability to compete against other fungi having the highest I_D scores against *P. verrucosum* and *A. ochraceus* and the lowest against *A. niger* and *A. flavus*. The lowest water activity gave (0.95 a_w) was the most conducive for fumonisin production with significant differences to 0.98 and 0.99 a_w. In a co-inoculation experiment, only FBs production from *P. verrucosum* was greater in the presence of the *F. verticilliodes* strains other fungi. The use of fungicides reduced Indices of Dominancy (I_D) for both *F. verticilliodes* strains. A significant reduction in *F. verticilloides* growth was observed when combining water stress and fungicide treatments. This information provides increased understanding of the colonisation patterns of *F. verticillioides* in relation to other mycobiota and to both environmental and chemical stresses, and has implications in relation to future climate change scenarios.

Key words: fungicides, water activity, competition, maize.

Introduction

Maize is a commodity colonised by a diverse community of spoilage fungi pre- and post-harvest. The dominant species depends on several abiotic and biotic factors and their interactions. In particular, air temperature and rain, and the water activity (a_w) dynamics in maize kernels during silking (Battilani *et al.*, 2011) all play crucial roles in determining the dominance of groups of fungi in the maize grain ecosystem (Lacey, 1980; Magan and Lacey, 1984a,b). *Fusarium* of the *Gibberella fujikuroi* species complex, which cause *Fusarium* ear rot in maize as well as contamination with fumonisins, are of particular importance (Bullerman and Draughon, 1994). *Fusarium verticillioides* and *F. proliferatum* are two of the most common *Gibberella fujikuroi* species complex isolated from corn (Bacon and Nelson, 1994) but abiotic factors, in particular water availability, might be responsible of the prevalence of *F. verticillioides* over *F. proliferatum* (Kommendahl and Windels, 1981).

To become dominant, *Fusarium* species must compete effectively against other non-toxigenic and toxigenic fungi, especially in the genera *Aspergillus* and *Penicillium.* Thus, understanding the complex interactions which occur between abiotic and biotic factors, and their impacts on growth and mycotoxin production in co-occurring *Fusarium* spp. and other fungi, is

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ISSN (print): 0031-9465
ISSN (online): 1593-2095

crucial. This information will be useful for the development of predictive models, and the definition of effective crop management strategies to limit the colonisation of maize by these species (Marin *et al.*, 1998a,b).

Based on available studies regarding maize kernel infection, a negative correlation between *A. flavus* and *F. verticillioides* was previously found (Wicklow *et al.*, 1988), but this was before the discovery of fumonisins. Competitiveness of both *F. proliferatum* and *F. verticillioides* strains have been demonstrated *in vitro* against a wide range of other fungi colonizing maize and over a range of environmental conditions (Marin *et al.*, 1998a,b). However, these studies did not include *P. verrucosum,* a known producer of ochratoxin, also found on maize kernels (Reddy *et al.*, 2013).

Fungal communities growing in ripening maize grain often exert some influence on each other, especially if they are competing for the same ecological niche (Marin *et al.*, 2001). Magan and Lacey (1984a; 1985) established five different types of interaction scores (scored 1–5) when hyphae from different fungi interacted with each other. These scores helped define an Index of Dominance (I_D), which can be used to compare the competitive capacities of species under different environmental conditions. They showed that a_w by temperature interactions and nutritional sources significantly influenced the relative I_D of a group of fungal species *in vitro*. Interactions between species has also been shown to influence the overall production of mycotoxins in cereals (Wicklow *et al.*, 1980; Cuero *et al.*, 1987; Ramakrishna *et al.*, 1993).

Other inputs into grain ecosystems include fungicide applications. It has been shown that the use of fungicides can influence both the dominance of fungal species and their production of mycotoxins (Folcher *et al.*, 2009; Mazzoni *et al.*, 2011; Formenti *et al.*, 2012). Studies of wheat grain showed that when sub-optimal concentrations of fungicides were used, stimulation of deoxynivalenol (type B trichothecene) production occurred for strains from different parts of Europe (Ramirez *et al.*, 2004). There is less information in maize on how interactions between a_w, temperature and fungicides may impact on mycotoxigenic fungi and on mycotoxin production.

The objectives of this study were to evaluate (a) the competitiveness of *F. verticillioides* strains against different fungal species commonly present in maize, under different a_w regimes, regarding both growth capacity and FBs production, and (b) the impact of sub-optimal concentrations of commercial fungicides, known to be active against Fusaria, on interspecific fungal interactions on a maize-based medium and on the overall I_D scores.

