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Will Triazoles Still Be of Importance in Disease Control of *Zymoseptoria tritici* in the Future?

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Abstract: Septoria tritici blotch (STB), caused by *Zymoseptoria tritici*, is one of the most important foliar wheat diseases worldwide. Current control strategies of STB rely mainly on fungicides, whereby triazoles (demethylation inhibitors; DMIs) have been the backbone in the control of *Z. tritici* in the last decades. However, in recent years a gradual loss of sensitivity of *Z. tritici* to several active ingredients of the triazole group has been reported in several European wheat-growing areas. Nevertheless, a new triazole fungicide, namely, mefentrifluconazole, has recently become available in disease management of STB, which belongs to a completely new triazole subclass, the so-called isopropanol triazoles. In this study, the trend in sensitivity development of *Z. tritici* towards older triazoles (tebuconazole, prothioconazole, and propiconazole) and the new isopropanol triazole mefentrifluconazole was determined in microtiter assays using *Z. tritici* field populations isolated in 1999, 2009, 2014, and 2020 in a high-disease-pressure and high-fungicide-input area in northern Germany in order to investigate whether the loss of sensitivity of *Z. tritici* to older triazoles also applies to mefentrifluconazole. For the three triazole fungicides tebuconazole, prothioconazole and propiconazole, a significant shift towards decreasing sensitivity of *Z. tritici* field populations was observed from 1999 to 2020, whereas the efficacy of mefentrifluconazole in reducing the in vitro fungal growth by 50% (EC_{50}) remained unchanged over the investigated period, demonstrating a stable sensitivity of *Z. tritici* towards mefentrifluconazole. Although older triazoles are suffering from a loss of sensitivity of *Z. tritici* field populations due to the selection and spread of less triazole sensitive strains within the *Z. tritici* population, the efficacy of the new triazole mefentrifluconazole with its unique isopropanol unit was not affected by these changes within the *Z. tritici* population. Thus, the introduction of such new molecular units could also represent an important contribution for older groups of active ingredients, which previously suffered from a loss of sensitivity.

Keywords: *Zymoseptoria tritici*; Septoria tritici blotch; wheat; fungicide sensitivity; resistance; demethylation inhibitor; triazole; mefentrifluconazole; isopropanol; microtiter assay

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1. Introduction

Fungal disease control in cereals relies upon the presence of effective fungicides [1]. These agrochemical compounds are also intensively used in wheat to reduce yield losses caused by *Z. tritici*, the causal agent of Septoria tritici blotch (STB). STB is one of the most important foliar wheat diseases worldwide [2]. Especially in maritime climatic regions of Europe, STB is the most yield-reducing wheat disease [3,4]. In Europe, 70% of the annual fungicide usage in wheat is linked to disease control of *Z. tritici* [4]. However, the frequent use of fungicides in disease management of *Z. tritici* gives rise to selection of fungicide resistance. One example is the emergence of resistance to quinone-outside inhibitors (strobilurins; QoIs), which no longer provide reliable field control of *Z. tritici* in most European countries [5–7]. Currently, the control of STB depends on the application of

single site demethylation inhibitors (azoles; DMIs) and succinate dehydrogenase inhibitors (carboxamides; SDHIs) as well as protective multi-site inhibitors as mixing partner for DMIs and SDHIs [8–11].

DMIs, mainly represented by the triazoles (e.g., tebuconazole, prothioconazole, and epoxiconazole) and the imidazole prochloraz, have been extensively used in disease management of *Z. tritici* since the 1980s [12], being the backbone in the control of STB since the development of QoI resistance in the mid-2000s [13]. However, a declining sensitivity of *Z. tritici* field populations towards DMI fungicides (shifting) has been documented in several European wheat growing areas since the early 2000s (e.g., United Kingdom, Germany, Ireland, and France) with a general increase of EC_{50} values for several active ingredients within the group of DMIs [11,13–19]. Due to the reduced field efficacy of DMIs in controlling STB, higher doses are now required to achieve effective disease control [10,17].

The reduction in sensitivity of *Z. tritici* to DMIs is mainly associated with mutations or mutation combinations in the CYP51 gene leading to amino acid changes of the CYP51 enzyme (C_{14}-demethylase), the molecular target of DMIs. More than 30 alterations have been found in the CYP51 enzyme, altering the CYP51 protein structure, and hence the binding capacity of DMI molecules [20–24]. Further molecular changes such as inserts in the promoter region of the CYP51 gene leading to an overexpression of the CYP51 enzyme or an enhanced efflux of DMIs reducing the accumulation of fungicidal compounds in the fungal cell can contribute to the decline in sensitivity towards DMIs [23,25–28]. Within the group of DMI active ingredients several incomplete cross-resistance relationships exist [9,16,20,22,24,26,29].

Nevertheless, DMIs still play an important role in the control of STB [30], including the direct control of STB as well as its usage as mixing partner for the very effective SDHIs by delaying resistance development of SDHIs. In fact, the mixture of DMI and SDHI fungicides is currently the most effective strategy to control STB and helps to slow down resistance build-up [31–33]. However, a further loss of sensitivity of *Z. tritici* to DMIs might increase the risk of resistance development of SDHIs. Unfortunately, the increased use of SDHIs in the last decade has also led to decreased sensitivities of *Z. tritici* towards that group of active ingredients in recent years [19]. In addition, fewer active ingredients are available for the control of *Z. tritici*, making a foresighted resistance management difficult. This is due to the progressing (e.g., DMIs) or complete (e.g., QoIs) fungicide resistance development and the ban of several fungicides within the European Union formerly used for management of STB such as DMI products containing epoxiconazole [34] and propiconazole [35] or most protective multi-site inhibitors (e.g., chlorothalonil, mancozeb) [36,37]. With the exception of SDHIs, hardly any new active ingredients have been launched on the market in the past, especially DMIs.

