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Comparison of the declining triazole sensitivity of *Gibberella zeae* and increased sensitivity achieved by advances in triazole fungicide development

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Abstract

Ascospores of *Gibberella zeae* are part of the primary inoculum for *Fusarium* head blight in wheat, a disease that causes yield losses and contamination of the grain by mycotoxins. Applications of fungicides containing triazoles are particularly effective during wheat anthesis, the time of primary infection. Effects of concentrations ranging from 0 to 10^{-3} M of the active ingredients of epoxiconazole, prothioconazole, metconazole and tebuconazole on germination of *G. zeae* ascospores were studied using strains that were isolated between 1987 and 2004. Prothioconazole, metconazole and tebuconazole inhibited ascospore germination, whereas epoxiconazole had only a minor effect within the range of concentrations tested. Average concentrations that inhibited germination of 50% of the spores (EC_{50}) were $0.58 \pm 0.19 \times 10^{-4}$ M for prothioconazole, $2.14 \pm 0.20 \times 10^{-4}$ M for metconazole, $3.79 \pm 0.31 \times 10^{-4}$ M for tebuconazole and 0.77 ± 0.76 M (value extrapolated) for epoxiconazole. Epoxiconazole (10^{-4} M) reduced mycelial growth of seven tested *G. zeae* isolates by more than 95% but it failed to suppress growth completely. The inhibitory effect of epoxiconazole on mycelial growth was strongest in strains isolated in 1987 and decreased with time at an average rate of 0.22% per year. The initial efficacy of the triazoles against *G. zeae* ascospores at their market introduction increased by a factor of 2.950 within 10 years (epoxiconazole excluded). During the first 10 years following their introduction, the EC_{50}s for metconazole and tebuconazole increased by factors of 1.391 and 1.393, respectively. However, our data suggest that the decline in *G. zeae* sensitivity against triazoles was slower in the past decades than the increase in sensitivity achieved by the development of new and more efficient triazole analogues.

Keywords: Co-evolution; Cross resistance; Fungicide; Fungicide efficacy; *Fusarium* head blight; *Fusarium graminearum*; Resistance

1. Introduction

Fungi of the genus *Fusarium* colonize various host plants, including crops that are essential for human nutrition such as wheat. In wheat, *Fusarium* species cause *Fusarium* head blight (FHB), a disease that is accompanied by yield losses and contamination of the grain by various mycotoxins with adverse effects on animal and human health.

Within the *Fusarium* complex, *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* ([Schw.] Petch) was reported to be the dominant species causing FHB in major wheat-producing areas of the world (Markell and Francl, 2003; Waalwijk et al., 2003). For inoculum dispersal, the fungus produces ascospores as well as macroconidia. Ascospores are formed in perithecia that are able to discharge spores forcibly into the air and—supported by wind—up to the wheat heads. In contrast, dispersal of macroconidia depends largely on the kinetic energy of raindrops. The susceptibility of wheat heads to *Fusarium* species is restricted to the period of anthesis and ascospores were sampled in larger numbers than macroconidia above wheat plots during this critical period (Fernando et al., 2000; Markell and Francl, 2003). Hence, airborne ascospores seem to be more important as primary inoculum

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than macroconidia. Effects of environmental factors on ascospore germination in *G. zeae* were studied previously (Beyer and Verreet, 2005) but information on fungicide effects on the ascospores is missing.

Fungicides containing triazoles as active ingredients are the most effective plant protection agents against *Fusarium* species at present (Mesterhazy et al., 2003). Their mode of action is the inhibition of 14α demethylase, an enzyme that is essential for biosynthesis of ergosterol. Efficacies of triazole fungicides ranged from 25% to 89% in experimental field studies (Haidukowski et al., 2005) and depended on the time of application. The best control of FHB pathogens by fungicides was observed following applications at wheat anthesis, whereas efficacies declined rapidly with time after or before this growth stage (Homdork et al., 2000; Wiersma and Motteberg, 2005). Information currently available suggests that fungicides may be particularly effective against FHB pathogens at early stages of the infection process (Schnieder et al., 2004), when spore germination and germ tube growth are required for a successful infection (Sutton, 1982). On the other hand, some authors note that inhibitors of sterol biosynthesis fail to suppress spore germination (Siegel, 1981; Ramirez et al., 2004).

