Eur J Plant Pathol (2008) 120:157–166
DOI 10.1007/s10658-007-9204-y

FULL RESEARCH PAPER

Effect of fungicides on the complex of *Fusarium* species and saprophytic fungi colonizing wheat kernels

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Received: 8 December 2006 / Accepted: 31 July 2007 / Published online: 19 September 2007

Abstract *Fusarium* head blight of wheat, often associated with mycotoxin contamination of food and feed is caused by various *Fusarium* species. The efficacy of fungicide sprays for the control of the disease and mycotoxins varies from being highly effective to even increasing mycotoxin levels. The potential role of antagonistic fungi in this variability was investigated assessing sensitivity of *Fusarium* species and saprophytic fungi colonizing wheat kernels to fungicides. Saprophytes were tested for their antagonistic activity to the prevalent *Fusarium* species *Fusarium avenaceum, Fusarium culmorum, Fusarium graminearum*, and *Fusarium poae*. Fungal isolates from mature winter wheat kernels were *Alternaria alternata, Arthrinium* sp., *Aspergillus niger, Epicoccum* sp., *Microdochium* spp., *Rhizopus oryzae* and *Trichoderma* sp. In dual culture *A. niger, R. oryzae* and *Trichoderma hamatum* were more effective in reducing mycelial growth of *Fusarium* species than *Microdochium majus; A. alternata* and *Epicoccum* sp. were ineffective because of slow growth rates. Saprophytic fungi were sensitive to triazoles; however, prothioconazole and tebuconazole had stronger effects on mycelial growth of *Fusarium* species. ED_{50} values also indicated significant differences in the sensitivity of *Fusarium* species to triazoles (range 0.1–1.7 mg l^{-1}). Azoxystrobin and fluoxastrobin were largely ineffective in inhibiting *in vitro* growth of *Fusarium* spp.; sensitivity of the other fungi was generally lower, except for *M. majus* which was highly sensitive. Due to differences in fungicide sensitivity among *Fusarium* spp. and ear-colonizing fungi antagonistic to *Fusarium* spp. fungicides are likely to modify the balance within the mycoflora of wheat ears which may also affect the mycotoxin contamination of grain.

Keywords Antagonistic interactions · *Fusarium* head blight · Strobilurin · Triazole

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Introduction

Fusarium head blight (FHB) is an important disease occurring in various cereal-growing areas all over the world (Parry et al. 1995). It is often associated with considerable yield losses because of premature senes-

cence and reduced grain filling, and contamination of kernels with various mycotoxins, i.e. trichothecenes, zearalenone, moniliformin and fumonisins (Botallico and Perrone 2002).

FHB of wheat is caused by a complex of *Fusarium* species including *F. graminearum, F. culmorum, F. avenaceum, F. poae*, and *F. sporotrichioides* (Parry et al. 1995). These *Fusarium* species produce a range of mycotoxins, whereas *Microdochium majus* and *M. nivale*, fungi also producing FHB symptoms, apparently do not produce mycotoxins (Xu et al. 2005). The distribution and predominance of FHB pathogens vary between regions and within regions from year to year; they are determined by the availability of inoculum sources and by climatic factors (Doohan et al. 2003), particularly temperature and moisture in the period heading to soft dough. It is of great importance to identify the exact *Fusarium* species involved in FHB, as they may differ in their response to environmental conditions as well as in their sensitivity to fungicides (Jennings et al. 2000; Rossi et al. 2001).

Chemical control is an important part of integrated FHB control in production areas with an overall high risk of infection. Fungicides applied to wheat at anthesis are used to reduce quantitative yield losses and mycotoxin contamination of kernels associated with FHB. Numerous studies have documented the effect of fungicide application on FHB and mycotoxin contamination in the field; however, reports on the efficacy are often conflicting. Some triazole fungicides, e.g. metconazole and tebuconazole, were shown to be effective, particularly in experiments with additional *Fusarium* inoculation resulting in reductions of head blight severity and mycotoxin contamination by 50–80% and 5–90%, respectively (Matthies and Buchenauer 2000; Pirgozliev et al. 2002; Chala et al. 2003; Haidukowski et al. 2005). In other experiments, however, fungicide applications resulted in an increased trichothecene accumulation (Gareis and Ceynowa 1994; Obst et al. 2000). Strobilurin fungicides have been reported several times to increase DON production, although they can partially control FHB (Simpson et al. 2001; Lienemann et al. 2002; Menniti et al. 2003; Mesterhazy et al. 2003). In contrast, Siranidou and Buchenauer (2001) and Cromey et al. (2001) observed no effects of azoxystrobin on DON concentrations or even a decrease.