Materials and methods

Strains

Two strains of *F. verticillioides* and one strain each of *F. proliferatum, Aspergillus flavus*, *A. ochraceus*, *A. niger* and *P. verrucosum* were used in this study (Table 1). They were all isolated from maize in different Eu-

Table 1. List of fungal strains used in this study, their codes in fungal collections, mycotoxin produced and papers where they were previously described or used.

Species	Strains	Origin	Mycotoxins produced	Citation
Fusarium verticillioides	MPVP 294 (ITEM 10027)	Italy	Fumonisins B	Lazzaro *et al.*, 2012
Fusarium verticillioides	MPVP 289 (ITEM 10026)	Italy	Fumonisins B	Lazzaro *et al.*, 2012
Fusarium proliferatum	ITEM 7595	Kansas (US)	Fumonisins B	Lazzaro *et al.*, 2013
Aspergillus flavus	MPVP A 2092 (ITEM 8069)	Italy	Aflatoxins B	Giorni *et al.*, 2011
Aspergillus ochraceus	LKN 14027	Denmark	Ochratoxin A	
Aspergillus niger	MPVP A 2350	Italy	Ochratoxin A	
Penicillium verrucosum	BFE 500	Germany	Ochratoxin A	Bogs *et al.*, 2006

Codes reported refer to the fungal collections: MPVP=Institute of Entomology and Plant Pathology, UCSC, Italy; ITEM=ISPA-CNR, Italy; LKN= Denmark; BFE= Bundesforschungsanstalt fur Ernahrung (Federal Research Centre for Nutrition), Germany.

ropean Countries and all were confirmed to produce their respective mycotoxins.

Media

Maize based medium (MA): maize kernels were milled and a subsample of the resulting flour (20 g) was added to agar (2%; Oxoid®) and bi-distilled water (1 L). Ingredients were mixed using a magnetic stirrer and the medium obtained was autoclaved at 120°C for 15 min and then poured into 90 mm diam. Petri dishes. The surface of this medium was overlaid with sterile discs of dark polyester fibre before inoculation to enable measurements of interacting colonies.

Water activity (a_w) modified media: The MA basal medium a_w was 0.99. Standard amounts of glycerol (Dallyn and Fox, 1980) were added to the medium to achieve 0.98 and 0.95 a_w treatment levels. An Aqualab Series 3 (Labcell Ltd., Basingstoke, Hants, UK) was used to measure a_w of the media.

Fungicide-modified media: before pouring the media into 90 mm Petri dishes, they were cooled to approximately 50°C and three different fungicides were incorporated separately: Folicur SE®, Sportak® 45EW and Proline®. These products are considered effective against Fusaria, as confirmed by Formenti *et al.* (2012). Their respective active ingredients (tebuconazole, procloraz and prothioconazole) were added in quantities corresponding to ED_{50} concentrations, i.e., the amount required to reduce fungal growth by 50% (Table 2).

Inter-specific interactions between fungi

Fungal spore suspensions (10^6 spores mL^{-1}) were prepared from 14-d-old colonies of each strain grown on MA; 0.25 mL of fungal suspension were centrally inoculated on each Petri dish containing MA and incubated at 25°C for 24 h. Agar discs (5 mm diam.) were cut from microcolonies and used to inoculate MA treatments and replicates.

Two discs were put into each Petri dish approx. 4 cm apart; one disc was always of *F. verticillioides* which was paired with each of the other fungi considered. Control plates were centrally inoculated with each of the fungi considered in the experiments.

Experiments were carried out on both a_w modified MA medium and the fungicide modified treatments.

Inoculated plates were grouped by a_w level, sealed in plastic bags and incubated at 25°C for 14 d. The diameters of all colonies were measured daily, in two orthogonal directions. Each treatment and condition was applied in triplicate.

Table 2. Fungicides used to modify the maize-based medium, their active ingredients, dosage of active ingredient and ED_{50} concentrations (Formenti *et al.*, 2012).

Fungicide	Active ingredient	ED_{50} µg/kg
Folicur SE®	Tebuconazole	6
Sportak® 45EW	Procloraz	0.0025
Proline®	Prothioconazole	6

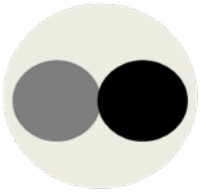
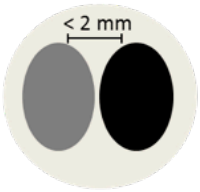
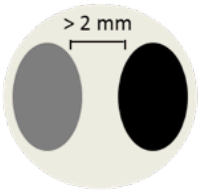
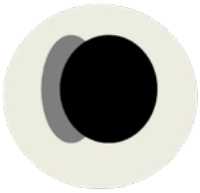
Numerical evaluation of fungal interactions and fumonisin detection

The mean diameters were computed at the incubation time when the first fungal strain covered the whole plate. The interaction between mycelia of dual cultures was determined by macroscopic and microscopic analysis according to Magan and Lacey (1984b), and a score given to each fungal species in the interaction as detailed in Table 3. Scores for each interacting species were added to obtain an overall Index of Dominancy (I_D) as a measure of competitiveness of each fungal species.