Since the introduction of target site specific plant protection agents, resistance of various phytopathogenic fungi against fungicides of different chemical classes has been described (Ma and Michailidides, 2005). A prominent example is the rapid development of *Blumeria graminis* resistance towards quinone outer binding site inhibitors (QoIs) that block electron transfer in mitochondrial respiration (Fraaije et al., 2002; Gisi et al., 2000). Furthermore, resistance against 14α-demethylase inhibitors (DMIs) was reported in *Venturia inaequalis* (Kunz et al., 1997) and *Mycosphaerella graminicola* (Zwiers et al., 2002; Stergiopoulos et al., 2003). However, development of fungal resistance towards fungicides depends on factors such as their application frequency, the mode of action of active ingredient(s), the rate of mutations within a pathogen population, the propagation rate and the fitness cost of an acquired resistance (Anderson, 2005; De Waard et al., 2006). Total inefficacy of a fungicide can be caused by the alteration of a single amino acid (e.g. QoI fungicides: monogenic resistance) while polygenic resistances (e.g. DMI fungicides, Brent and Hollomon, 1998) are usually characterized by the accumulation of different mechanisms of adaptation over an extended period time. Fungal mutants selected in the laboratory for resistance to sterol biosynthesis inhibitors also showed resistance to other sterol biosynthesis inhibitors (Siegel, 1981), a phenomenon termed cross resistance. To our knowledge no information on triazole resistance or cross-resistance in *G. zeae* is available.

The objectives of this study were to test if triazoles inhibit germination of the primary inoculum in *G. zeae* and if a decline in triazole sensitivity over time could be compensated for by advances in the development of new active ingredients in recent years.

2. Materials and methods

2.1. Fusarium isolates

Isolates of *G. zeae* (Schw.) Petch (anamorph *F. graminearum* Schwabe) were kindly provided by M. Goßmann (Humboldt University Berlin, isolates 37, 102, 106, and 123) and J. Lepschy (LfL Freising, isolates 137 and 4528b) or were isolated from infected wheat heads by our group (isolate 74B). Strains were isolated in 1987 (137, 4527b [both isolated from maize in Bavaria, Germany]), 1994 (37 [isolated from *Miscanthus* spec. at Forchheim, Germany]), 1999 (102, 106, 123 [all isolated from wheat grain at Frontenhausen, Germany]) or 2004 (74b [isolated from wheat head at Ahrensfelde, Germany]) and subsequently stored as macroconidial suspensions at −70 °C. The identity of isolates as *G. zeae* was confirmed by polymerase chain reaction (PCR) using species-specific primers (Ludewig, 2003; Klix, unpublished) and their morphological traits according to Nelson et al. (1983).

2.2. In vitro production of ascospores

Ascospores of *G. zeae* were produced according to the procedure described by Beyer et al. (2005). Fungal isolates were grown on solid SNA medium (Nirenberg, 1976) and incubated at 25 °C in the dark. Macroconidia were harvested from plates with sterile deionised water. One droplet of spore suspension was used to inoculate solid carrot agar (Klittich and Leslie, 1988) and plates were incubated at 20 °C and a 10 h photoperiod (photon flux density; $205 \pm 14\,\mu mol\,m^{-2}\,s^{-1}$ PAR; light-meter LI-250 equipped with a light quantum sensor LI-190SA, LI-COR Bioscience, Lincoln, USA). After 5 days, formation of the perithecia was induced by flattening the mycelium using a stainless steel rod after addition of 1 ml 2.5% Tween 20 (Carl Roth GmbH, Karlsruhe, Germany). Perithecia developed about 12 days after induction. Ascospores were harvested from condensation droplets at the inner side of the Petri dish lids at the earliest 19 days post induction. The lids of Petri dishes were scanned daily for discharged ascospores. Ascospores utilized in the present study were discharged within the last 24 h, because ascospore viability decreased with time after discharge (Beyer and Verreet, 2005). The ascospores were taken up from the lids using sterile deionized water and stored at −70 °C until further use. Freezing the ascospores had no significant effect on the percentage of viable spores (Beyer and Verreet, 2005). After harvesting the ascospores, Petri dish lids were replaced by new ones and the perithecia were wetted by adding 500 µl of sterile deionised water. However, ascospore production of the isolates varied greatly. Therefore, only isolates that produced enough spores for all relevant treatments and replicates could be used for experimental series.