Since cultural measures, use of partially resistant cultivars and use of fungicides give only partial FHB control, biological control is being explored as an additional tool in the integrated management of this disease. Screening for micro-organisms to control FHB has gained momentum in the past and various fungi with antagonistic activity to *Fusarium* spp. have been identified (Khan et al. 2001; Dawson et al. 2004; Luongo et al. 2005). Investigations, however, focus on antagonistic effects in soil, on crop debris, or for storage purposes.

Knowledge on the significance of non-*Fusarium* fungi growing on the wheat surface on the development of *Fusarium* spp., FHB severity and mycotoxin contamination of grain is very limited. Jennings et al. (2000) and Pirgozliev et al. (2003) concluded from field experiments that strobilurin fungicides, by preferentially controlling the FHB pathogen *Microdochium* spp., also eliminate its antagonistic activity against mycotoxin-producing *Fusarium* species. Poor performance of fungicides under field conditions and increased mycotoxin concentration in wheat grain after application of fungicides such as azoxystrobin may be due to the presence of non-target species such as *Alternaria* spp., *Cladosporium* spp., or the non-toxin producing FHB species *Microdochium* spp. Information on the impact of saprophytic fungi on ears and their interactions with *Fusarium* spp is limited.

The objective of this study was to investigate the potential role of saprophytic fungi in the FHB development of wheat treated with fungicides. *Fusarium* species and saprophytic fungi colonizing wheat kernels were assessed on field-grown plants. The most frequent species were tested *in vitro* for their antagonistic potential against four *Fusarium* species. As fungicides applied for the control of leaf diseases and FHB also influence saprophytic fungi on the plant surface, the sensitivity of both *Fusarium* species and saprophytic fungi to fungicides from two chemical groups, triazoles and strobilurins, was investigated *in vitro* in order to explain the variability of fungicide activity in FHB control in the field.

Materials and methods

Field experiments

The FHB susceptible winter wheat (*Triticum aestivum* L.) cullivars Bandit, Complet and Ritmo were grown at Kerpen–Buir and Meckenheim, Germany, in 2001/

2002 (plots 1.5×8 m, 4 replicates). The plots were harvested with a combine harvester on August 13 and the kernels were stored at −20°C. The frequency of fungal infection was assessed for 50 wheat kernels per plot.

Isolation and identification of fungi

For isolation of *Fusarium* species, wheat kernels were surface-sterilized using NaOCl (1.3% available chlorine) for 2 min and placed into Petri dishes (5 kernels per dish) containing Czapek-iprodione-dicloran agar (CZID-agar) prepared according to Abildgren et al. (1987). After incubation at 22°C for 7 days under near-UV light mycelium growing from the kernels was transferred onto SNA (Nirenberg 1976) and PDA and grown at 22°C for 7 days. Pure cultures were obtained by transferring single hyphal units produced by the streak plate method. *Fusarium* isolates used in the *in vitro* experiments are given in Table 1.

Saprophytic fungi were isolated by placing wheat kernels without surface-sterilization into Petri dishes (5 kernels per dish) containing CZID-agar, PDA containing antibiotics (50 mg l^{-1} penicillin, 50 mg l^{-1} chlortetracycline, 50 mg l^{-1} streptomycin) and vegetable juice (V8) agar (160 ml vegetable juice [Krings, Lüdinghausen, Germany], 2.4 g $CaCO_3$, 12 g agar, 800 ml distilled water). Petri dishes were incubated at 22°C for 7 days under near-UV light. Pure cultures were obtained as described for *Fusarium*; they were examined macroscopically for the identifications of fungal growth characteristics using a Leitz DMRB microscope (Leitz, Wetzlar, Germany). *Fusarium* species were identified using the key of Nelson et al. (1983); for other species the keys of von Arx (1987) and Watanabe (2002) were used. Identification of isolates was checked by sequencing the ITS region of fungal DNA according to White et al. (1990).