For fumonisin analysis, an aliquot of the content of each Petri dishe (1.8 g) was taken from different points along the radius of the colony by cutting agar plugs, which were weighed and transferred to a flask. Fumonisins were extracted for 60 min with 20 mL of methanol:acetonitrile:water (25:25:50 vv) using a magnetic stirrer (Visconti *et al.*, 2001). The solution was then poured into a glass vial and centrifuged at 3000 g for 5 min, diluted (0.1 mL brought to 1 mL) with acetonitrile:water (25:75 vv), and filtered (Millex HV 0.45 µm) before HPLC analysis.

The analyses were performed at the end of the incubation period (14 d), only in media not amended with fungicides, using HPLC as described elsewhere (Pietri and Bertuzzi, 2012). A recovery experiment on media used was performed in triplicate with two different levels of contamination (500 and 5000 ng g^{-1}) resulting in a mean recovery of 95.8% (rsd ±

Table 3. Scores, visual appearance and description used to classify interactions between fungal colonies on agar media (Adapted from Magan and Lacey, 1984b).

Score	Visual appearance	Description
1		Mutual intermingling Score: 1/1
2		Mutual inhibition on contact or space between colonies small (< 2 mm) Score: 2/2
3		Mutual inhibition at a distance (> 2 mm) Score: 3/3
4		Inhibition of one organism on contact, the inhibior species continues to grow unchanged or at a reduced rate through the inhibited colony Score: 0/4
5		Inhibition of one organism at a distance, the inhibitor species then continuing to grow through the resulting clear zone and the inhibied colony, perhaps at a reduced rate Score: 0/5

4.1%). Because of similar fumonisin production levels shown by the two selected strains of *F. verticillioides* in previous studies (Lazzaro *et al*., 2012, 2013), fumonisin B_1+B_2 content was only analysed for one strain (ITEM 10026).

Data analyses

Analysis of variance (ANOVA) was carried out on fungal growth rate data from normal and fungicide modified media. Means were compared using the Tukey's test. The statistical package PASW statistics (ver. 19, SPSS Inc., Chicago, USA, 2009) was used for data analyses.

Results

Fungal growth at different a_w levels

The seven fungal strains considered showed statistically significant differences in growth on

MA medium ($P \leq 0.01$). *Penicillium verrucosum* had the least growth followed by *F. verticillioides* (ITEM 10026). *Fusarium proliferatum* and *A. flavus* were the most rapid colonisers of the maize-based medium. No significant differences were found between *F. verticillioides* (ITEM 10027), *A. ochraceus* and *A. niger* (Table 4, Figure 1). For all species examined, fungal growth was optimum at 0.98 a_w with significant decreases under increasing water stress (0.95 a_w; Table 4, Figure 1).

Strains of *F. verticillioides*, *F. proliferatum* and *A. niger* colonised the MA medium completely after 9 d at 25°C with freely available water (0.99 a_w). However, *A. niger* colonised the whole medium surface over this period also at 0.98 a_w (Figure 1).

Inter-specific interactions between fungi grown on fungicide-containing media

When fungicide was added to the MA medium, all the active ingredients significantly reduced fungal growth ($P \leq 0.01$). *Fusarium verticillioides*, together with *F. proliferatum*, *A. flavus* and *A. niger,* were more affected by fungicides than *A. ochraceus* and especially *P. verrucosum* (Table 5). The effects of the fungicides were also enhanced by lower a_w levels (0.95 and 0.98 a_w) (Table 5).

Prothioconazole and prochloraz were more effective than tebuconazole in limiting fungal growth. All the fungal species were affected by the fungicide-modified media, with reductions in growth varying from about 9 to 66% (Table 5).

In general, *F. verticillioides* and *A. niger* were the most susceptible to the fungicides. Growth of *F. verticillioides* was reduced by 60–70% with prothioconazole and prochloraz (Figure 2). Growth of *A. niger* was reduced by about 66%, *F. proliferatum* had a mean reduction in growth of 60%, followed by *A. flavus* (48%) and *A. ochraceus* (46%). There was only a statistically significant effect of the fungicides on *P. verrucosum* (P=0.01), because of its relative insensitivity to the fungicides used (Table 5, Figure 2).