2.3. Sensitivity tests

The inner sides of Petri dish lids were coated with 1.5% (w/v) water agar (WA) amended with antifungal agents (concentration range from 0 (control) to 10^{-3} M). Active compounds tested were epoxiconazole, metconazole, tebuconazole (all Sigma-Aldrich, Seelze, Germany) and prothioconazole (Bayer CropScience, Monheim, Germany) that were introduced to the markets in 1993, 1994 and 1988 (Tomlin, 2000) and 2004, respectively (for further details see Table 1). Active compounds were dissolved in methanol and added to warm (≈60 °C) WA. The final methanol content in the plates was 10% (v/v) but there was no significant effect of methanol concentrations up to 10% on the time course of ascospore germination ($P = 0.12$, Fig. 1). WA amended with methanol (10%) only served as control. Spore germination experiments were carried out one day after the preparation of the WA plates amended with triazoles as described by Beyer et al. (2004). Briefly, droplets of defrosted spore suspension were transferred to Petri dish lids coated with fungicide amended WA. Relative humidity was adjusted to 100% by placing a wet filter paper on the bottom of the Petri dish. Petri dishes were sealed with parafilm, and, subsequently incubated at 20 °C in darkness. The percentage of germinated ascospores was determined every hour for 20 ascospores per plate ($n = 3$–4 plates per experiment) using a light microscope. The reproducibility of the ascospore germination assay was tested by comparing two experiments with four replicates using isolate 37. Differences between experiments one and two were non-significant at the 5% level ($P_{\text{epoxiconazole}} = 0.95$, $P_{\text{prothioconazole}} = 0.07$, $P_{\text{metconazole}} = 0.27$, $P_{\text{tebuconazole}} = 0.08$). For isolates 123 and 74b, data were obtained using four replicates (plates). Unless specified otherwise, eight concentrations including the control treatment were used per fungicide. The comparison of isolates was carried out on the same WA plates by placing 2 µl droplets of spore suspension from different isolates next to each other.

For selected fungicide–isolate combinations, WA discs (5 mm diameter) colonized by mycelium were transferred to the middle of the Petri dish lids coated with WA containing the fungicides. Radial growth of the colony was measured periodically for up to 14 d at 20 °C and a relative humidity of 100%. Colonies grown on WA without fungicides served as control.

2.4. Statistics

Time courses of ascospore germination (Figs. 1 and 2) were compared at $P = 0.05$ using the REPEATED option of the Statistical Analysis System software package (version 8.02; SAS Institute Inc., Cary, NC, USA) GLM procedure. The radial growth rate of mycelium was estimated from the slope of a linear regression where the radius of the colony was the dependent variable and time was the independent variable. The relationships between dependent variable germination and independent variable concentration were described using the sigmoid model $y = a/(1 + e^{-(x-EC_{50})/b})$, where y is the dependent variable, a the maximum value, x the concentration, EC_{50} the concentration where fungicide efficacy = 50% and b the

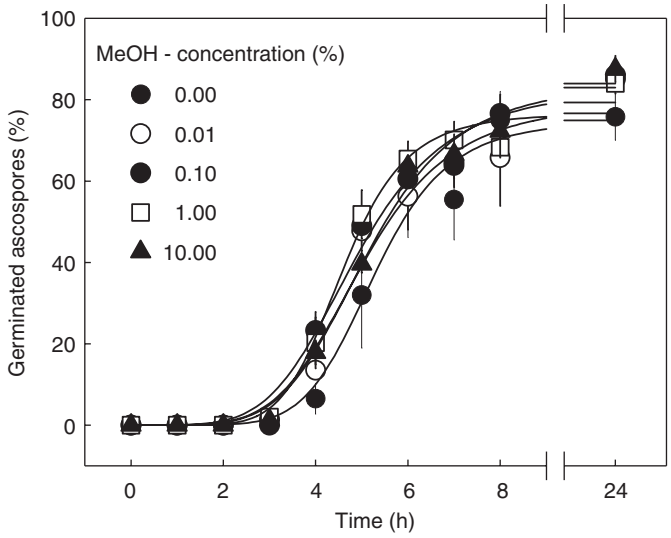

Fig. 1. Effect of methanol concentration (v/v) in water agar (1.5% w/v) on the time course of *G. zeae* ascospore germination (isolate 37). Ascospores were incubated at 20 °C and 100% RH in the dark.