Test of fungi for antagonism in dual culture

Isolates of *Alternaria alternata, Arthrinium phaeospermum, Aspergillus niger, Epicoccum nigrum, M. majus, Rhizopus oryzae* and *Trichoderma hamatum* were selected for screening of antagonistic effects against the pathogens *F. avenaceum, F. culmorum, F. graminearum* and *F. poae* isolated from wheat kernels (Table 1). Mycelial plugs (∅ 5 mm) of one *Fusarium* species and one potential fungal antagonist were placed onto half-strength PDA with the mycelium facing down. The distance between mycelial plugs was 60±2 mm. The dual cultures were incubated for 10 days at room temperature and with a day–night cycle of 14/10 h. The radial growth of the mycelia of the *Fusarium* species and the potential antagonistic fungus was measured after 3, 7 and 10 days; for asymmetrical colonies the minimum and maximum diameter were measured. The experiments were repeated twice.

Tests on fungicide sensitivity

Mycelial plugs (∅ 5 mm) of each fungus were placed with the mycelial side facing up into the centre of

Table 1 List of fungal isolates used in the *in vitro* assays

Species	Isolate	Origin	Year
Alternaria alternata	Asp 3	Grapes, Veitshöchheim, D	1999
Arthrium phaeospermum	DSMZ 62039	DSMZ, Braunschweig, D	2005
Aspergillus niger	ASP 2	Wheat kernels, Meckenheim, D	2002
Epicoccum nigrum	DSMZ 2586	DSMZ, Braunschweig, D	2005
Fusarium avenaceum	F1.14	Wheat kernels, Kerpen-Buir, D	2002
Fusarium crookwellense	F2.8	Wheat kernels, Kerpen-Buir, D	2002
Fusarium culmorum	F3.34	Wheat kernels, Kerpen-Buir, D	2002
Fusarium graminearum	F5.16	Wheat kernels, Kerpen-Buir, D	2002
Fusarium. Poae	F7.14	Wheat kernels, Kerpen-Buir, D	2002
Fusarium sporotrichioides	F9.8	Wheat kernels, Kerpen-Buir, D	2002
Fusarium tricinctum	F10.10	Wheat kernels, Kerpen-Buir, D	2002
Microdochium majus	Fus 2	Rye, Bayer AG, Monheim, D	1992
Penicillium chrysogenum	Pen 3	Grapes, Veitshöchheim, D	1999
Rhizopuz oryzae	CM 12	Wheat kernels, Meckenheim, D	2002
Trichoderma hamatum	TRI 7	Wheat kernels, Meckenheim, D	2002

half-strength PDA medium amended with the fungicides azoxystrobin (Amistar®, 250 g l^{-1} azoxystrobin, Syngenta, Basle, Switzerland), fluoxastrobin (HEC® 480 SC, Bayer CropScience, Monheim, Germany), prothioconazole (Proline®, 250 g l^{-1} prothioconazole, Bayer CropScience), and tebuconazole (Folicur®, 251.2 g l^{-1} tebuconazole, Bayer CropScience) in concentrations of 0.1, 0.3, 1, 3, 10, 30, and 100 mg l^{-1}, respectively. For every concentration four replicates were incubated at room temperature with a day/night cycle of 14/10 h for 7 days. Radial growth of mycelium was measured after 3 and 7 days. The experiments were repeated twice.

Statistical analysis

Analysis of variance and subsequent comparison of means at the 5% level of significance were performed using SPSS (Vers. 11.0 for Windows, Apache Software Foundation, USA). ED_{50} values of fungicide tests were determined using the model $f_{growth}=k\ (1+(conc/ED_{50})^{**}b)$ in the NLIN procedure, SAS programme (SAS Version 8.0, SAS Institute Inc., Cary, NC, USA).