Water stress had significant impacts on the efficacy of the fungicides. The fungicides were less effective with freely available water (0.99 a_w), with a mean reduction in fungal growth of 36%. At 0.98 a_w and 0.95 a_w there were no significant differences between the fungicides, but the mean reduction in fungal growth was greater, at between 50 and 61%.

Table 4. Analysis of variance results for growth (mean colony radius) at 25°C for the seven fungal strains tested on MA agar at three different levels of a_w, from 0.95 to 0.99.

Factors	Mean radius (mm)[a]
Strain	
F. verticillioides (ITEM 10027)	3.06 bc[b]
F. verticillioides (ITEM 10026)	2.53 e
F. proliferatum	2.87 d
A. flavus	3.25 a
A. ochraceus	3.13 b
A. niger	2.98 c
P. verrucosum	1.42 f
a_w	
0.95	2.62 b
0.98	2.99 a
0.99	2.63 b

a Data are to mean colony radiis (mm) obtained for fungi at the three a_w levels considered (0.95, 0.98 and 0.99) and measured at day 9.

b Different letters indicate significant differences according to the Tukey test (P<0.01).

Fungal interactions, Index of Dominance scores and production of fumonisins

MA media

The interaction scores for *F. verticillioides* ITEM 10027 and *F. verticillioides* ITEM 10026 were calculated in comparison with all the other fungi (Table 6). These two strains of *Fusarium* behaved similarly at 0.99 a_w, exhibiting mutual antagonism on contact with *F. proliferatum*, *A. flavus* and *A. ochraceus*. Only *A. niger* was able to dominate both strains of *F. verticillioides* at 0.95 a_w.

The sum of the I_D values indicated that both *F. verticillioides* strains were in general more competitive at 0.99 a_w while at 0.98 a_w and 0.95 a_w *F. verticillioides* ITEM 10026 was more competitive than *F. verticillioides* ITEM 10027, always having a higher overall I_D at these water availability values.

Considering fumonisins content (FB_1+FB_2), *F. verticillioides* ITEM 10026 produced mycotoxins in the presence of all the other fungi and at all the a_w