Table 1
Selected properties of the triazoles used in the present study

Compound	Formula	CAS-no.	MW	Solubility in water $\times 10^{-3}$ (g/100 g at 20 °C)	LD 50[a] (mg/kg)	Manufacturer
Tebuconazole	$C_{16}H_{22}ClN_3O$	107534-96-3	307.82	3.6	1700	Sigma-Aldrich
Metconazole	$C_{17}H_{22}ClN_3O$	125116-23-6	319.80	3.0[b]	660	Sigma-Aldrich
Epoxiconazole	$C_{17}H_{13}ClFN_3O$	106325-08-0	329.76	0.7	3160	Sigma-Aldrich
Prothioconazole	$C_{14}H_{15}Cl_2N_3OS$	178928-70-6	344.26	n.a.[c]	6200	Bayer CropScience

[a]Oral, rat. Whenever differences between male and female rats were reported, the lower LD_{50} is given. Data for prothioconazole were taken from www.kemi.se, all others from Industrieverband Agrar (2000), see reference list).
[b]At pH 7.
[c]No data available, yet.

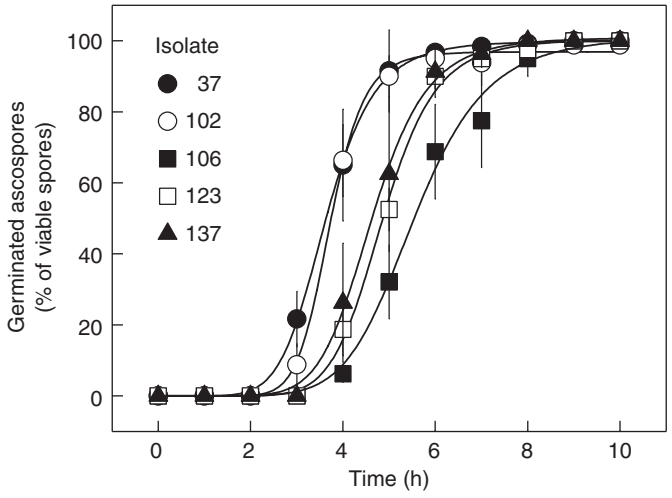

Fig. 2. Time course of ascospore germination of five *G. zeae* isolates. Ascospores were incubated on 1.5% WA at 20 °C and 100% RH in the dark.

slope parameter (Fig. 3). Unless specified otherwise, data are presented as mean ± standard error (SE).

3. Results

Germ tube growth of *G. zeae* ascospores was observed 3 h after inoculation on WA at 20 °C and 100% RH, in darkness (Fig. 2). Eight hours post inoculation, all viable spores germinated irrespective of isolate. The percentage of germinated spores was assessed after more than 8 h in subsequent experiments with fungicides. The differences between the time courses of ascospore germination of five isolates tested were non-significant ($P = 0.17$) in the absence of fungicides (Fig. 2).

Prothioconazole, metconazole and tebuconazole suppressed ascospore germination while epoxiconazole had only a minor effect within the range of concentrations tested (up to 10^{-3} M, Fig. 3). Depending on the isolate, the EC_{50} values of ascospore germination ranged from 0.32×10^{-4} to 0.94×10^{-4} M for prothioconazole, from 1.77×10^{-4} to 2.47×10^{-4} M for metconazole, from 3.27×10^{-4} to 4.34×10^{-4} M for tebuconazole and from 1.08×10^{-2} to 2.30 M for epoxiconazole. Since epoxiconazole had only a small effect on ascospore germination, the EC_{50} had to be extrapolated in this case. Results obtained by extrapolation are somewhat uncertain and therefore changes in epoxiconazole sensitivity over time were additionally studied using a mycelium assay. Mycelial growth of the seven *G. zeae* isolates tested was retarded by epoxiconazole, but it failed to entirely suppress growth (Table 2). In contrast, the growth of mycelium was completely inhibited at tebuconazole concentrations $\geqslant 0.001$ M (data not shown). A negative relationship between the inhibitory effect of epoxiconazole on mycelial growth and the year the *G. zeae* strains were isolated, was found (Table 2, regression equation: Inhibition (%) = 553.64−0.22 × year of isolation, $r^2 = 0.70^*$).