Results

Incidence of *Fusarium* spp. and saprophytic fungi in the field

Most prevalent *Fusarium* head blight-producing species as well as the spectrum of saprophytic fungi on wheat kernels were identified in field experiments in 2002. Depending on the cultivar, 15 to 40% of wheat kernels were infected by *Fusarium* species. The complex of *Fusarium* species was dominated by *F. avenaceum* with *F. culmorum* being the second most common species, followed by *F. poae* and *F. graminearum*. Additionally, *Fusarium tricinctum, Fusarium crookwellense* and *F. sporotrichioides* were isolated. Depending on the field site, 65 to 95% of untreated kernels were colonized by at least one saprophytic fungus. The most frequent saprophytes were – in order of isolation frequency – *A. alternata, Epicoccum* sp., *Rhizopus* sp., *Trichoderma* sp., *A. niger*, *Arthrinium* sp., and *Microdochium* spp. Isolates of these fungi were used for the subsequent *in vitro* assays (Table 1).

Interactions between saprophytic fungi and *Fusarium* species

Effects of saprophytes isolated from wheat kernels and *M. majus* on mycelial growth of the *Fusarium* species *F. avenaceum, F. culmorum, F. graminearum*, and *F. poae*, and *vice versa* were examined in dual culture. The level of mycelial inhibition of *Fusarium* species varied with the test organisms. *Alternaria alternata* and *E. nigrum* had no effect on mycelial growth of *F. avenaceum, F. culmorum, F. graminearum* or *F. poae* after 10 days (data not shown). The growth rate of *A. alternata* and *E. nigrum* was very slow; therefore, no contact between mycelia of these fungi and mycelia of *F. avenaceum* and *F. graminearum* was observed.

Arthrinium phaeospermum showed intensive mycelial growth and significantly reduced the growth of *F. poae* after 7 days of incubation. An effect on the growth of *F. culmorum* and *F. graminearum* was shown after 10 days of growth in dual culture (Fig. 1). *Fusarium avenaceum* was not significantly influenced by the presence of *Arthrinium* sp.

In contrast to other fungi tested, mycelial growth of *A. niger* was relatively sparse while producing large amounts of spores, and measurements for antagonistic activity were based on the area of Petri dishes covered with *A. niger* spores. In dual culture with *A. niger* all *Fusarium* species revealed a reduction in growth after 7 days, especially *F. culmorum* and *F. poae*, species with rapid growth in single culture, which were inhibited significantly (Fig. 1). The development of *F. graminearum* and *F. poae* was reduced already after only 3 days of incubation.

Mycelial growth of *M. majus* was rather slow compared to the saprophytic fungi tested. After 7 days of incubation there was still no contact between mycelia of *M. majus* and *F. avenaceum* and *F. graminearum*, respectively. However, mycelial growth of both *Fusarium* species was slightly reduced after 10 days (Fig. 1). Effects on the development of *F. culmorum* and *F. poae* also became obvious only at this late stage of the experiments. Mycelium of *M. majus* had direct contact with these *Fusarium* species and the reduction in growth of both antagonist and *Fusarium* species was 40–50%.

Rhizopus oryzae had no significant effect on mycelial growth of any *Fusarium* species grown in dual culture after 3 days. But within 7 days, *R. oryzae*