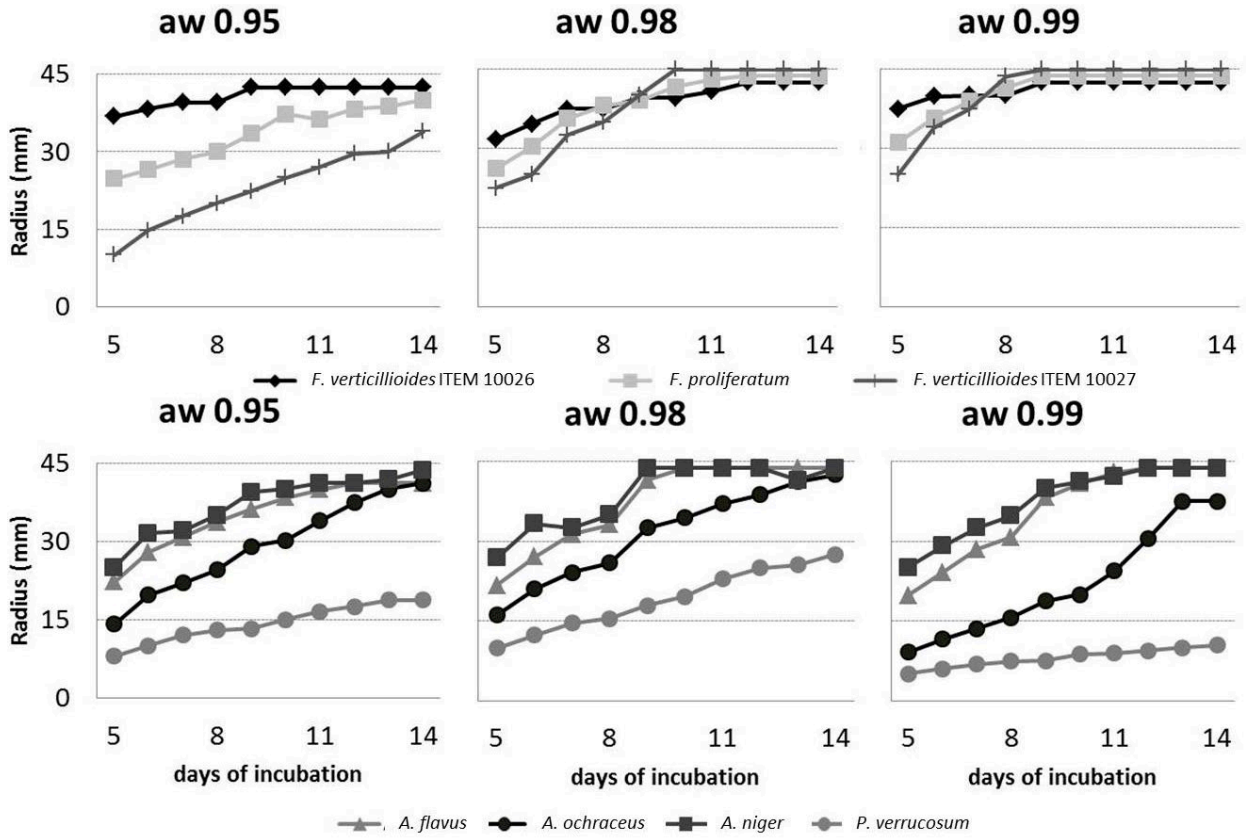


Figure 1. Fungal growth registered for *Fusarium verticillioides*, *F. proliferatum*, *Aspergillus flavus*, *A. ochraceus*, *A. niger* and *Penicillium verrucosum* during incubation of 14 d at 25°C.

Table 5. Analysis of variance results for six fungal strains tested on MA agar amended with three fungicides and incubated at 25°C for 9 d at three different levels of a_w from 0.95 to 0.99.

Fungicide	Fungal growth reduction (%)	Strain	Fungal growth reduction (%)	Aw	Fungal growth reduction (%)
Tebuconazole	33.3 b[a]	*F. verticillioides*[b]	64.3 a	0.95	60.8 a
Prothioconazole	53.9 a	*F. proliferatum*	60.4 ab	0.98	50.1 a
Prochloraz	59.3 a	*A. flavus*	47.5 ab	0.99	35.6 b
		A. ochraceus	45.8 c		
		A. niger	66.4 a		
		P. verrucosum	8.7 d		

[a] Different letters indicate significant differences according to the Tukey test ($P<0.01$). Each datum reported in the table is a mean of several values: Fungicides = mean of three a_w and seven fungal strains; Strain = mean of three fungicides and three a_w; Aw = mean of three fungicides and seven fungal strains.
[b] Mean of ITEM 10027 and ITEM 10026.