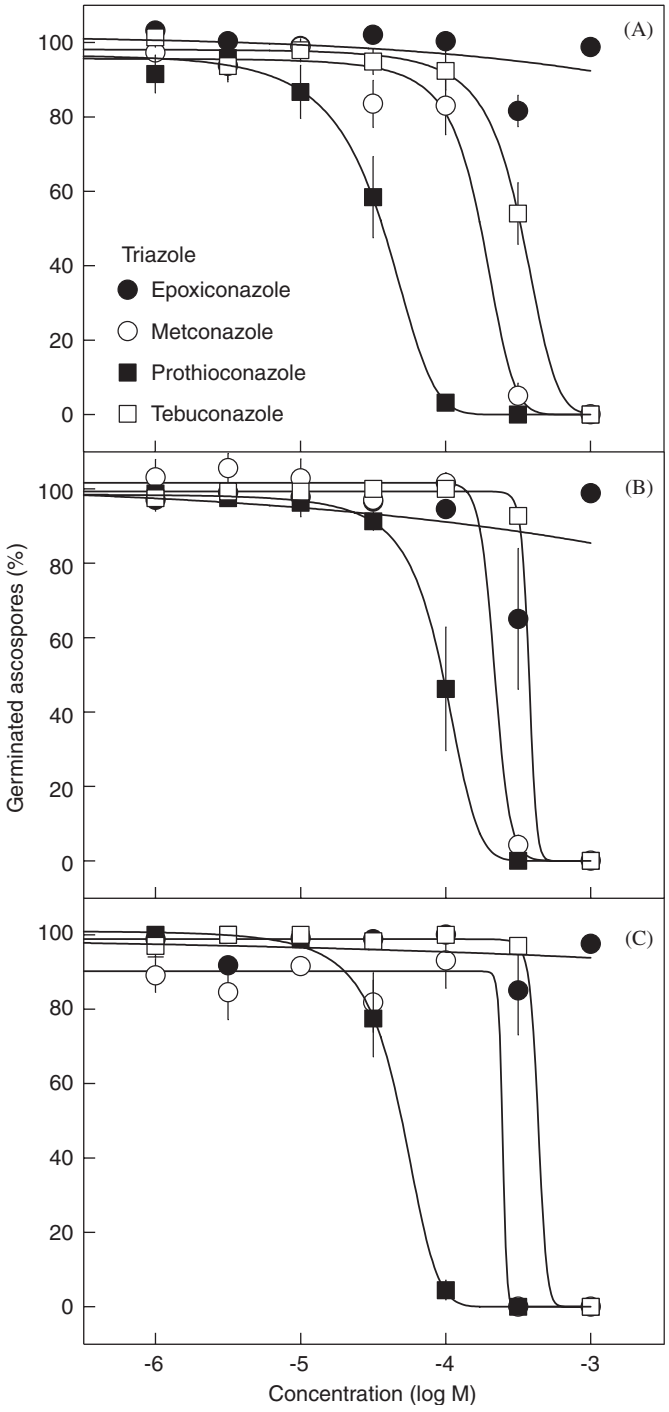

Fig. 3. Effects of epoxiconazole, prothioconazole, metconazole and tebuconazole at concentrations ranging from 0 to 10^{-3} M on the percentage of germinated ascospores of *G. zeae*. (A) Isolate 37 (isolated in 1994). (B) Isolate 123 (isolated in 1999). (C) Isolate 74b (isolated in 2004). Ascospores were incubated at 20 °C and 100% RH in the dark.