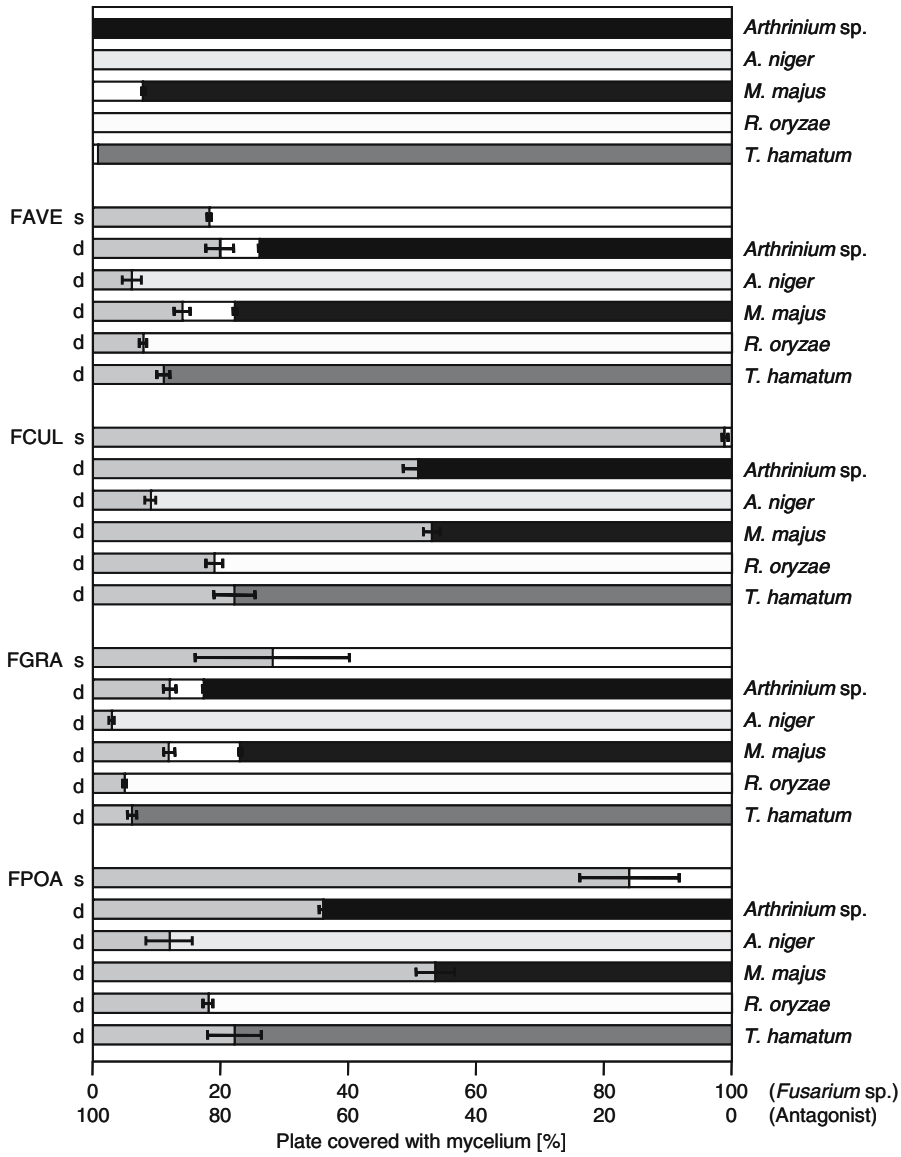

Fig. 1 Antagonistic effect of *Arthrinium phaeospermum*, *Aspergillus niger*, *Microdochium majus, Rhizopus oryzae*, and *Trichoderma hamatum* on *in vitro* growth of *F. avenaceum* (FAVE), *F. culmorum* (FCUL), *F. graminearum* (FGRA), and *F. poae* (FPOA) on PDA. The fungi were grown in single (*s*) and dual (*d*) culture for 10 days (*bars* represent SE)

had covered most of the agar medium, resulting in a significant inhibition of all species, especially the growth of *F. culmorum* and *F. poae* (Fig. 1). After 10 days the effect of *Fusarium* spp. on growth of the zygomycete was smaller than the effect on *M. majus; R. oryzae* had overgrown the mycelium of *Fusarium* spp. to some extent.

After 3 days of incubation, *T. hamatum* had no effect on mycelial growth of the *Fusarium* species tested. Growth of *F. culmorum, F. graminearum* and *F. poae* was significantly inhibited after 7 and 10 days of incubation (Fig. 1). After 7 days, growth of *T. hamatum* was significantly stronger in dual culture than in single culture. Microscopic observations revealed that hyphae of *Trichoderma* sp. and *Fusarium* spp. were in contact indicating mycelial interactions may have occurred.

Effect of fungicides on in vitro growth of fungi

Isolates of the *Fusarium* species and other fungal species isolated from wheat kernels were tested for their sensitivity to triazoles and strobilurins by growing the fungi on PDA containing the active ingredients in a concentration range 0 to 100 mg a.i. l^{-1} for 7–10 days. The triazole prothioconazole was