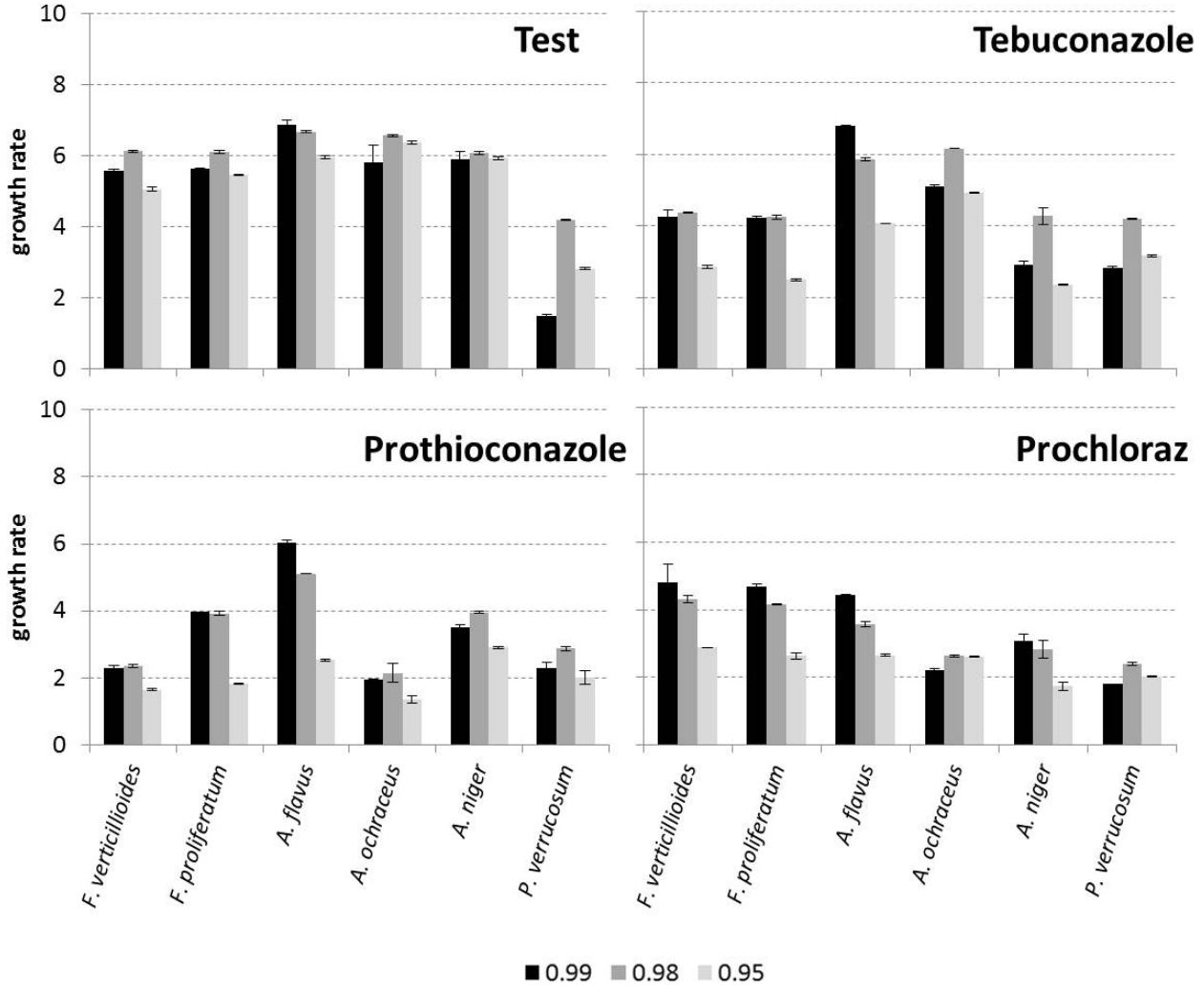


Figure 2. Calculated growth rates (at 9 d) of *Fusarium verticillioides* , *F. proliferatum*, *Aspergillus flavus*, *A. ochraceus* , *A. niger* and *Penicillium verrucosum* on maize agar medium (MA: test), or media modified with sub-optimal levels of three fungicides, and at different a_w levels (0.99, 0.98, 0.95) at 25 °C.

levels tested. In particular, the maximum and minimum fumonisin production were obtained respectively when medium was co-inoculated with *P. verrucosum* at 0.95 a_w (93,667 ppb) and *A. ochraceus* at 0.99 a_w (3,920 ppb). Water availability of 0.95 was the most conducive for fumonisin production, with significant differences with respect to 0.98 and 0.99 a_w ($P \leq 0.01$). Regarding co-inoculation, only fumonisin production obtained with *P. verrucosum* was significantly different ($P \leq 0.01$) from production obtained in the presence of the other fungi (data not shown).

Effects of fungicides on fungal interactions

The addition of fungicides to MA medium changed the relative I_D scores of the fungal strains examined. In particular, considering both *F. verticillioides* strains, their I_D scores were reduced with all three active ingredients examined, and at all the a_w levels considered.

Table 7 shows the effects of interactions between *F. verticillioides* and the other fungi in the presence of the fungicides under different a_w regimes. *Fusarium verticillioides* ITEM 10027 was more affected by the fungicides and had, respectively, total I_D scores of 12,

Table 6. Interaction and Index of Dominance (I_D) scores for *Fusarium verticillioides* (ITEM 10027) and *F. verticillioides* (ITEM 10026) *versus* other fungi frequently isolated from maize. Fungi were grown on maize based agar (MA) at three a_w conditions and incubated at 25°C for 14 d.

Fungal species	0.99			0.98			0.95			I_D		
	ITEM 10027	ITEM 10026	FB_1+FB_2	ITEM 10027	ITEM 10026	FB_1+FB_2	ITEM 10027	ITEM 10026	FB_1+FB_2	ITEM 10027	ITEM 10026	FB_1+FB_2 [a]
F. proliferatum	2	2	8162	2	2	8169	2	2	34017	**6**	**6**	16783
A. flavus	2	2	5691	2	2	15897	0	2	39228	**4**	**6**	20272
A. ochraceus	4	4	3920	2	2	8225	2	4	41909	**8**	**10**	18018
A. niger	2	2	10591	2	2	9513	0	0	15470	**4**	**4**	11858
P. verrucosum	4	4	2737	4	2	15099	2	4	93667	**10**	**10**	37168
Total (I_D)	14	14		8	10		6	12		32	36	

[a] Corresponds to mean of FB_1+FB_2 values obtained at the three different a_w levels tested for each fungus.