EC_{50}s were lower for *G. zeae* strains isolated in 1994 than those of strains isolated in 1999 or 2004 except for prothioconazole, where no consistent trend over time was

Table 2
Radial growth rates of mycelium of seven *Fusarium graminearum* strains isolated between 1987 and 2004 as affected by epoxiconazole (10^{-4} M)

Isolate	Year of isolation	Growth rate of mycelium (mm d^{-1})		Inhibition (%)
		Control	Epoxiconazole	
37	1994	7.400±0.677	0.098±0.044	98.7
102	1999	5.990±1.074	0.172±0.035	97.1
106	1999	3.963±0.252	0.184±0.033	95.4
123	1999	4.224±0.808	0.172±0.035	95.9
137	1987	6.283±1.291	0.023±0.016	99.6
4527b	1987	6.937±0.626	0.136±0.072	98.0
74B	2004	5.123±0.620	0.221±0.052	95.7

Fusarium isolates were grown on 1.5% (w/w) water agar amended with epoxiconazole at 20 °C and the diameter of the developing colony was measured periodically. Growth rates were estimated from a plot of colony radius vs. time using the slopes of linear regressions. Data are presented as means±SE of four replicates.

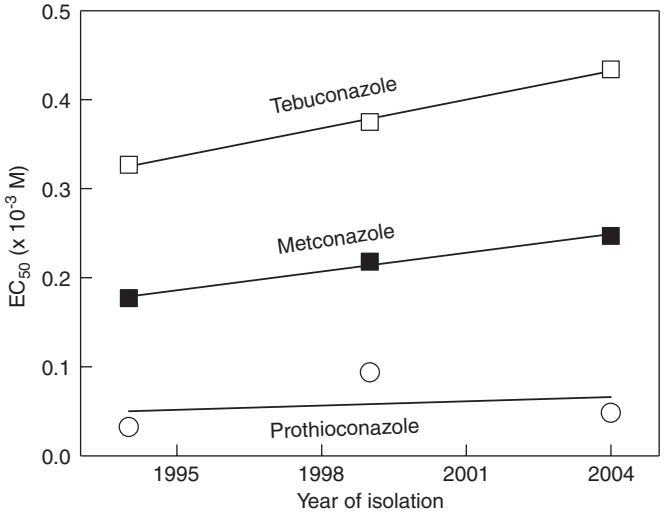

Fig. 4. Relationship between EC_{50} values and time expressed as years that fungal strains were isolated. EC_{50} values were obtained from the interpolations depicted in Fig. 3.

observed (Fig. 4). Regression equations were: EC_{50} (M) $= -0.021 + 1.07 \times 10^{-5} \times$ year of isolation ($r^2 = 0.99$, $P = 0.04$) for tebuconazole, EC_{50} (M) $= -0.014 + 7.00 \times 10^{-6} \times$ year of isolation ($r^2 = 0.99$, $P = 0.06$) for metconazole and EC_{50} (M) $= -0.003 + 1.60 \times 10^{-6} \times$ year of isolation ($r^2 = 0.06$, $P = 0.84$) for prothioconazole.

4. Discussion

4.1. Inhibition of ascospore germination by triazoles

Triazoles are inhibitors of the ergosterol biosynthesis pathway (Siegel, 1981). According to Ramirez et al. (2004), fungicides inhibiting the biosynthesis of ergosterol fail to inhibit spore germination, which is in agreement with the results we obtained for epoxiconazole. However, all other triazoles we tested prevented ascospore germination in *G. zeae*. Hence, some inhibitors of ergosterol synthesis fail to inhibit fungal spore germination, while others do not.

4.2. Considerations on cross resistance

Given the fact that the mode of action should be the same for all triazoles tested, the large difference between the effect of epoxiconazole and the other triazoles is remarkable. Fungal mutants selected in the laboratory for resistance to sterol biosynthesis inhibitors also showed resistance to other sterol biosynthesis inhibitors (Siegel, 1981; Zwiers et al., 2002), a phenomenon termed cross resistance. If the low efficacy of epoxiconazole was related to fungal resistance against this active ingredient, cross-resistance does not seem to play a dominant role with regard to the other triazoles tested. The fact that epoxiconazole did not kill strains that were isolated before its market launch in 1993, suggests that epoxiconazole was fungistatic rather than fungicidal for *G. zeae* from the very beginning. Furthermore, a 0.22% loss of efficacy per year is small. Hence, the large difference of efficacies between epoxiconazole and the other triazoles tested seems to be primarily caused by a low baseline sensitivity of *G. zeae* to epoxiconazole and not by resistance. Since the declines in sensitivity observed in the present study were rather small, cross-resistance phenomena can also be expected to be small in the system described.