highly effective in reducing the mycelial growth of all *Fusarium* species tested (Table 2); ED_{50} values of the various *Fusarium* spp. varied from 0.1 to 3.2 mg l^{-1} with *F. crookwellense* being the least sensitive fungus and the only one with an ED_{50} value >1 mg l^{-1}. The other triazole tebuconazole showed very similar efficacy, but at some higher concentrations; in decreasing order, *F. crookwellense, F. tricinctum* and *F. culmorum* had the lowest tebuconazole sensitivity of the *Fusarium* species (ED_{50} values 1.1–5.5 mg l^{-1}). The effect of azoxystrobin on *in vitro* growth of *Fusarium* species was very low; only the ED_{50} value of *F. avenaceum* was <100 mg l^{-1}. The sensitivity of all *Fusarium* species to the strobilurin differed by a factor of > 100 from that of the triazoles. Sensitivity of *Fusarium* spp. to fluoxastrobin also belonging to the group of strobilurins was somewhat higher, particularly for *F. tricinctum* and *F. graminearum*. However, ED_{50} values of 14 and 31 mg l^{-1} of the most sensitive *Fusarium* species also demonstrated an overall low strobilurin sensitivity of this genes.

Mycelial growth of the other ear-colonizing fungi was likewise affected by prothioconazole (Table 2). Sensitivity among fungi differed from <0.1 to 6.2 mg l^{-1} and 26 mg l^{-1} for the zygomycete *R. oryzae*, respectively. Sensitivity of mycelial growth to tebuconazole was in the same range; most fungi were less sensitive to tebuconazole than to prothioconazole; however, *E. nigrum* and *R. oryzae* were remarkable exceptions. The sensitivity to strobilurin compounds differed among fungi. With fluoxastrobin being more active than azoxystrobin, ED_{50} values varied from < 0.1 mg l^{-1} for *M. majus* to >100 mg l^{-1} for *Penicillium chrysogenum*. ED_{50} values for strobilurins were generally higher than for triazoles; *M. majus* was the only exception showing higher sensitivity to strobilurins compared to triazoles and very similar ED_{50} values for both compounds within the two classes of fungicidal compounds.

Discussion

Saprophytic fungi were isolated from wheat kernels, not for a screening of their potential as biopesticides, but to investigate their potential role in the variability of fungicide efficacy in FHB control and mycotoxin contamination of kernels due to their antagonistic properties. The frequency of microbial colonization of mature wheat kernels was high. Most of the kernels were colonized by one or two fungal isolates;>1% of kernels yielded three or more fungi. Colonization was not restricted to the kernel surface, but saprophytes could also be isolated after surface-sterilization (data not shown). It seems likely that

Table 2 Sensitivity of head blight-producing *Fusarium* species and other ear-colonizing fungi to the fungicides prothioconazole, tebuconazole, azoxystrobin and fluoxastrobin

Species	ED_{50} (mg l^{-1})			
	Triazoles		Strobilurins	
	Prothioconazole	Tebuconazole	Azoxystrobin	Fluoxastrobin
Fusarium sp.				
F. avenaceum	0.27±0.01[a]	1.7±0.2	69±11	43±6
F. crookwellense	3.2±0.8	6.5±1.0	>100	>100
F. culmorum	0.19±0.00	2.1±0.5	>100	64±15
F. graminearum	0.40±0.05	0.57±0.06	>100	>100
F. poae	0.09±0.04	1.5±0.1	>100	>100
F. sporotrichioides	0.23±0.04	0.24±0.03	>100	>100
F. tricinctum	0.19±0.01	3.5±0.3	>100	14±4
Other ear-colonizing fungi				
A. alternata	0.41±0.14	1.3±0.2	>100	12±2
A. phaeospermum	0.21±0.01	2.4±0.2	81±9	4.3±0.3
E. nigrum	6.3±0.9	2.5±0.4	>100	24±4
M. majus	1.9±0.2	1.8±0.2	<0.1	<0.1
P. chrysogenum	0.36±0.03	14±2	>100	>100
R. oryzae	26±1	1.5±0.1	>100	12±0.1
T. hamatum	<0.1	2.3±0.2	>100	18±0.7

[a] Mean±SE

kernel colonization by saprophytic fungi prevents infection of kernels by pathogens.

Fungal colonization of plant surfaces is strongly influenced by external factors such as weather conditions, microclimate and availability of nutrients. Relative humidity and UV light especially influence growth and survival of saprophytes on the plant surface. External factors, therefore, contribute to the diversity of species colonizing plant surfaces. Most of the saprophytic fungi identified on wheat kernels have also been reported from the cereal phylloplane (Southwell et al. 1999; Perello et al. 2002). *Epicoccum* sp. and *A. alternata* were the predominant species on wheat kernels in our investigations, whereas these species were detected less frequently on wheat leaves in Argentina where *Cryptococcus* sp. and *Chaetomium globosum* prevailed. According to Southwell et al. (1999), *A. alternata, C. albidus* and *E. niger* are common in the fungal population of wheat and barley phylloplanes.