14 and 20 with tebuconazole, prothioconazole and prochlorazagainst all the interacting species. Strain ITEM 10026 showed greater I_D scores (24, 24 and 28, respectively, for tebuconazole, prothioconazole and prochloraz) confirming a stronger ability to interact with other fungal species in sub-optimal environmental conditions (Table 7).

In general, the combination of fungicide and reduced a_w level decreased the competitive ability of *F. verticillioides* strains against the other species, especially for ITEM 10027.

In the presence of fungicides, both strains of *F. verticillioides* were unable to compete with *A. flavus* under all the a_w levels considered. *Fusarium verticillioides* ITEM 10026 was never able to inhibit *A. niger* in tebuconazole modified media, regardless of the a_w level; this was similar for *F. verticillioides* ITEM 10027, in the presence of prothioconazole or prochloraz.

Aspergillus ochraceus was able to inhibit *F. verticillioides* ITEM 10027 in media containing Tebuconazole at all the a_w levels considered. In contrast, an opposite effect was observed with the strain ITEM 10026 of *F. verticillioides*. However, *F. verticillioides* ITEM 10026 was able to inhibit *A. ochraceus* in the presence of prochloraz at all the a_w levels tested.

Discussion

Fusarium species exhibited dominance on contact towards *A. ochraceus* and *P. verrucosum* at 0.99 a_w. At lower a_w levels (0.98 and 0.95), however, mutual antagonism was more common and sometimes the *Fusarium* species were dominated by other species, especially *A. flavus* and *A. niger* at 0.95 a_w. Our results contrast with those from other studies. In particular, Wicklow *et al*. (1988), in a trial where maize kernels were artificially inoculated with common fungi present in the field, reported that *F. verticillioides* inhibited infection by *A. flavus.* In another study on niche overlap reported by Giorni *et al.* (2009), *F. verticillioides* was always dominant against *A. flavus* isolates at 0.95 a_w and 20°C. At 0.95 a_w and at 0.99 a_w FB_1+FB_2 production was, respectively, the greatest and least for all the fungal interactions tested. This is in contrast with other studies where 0.99 a_w was the most conducive for fumonisin production by *F. verticillioides* (Lazzaro *et al*., 2012; Fanelli *et al*., 2013). However, in these studies, *F. verticillioides* was considered alone, without competition with other fungi. The presence of other fungi is likely to influence the ability of *F. verticillioides* to produce fumonisins in different environmental conditions. In particular, in our study, differences in fungal interactions were noted only when *F. verticillioides* dominateed contact with other fungi (*A. ochraceus* and *P. verrucosum*).

Fungicides influenced the growth of all the fungal species tested. Since these chemicals are utilised to control *Fusarium* species in maize and wheat, the efficacy against growth of *F. verticillioides* and *F. proliferatum* was confirmed (Folcher *et al*., 2009; Formen-

ti *et al*., 2012). Only *A. flavus* and *A. niger* were able to dominate *Fusarium* species at sub-optimal levels of active ingredients tested. These results are in agreement with the previous study of Marin *et al.* (1998a) who reported that some *Aspergillus* species inhibited growth of some *Fusarium* species in a range of natural conditions. Moreover, the a_w levels tested in this study were relatively greater than the a_w levels of maize at harvest and in the field (Battilani *et al*., 2011) which are more conducive to *Fusarium* dominance.

Differences were found in the responses of the two *F. verticilliodes* strains we tested. In particular, strain ITEM 10027 was more sensitive than ITEM 10026 to the fungicides evaluated, with a resulting lower total I_D, even under water stress conditions. This confirms the possible combined effect of fungicides and low a_w levels to reduce *Fusarium* populations on maize (Parsons and Munkvold, 2010). Some differences in strains are normal but the overall effect of fungicides at low a_w levels were shown for both *F. verticillioides* isolates. The production of increased amounts of fumonisins under water stress was shown in our studies, indicating a response in this fungus to stress condirions (Battilani *et al*., 2005; Parsons and Munkvold, 2010).

Table 7. Interactions between *Fusarium verticillioides* (ITEM 10027) and other common maize grain fungi, on maize agar at various a_w levels and amended with tebuconazole, prochloraz or prothioconazole. Plates were incubated at 25°C for 14 d.