4.3. Comparison of in vivo and in vitro performance of epoxiconazole

Henriksen and Elen (2005) reported that a treatment with kresoxim methyl and epoxiconazole prior to wheat anthesis was hardly effective against FHB infections in a field experiment. They suggested that fungicide applications prior to flowering promote subsequent infections by *Fusarium* species due to the elimination of competing microflora on the ears. Our experiments indicated a poor performance of epoxiconazole too, but the timing of fungicide applications in relation to the growth stage of host plants or competing microflora can be excluded as explanations in our *in vitro* assays. Further evidence for a low efficacy of epoxiconazole to FHB pathogens was provided by Menniti et al. (2003), who found that epoxiconazole had no effect on the content of the *Fusarium* toxin deoxynivalenol (DON) in durum wheat samples while tebuconazole, prochloraz and bromuconazole reduced the DON content on average by 43%. Since we used active ingredients solely, we can rule out that the effects reported in the present study were biased by additives that are commonly used in commercial fungicide formulations. The extrapolated average EC_{50} (spore germination basis), for epoxiconazole (0.77 M $\approx$ 256 g l^{-1}) was several orders of magnitude larger than its solubility in water. Hence, effective epoxiconazole concentrations are hardly possible in aqueous solution.

4.4. Advances in fungicide development vs. declining fungal sensitivity

A negative relationship was observed between fungicide efficacy expressed as EC_{50} on an ascospore germination basis and the year of fungicide market launch (Fig. 5). As already shown, epoxiconazole represents an outlier. The fact that fungicide efficacy in our assay was negatively related to the number of years the fungicides were in use may be interpreted as evidence for emerging fungal resistance against triazoles. A stepwise decrease in sensitivity would be in agreement with the "quantitative" or "multi-step" resistance development model suggested for DMI fungicides by Brent and Hollomon (1998). The longer and the more often an active ingredient is used, the more likely is the selection of partially or totally resistant microbial strains. However, the lower efficacy of older triazoles is not necessarily related to fungal resistance but may indicate that skills and technologies of the manufacturers to identify and produce better active ingredients improved with time. This hypothesis is supported by Fig. 4, where the slope of the lines can be interpreted as changes in fungal sensitivity over time and the *y*-intercept of the lines at the time of introduction of the respective compound as an estimate of the baseline sensitivity. Using the relationships depicted in Fig. 4, baseline EC_{50}s were estimated to be 2.72×10^{-4} M for tebuconazole in 1988, 1.79×10^{-4} M for metconazole in 1994 and 6.64×10^{-5} M for prothioconazole in 2004. Hence, the initial efficacy of the triazoles increased by a factor of 4.096 within 16 years which corresponds to a factor of 2.950 within 10 years. This effect can be attributed to advances in fungicide development. Within the first 10 years after their introduction, the EC_{50}s for metconazole and tebuconazole increased by factor 1.391 and 1.393, respectively. This decline in sensitivity can be attributed to fungal adaptation to triazoles. However, advances in triazole development seemed to be faster in the system described herein than the decline in *G. zeae* sensitivity during the last decades. For prothioconazole we can rule out that the fungal isolates used in the present study were challenged by this compound before, because they were isolated in or before 2004, the year where prothioconazole was introduced. Hence, no consistent trend (slope) can be expected in Fig. 4 and therefore only the baseline sensitivity could be estimated for prothioconazole. The effect of epoxiconazole on mycelial growth decreased at a rate of 0.22% per year, which is almost negligible and illustrates that the phenomenon described above is not restricted to ascospores.