The predominant saprophytes on wheat kernels, *A. alternata* and *Epicoccum* sp., did not show any effect on *in vitro* growth of *Fusarium* species tested on complete media because of low growth rates. Coiling of *Epicoccum* sp. around the hyphae of *Fusarium* species, giving evidence for hyperparasitism of *E. nigrum* on *Alternaria tritimaculans* Perello et al. (2002) could not be observed. However, high numbers of colony-forming units of these species may be able to compete with *Fusarium* spp. for nutrients and space.

Arthrinium species have been described to produce antibiotics inhibitory to bacteria and fungi like *Aspergillus* and *Penicillium; A. phaeospermum* is able to produce arthrichitin (Larrondo et al. 1996). Since *Arthrinium* is also reported to be involved in the production of mycotoxins in foodstuffs, like *Alternaria, Aspergillus, Fusarium* and *Penicillium* (Blunden et al. 1991), the antagonistic activity of these fungi on wheat ears seems to be two-edged. However, neither species of all of these fungi nor all isolates of these species are potent mycotoxin producers.

Microdochium majus proved to be competitive against *F. culmorum, F. graminearum* and *F. poae*; nevertheless, effectiveness of *M. majus* and *A. phaeospermum* was lower than that of the other antagonists tested. *Microdochium majus* and *M. nivale* cause seedling blight, pink snow mould, stem rot, leaf diseases and ear blight of cereals (Parry et al. 1995; Hare et al. 1999) and prevail in regions with cool and wet conditions like the UK and Ireland (Xu et al. 2005). Although often reported to have a high antagonistic potential (Jennings et al. 2000; Pirgozliev et al. 2003), *M. majus* had only a small effect on mycelial growth of *Fusarium* spp. on artificial media. Since the fungus is known for its preferential growth at lower temperatures its antagonistic activity may be considerably higher at these temperatures. The impact of *Microdochium* spp. as competitors with *Fusarium* species in intensive wheat production is not yet clear.

Aspergillus niger, R. oryzae and *T. hamatum* were the saprophytic fungi with the highest antagonistic potential. *Aspergillus niger* and *R. oryzae* are mainly known for causing rotting of numerous fruits, vegetables and other food products and are commonly found in soils or as airborne spores. Fungi of the genus *Trichoderma* are well known for their antagonistic activity against plant pathogens, mainly against soilborne fungal plant pathogens. Their effect against other fungi is based on parasitism and the production of antifungal antibiotics, i.e. gliovirin and gliotoxin (Howell 2003). The promotion of *T. hamatum* growth in the presence of *Fusarium* species indicates hyperparasitic activity of this antagonist. Interactions with saprophytic fungi, observed in this study, do not clearly reveal the cause of *Fusarium* inhibition. Antagonism may be due to competition for nutrients, production of antibiotics, and or parasitism. The efficacy of antagonists relying on competition may be higher under *in vivo* conditions where nutrient supply and fungal growth rates are lower (Khan et al. 2001).

Differences in the sensitivity to fungicides commonly used in cereals for disease control in high-productivity systems are likely to affect the balance among fungi on wheat ears as well as on leaves. Mycotoxin production may be favoured by fungicide applications by (1) eliminating fungal species competitive to *Fusarium* spp., (2) affecting the balance between *Fusarium* species differing in fungicide sensitivity, and (3) by a direct stress effect on mycotoxigenic species increasing the activity of enzymes involved in toxin biosynthesis (Edwards et al. 2001).

The triazole fungicides prothioconazole and tebuconazole proved to be more effective against *Fusarium* species than the strobilurins tested, confirming earlier results from field experiments (Mauler-Machnik and

Zahn 1994; Simpson et al. 2001; Vanova et al. 2004). Prothioconazole had a higher intrinsic activity against all *Fusarium* species with the difference being significant for most species. Similarly, the new strobilurin fluoxastrobin was more effective in controlling mycelial growth of *Fusarium* species than azoxystrobin; however, ED_{50} values greater than 50 mg l^{-1} for most isolates indicate relative insensitivity of *Fusarium* species to strobilurins as described in field experiments (Ioos et al. 2005). Fungicide sensitivity differed markedly among *Fusarium* species; ED_{50} values for prothioconazole differed by a factor >30 for *F. poae* and *F. crookwellense*. The differences between species reported here do not, therefore, indicate absolute differences between *Fusarium* species, but the variability within the FHB complex.