Species	0.99		0.98		0.95		I_D	
	ITEM 10027	ITEM 10026	ITEM 10027	ITEM 10026	ITEM 10027	ITEM 10026	ITEM 10027	ITEM 10026
Tebuconazole								
F. proliferatum	2	2	2	2	2	2	**6**	**6**
A. flavus	0	0	0	0	0	0	**0**	**0**
A. ochraceus	0	4	0	4	0	2	**0**	**10**
A. niger	2	0	2	0	2	0	**4**	**0**
P. verrucosum	2	4	0	2	0	2	**2**	**8**
TOTAL (I_D)	**6**	**10**	**4**	**8**	**2**	**6**	**12**	**24**
Prothioconazole								
F. proliferatum	2	2	2	2	2	2	**6**	**6**
A. flavus	0	0	0	0	0	0	**0**	**0**
A. ochraceus	2	2	2	2	2	2	**6**	**6**
A. niger	0	2	0	2	0	2	**0**	**6**
P. verrucosum	2	2	0	2	0	2	**2**	**6**
TOTAL (I_D)	**6**	**8**	**4**	**8**	**4**	**8**	**14**	**24**
Prochloraz								
F. proliferatum	2	2	2	2	3	2	**7**	**6**
A. flavus	0	0	0	0	0	0	**0**	**0**
A. ochraceus	2	4	2	4	3	4	**7**	**12**
A. niger	0	2	0	0	0	2	**0**	**4**
P. verrucosum	2	2	2	2	2	2	**6**	**6**
TOTAL (I_D)	**6**	10	6	8	8	10	20	28

Previous studies have demonstrated that *F. verticillioides* and *F. proliferatum* shared their niches with *A. ochraceus*, based on both interaction experiments and niche overlap indices (Marin *et al.*, 1998a). Our study showed that when *F. verticillioides* shared its niche with *A. ochraceus* it was able to compete effectively in dual culture for the maize medium domain, although this dominance disappeared when fungicides *Fusarium* spp. were added.

Penicillium verrucosum was the only exception as it behaved differently in the presence of the fungicide treatments. While growing more slowly, this fungus was more tolerant to the treatments, with growth sometimes enhanced. Similar results were obtained with other *Penicillium* species where, for example, *P. implicatum* was dominant against *F. verticillioides*, *P. proliferatum* and other *Aspergillus* species at low a_w levels, even if they grew more slowly (Marin *et al.*, 1998b). The *Penicillium* species are xero-tolerant and thus may have competitive advantages over the *Fusarium* species. Furthermore, they are more likely to compete with other xero-tolerant and xerophilic species such as *A. flavus*. However, it has been demonstrated that growth rate *per se* is not a major criterion in competitiveness and niche occupation (Magan and Aldred, 2007). Other factors such as the rate of spore germination, hydrolytic enzyme production and the utilization of key substrate components may also be important. In a previous study, different fungal species were shown to use diverse carbon sources. *Aspergillus flavus* preferentially utilised carbohydrates while *F. verticillioides* used carbohydrates and amino acids equally (Giorni *et al.*, 2009).

In conclusion, we have demonstrated that *F. verticillioides* strains retain good competitiveness over a range of fungal species present in maize, even in sub-optimal environmental conditions (low a_w levels). In extreme conditions, some *Aspergillus* species can become dominant. However, it is important to emphasize that *F. verticillioides* is the most relevant fungus present in Italian maize, with incidence of infected kernels increasing from 30 to 60%, while *A. flavus* is found only in 5% of kernels (Mazzoni *et al.*, 2011). However, under drought stress, this situation could change.

The use of fungicides for the control of *Fusarium* modified the growth of *F. verticillioides* to a greater extent than non-target mycobiota (in the genera *Aspergillus* and *Penicillium*). Infection of maize by *F. verticillioides* starts from silk emergence and the silk browning stage optimises infection efficiency (Headrick *et al.*, 1990). In a previous study, the presence of *F. verticillioides* and FB_1 content were less if European Corn Borer control was effective (Mazzoni *et al.*, 2011).

The present study confirms that different mycobiota communities can develop on maize and interact with each other. During the maize growing season, several ecological parameters can change, including a_w and temperature, allowing one fungal species to dominate in a specific combination of parameters. In particular, *F. verticillioides*, which is the most important toxigenic fungus in maize grown in Italy, was shown to dominate over almost all the other fungal species considered in this study, especially in conditions of high a_w. However, the use of fungicides can play an important role since they have been shown to modify the natural balance amongst the colonising fungal communities. this information needs to be taken into account as the maize production system is impacted by ecosystem changes, particularly those intimated in current climate change scenarios.

Acknowledgements

We are grateful to the government of Emilia Romagna for supporting this research, and the collaboration of the Research Centre for Crop Production (CRPV). Silvia Formenti carried out this work within the Doctoral School on the Agro-Food System (Agrisystem) of Università Cattolica del Sacro Cuore (Italy).

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Accepted for publication: June 3, 2014
Published online: December 22, 2014