4.5. Ranking triazoles by efficacy and toxicity

The use of molar concentrations allowed the evaluation of fungicide efficacy on a molecule basis. For instance, comparison of EC_{50} values revealed that a single molecule of prothioconazole was 3.7 and 6.5 times more effective in inhibiting *G. zeae* ascospore germination than one molecule of metconazole or tebuconazole, respectively. Metconazole was 1.8 times more effective than tebuconazole. A good fungicide should be characterized by high efficacy against fungi as well as a low toxicity to other organisms such as mammals. Efficacies (EC_{50}), of the tested compounds may be standardized such that relative efficacies of 1.00, 0.27, 0.15 and 0.00 are obtained for prothioconazole, metconazole, tebuconazole and epoxiconazole, respectively. Toxicities ($LD_{50\ rat}$) may be standardized in the same way and relative toxicities of 0.10, 1.00, 0.35 and 0.19 are obtained for prothioconazole, metconazole, tebuconazole and epoxiconazole, respectively. Dividing the relative fungicide efficacy by its relative toxicity resulted in 10.33, 0.27, 0.44 and 0.00 relative units efficacy for prothioconazole, metconazole, tebuconazole and epoxiconazole per relative unit toxicity, respectively. Hence, prothioconazole produced the highest benefit at a given level of toxicological cost in this system, followed by tebuconazole, metconazole and epoxiconazole.

4.6. Estimating critical water volumes for commercial field applications

Numerous fungicide formulations are currently approved for use against *Fusarium* species in the field. In Germany, the list of approved fungicides in winter wheat consists of eight products (http://www.bvl.bund.de/pflanzenschutz/psmdbstart.htm). However, all of those eight products contain at least one of the four active ingredients covered by this study. Some products contain one active ingredient only and in those cases our data can be used to estimate if the EC_{50}s observed in the present study are exceeded in commercial fungicide applications. The products Folicur, Caramba and Proline contain the active ingredients tebuconazole, metconazole and prothioconazole at concentrations of 251.2, 60.0 and 250.0 $g\,l^{-1}$,

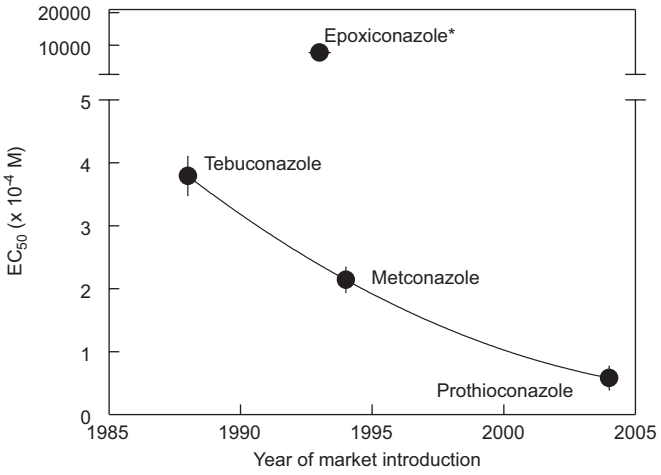

Fig. 5. Relationship between the year of introduction to the market and EC_{50} values on spore germination basis of the tested antifungal compounds. *The EC_{50} value for epoxiconazole was obtained by extrapolation.

respectively. Application rates of 1.0, 1.5 and 0.8 l ha^{-1} for Folicur, Caramba and Proline respectively were approved against *Fusarium* species in wheat. Concentrations of active ingredients would fall below the EC_{50} of *G. zeae* ascospores if 1.0 l ha^{-1} Folicur, 1.5 l ha^{-1} Caramba, or 0.8 l ha^{-1} Proline are applied in more than 2153, 1315 or 10017 l water ha^{-1}, respectively. Recommended water volumes used in fungicide applications in the field range from 200 (Frießleben, 2005) to 600 l ha^{-1} (Menniti et al., 2003). Thus, concentrations used in the field greatly exceed the EC_{50} level we calculated based on our experiments when following official and manufacturer's recommendations. However, concentrations in the field obviously also depend on factors such as dilution by rain or dew and evaporation. Reduced application rates or mixtures of plant protection agents are used from time to time in commercial production and our conclusions on critical concentrations do not necessarily hold for these situations. Finally, it should be noted that besides fungicides, agronomic factors such as management of previous crop and tillage are important in FHB control (Schaafsma et al., 2005).

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