Overall sensitivity of non-*Fusarium* fungi colonizing wheat ears to triazoles was lower than that of *Fusarium* species. More significantly, ED_{50} values, however, indicated large differences in the fungicide sensitivity among saprophytes from various fungal groups. Sensitivity of *M. majus* reported to be rather insensitive to azoles (Simpson et al. 2001), was moderate in this *in vitro* study. Similar to the results with *Fusarium* species, potential antagonistic fungi had a lower sensitivity to strobilurins; however, there was one important exception. As expected from field experiments (Bertelsen et al. 2001), *M. majus* proved to be highly sensitive to the Q_o inhibitors. *Trichoderma hamatum*, exhibiting antagonistic activity against *Fusarium* species involved in FHB (Kovacikova and Kudela 1990) was sensitive to azoles.

In experiments on the greening effect of strobilurins on wheat, mycelial growth of saprophytic fungi on leaves was reduced by azoxystrobin and epoxiconazole, with the strobliurin being more effective in increasing green leaf area duration and kernel yield in the field (Bertelsen et al. 2001). Being a Q_o inhibitor of fungal respiration (Godwin et al. 1992), azoxystrobin strongly reduced energy-dependent spore germination; in contrast, the sterol biosynthesis-inhibiting triazole had no effect on spore germination, but inhibited mycelial growth. Growth reduction of saprophytes *A. alternata* and *Cladosporium macrocarpum* by the strobilurin was stronger than by the triazole (Bertelsen et al. 2001).

Fungicides with low activity in *Fusarium* control, but inhibiting some saprophytes colonising the plant surface may promote the development of FHB and mycotoxin accumulation (Liggitt et al. 1997; D'Mello et al. 1998; Jennings et al. 2000). It is suggested that over the period of inoculum spread from sources on the soil to susceptible florets of the ear, *Fusarium* species depend on leaves as intermediate stations on their way to the ear. They must compete with other fungi – pathogens and saprophytes – on the plant surface. Fungicides eliminating leaf diseases and also changing the saprophytic mycoflora may facilitate the spread of *Fusarium* spp. by reducing the antagonistic potential of fungi competing with *Fusarium* spp. for nutrients and space.

In areas where FHB is caused by a complex of *Fusarium* species, fungicide applications may result in changes in the balance between species on leaves and ears as well as in the spectrum and amount of mycotoxins. *Fusarium culmorum* and *F. graminearum* are potent colonizers of wheat tissue and can compete with, and decrease, the development of other *Fusarium* species (Jones et al. 1997) or other toxigenic fungi such as species of *Aspergillus, Penicillium* or *Alternaria* (Lacey 1995). In screening tests on fungi antagonistic to trichothecene-producing isolates of *F. graminearum, F. equiseti* and *F. subglutinans*, both species also potent mycotoxin producers, were among the most effective species (Cooney et al. 2001; Dawson et al. 2004; Luongo et al. 2005). The role of competition among *Fusarium* species and isolates differing in mycotoxin production for the overall contamination of grain in this complex system is largely unknown. As fungicides are likely to modify this ecosystem directly, through their effect on *Fusarium* species and their activity on mycotoxin biosynthesis (Edwards et al. 2001), and indirectly, through effects on other pathogens and saprophytes, further investigation on the interactions are required in order to optimize cultural and chemical control of FHB. Direct interactions between saprophytes and pathogens as well as among pathogenic species on the ear should be demonstrated in order to evaluate their effects on the infection rate of FHB-causing fungi. These interactions have to be distinguished from the coincidence of different species which may result from similar ecological requirements for the production of inoculum or the infection of wheat ears.

Acknowledgements The assistance of Prof. R. Haverkamp, Mathematical Seminar, University of Bonn, in the statistical

analysis of data on fungicide sensitivity using nonlinear curve fitting is gratefully acknowledged.

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