However, in 2020, a new triazole fungicide, namely, mefentrifluconazole, was introduced to the European market [11]. According to Jørgensen et al. [38], mefentrifluconazole performs significantly better against STB in the field compared to older triazoles that were usually used for disease management of STB (e.g., tebuconazole, prothioconazole, and epoxiconazole). This new active ingredient is expected to replace some of the older triazoles due to its unique chemical structure with a new flexible isopropanol unit [39].

In the light of decreasing efficacy profiles of older triazoles commonly used for STB control in wheat, the special position of mefentrifluconazole within the triazole group is to be demonstrated. In our present study we investigate the efficacy of mefentrifluconazole with its flexible isopropanol unit in comparison to older triazoles in microtiter assays by using *Z. tritici* field populations isolated between 1999 and 2020 in a high-disease-pressure and high-fungicide-input area in northern Germany. The aim of our present study was to clarify the role of triazoles, which previously suffered from a loss of sensitivity, in future control strategies against *Z. tritici* due to innovations in their chemical structure.

2. Materials and Methods

2.1. Sampling Area and Isolation of Z. tritici

Wheat plants of the cultivar "Ritmo", characterized as moderately to highly susceptible to STB [40], were sampled in 1999, 2009, 2014, and 2020 in the northernmost federal state of Germany, Schleswig-Holstein. The region between the North and Baltic Sea is characterized by large acreages of winter wheat, accounting for 28.9% of arable land [41], and maritime weather conditions, with an average annual temperature of 8.9 °C and an annual precipitation of 823 L/m^2 [42], which are conducive for *Z. tritici* infections in wheat [43,44]. In each sampling year, 30 wheat plants with *Z. tritici* lesions were collected at growth stage 51 (begin of ear emergence) [45] from two reference locations, namely Kluvensiek (Coordinates: x = 1092115, y = 7234219; EPSG-Code: 3857) and Futterkamp (Coordinates: x = 1183896, y = 7225601), which were part of a regional monitoring for leaf pathogens (IPM wheat model) [43] and Fusarium head blight (FHB) in wheat [46,47]. Wheat plants were taken from three fungicide untreated control plots (10 plants per plot; plot size 2 × 5 m) per location and year within the IPM and FHB monitoring and were stored at −20 °C until *Z. tritici* isolation.

For the preparation of *Z. tritici* field populations [19], leaf pieces (3 cm in length) from the upper three leaves with typical *Z. tritici* necroses containing pycnidia were washed in running tap water, surface sterilized (2 min in 2% sodium hypochlorite), triple rinsed with sterile deionized water, and dried on tissue paper. During incubation of leaf pieces on water agar (15 g in 1 L water; Carl Roth, Karlsruhe, Germany) for 24 h in the dark at 20 °C, cirrhi were discharged from pycnidia. Cirrhi from individual pycnidia were transferred to a Petri dish containing malt yeast agar (MYA; 4 g of yeast extract, malt extract, glucose plus 15 g of agar in 1 L water; all obtained from Carl Roth) as well as penicillin and streptomycin (each 50 mg/L; both obtained from Carl Roth) to prevent bacterial growth. In total, 15 Petri dishes, originating from 15 individual pycnidia, were prepared for each field plot per location and year, resulting in 45 plates per location and year. Spores produced on MYA plates were washed off using 10 mL sterile skim milk (100 g of skim milk powder in 1 L of water; Sigma-Aldrich, Schnelldorf, Germany) per plate. Spores of each plate, representing individual pycnidia isolates, were stored separately at −70 °C (stock solution) until further use. For the fungicide sensitivity tests, spores from the stock solution were transferred to MYA plates, distributed over the medium surface, and grown for 4 days at 20 °C. Subsequently, spores were washed off using sterile deionized water and spore concentrations of the solutions originating from individual pycnidia isolates were determined using a Fuchs Rosenthal haemocytometer and a light microscope. Spore solutions were diluted with sterile deionized water to approximately 2.5 × 10^5 spores/mL. Mixtures of 15 spore solutions originating from 15 isolated individual pycnidia isolates per field plot (replication) within the same location and year were prepared, representing *Z. tritici* field populations, which were used for the in vitro fungicide sensitivity bioassays described below.

2.2. Fungicide Sensitivity Testing

The in vitro fungicide sensitivity of *Z. tritici* field populations isolated in Northern Germany in 1999, 2009, 2014, and 2020 was determined by using microtiter assays as described by Birr et al. [19]. Analytical grade compounds (>98% purity) of tebuconazole, propiconazole, prothioconazole (all purchased from Sigma-Aldrich), and mefentrifluconazole (HPC Standards GmbH, Cunnersdorf, Germany) were used in six test concentrations of 125, 12.5, 1.25, 0.125, 0.0125, and 0 mg/L, respectively, which were obtained via serial dilutions with ethanol (HPLC grade). For each fungicide, triplicates of each test concentration were added to clear, flat-bottomed 96-well microtiter plates (Sarstedt, Nümbrecht, Germany) with 100 µL per well for each year of *Z. tritici* isolation. After evaporation of solvents overnight, 100 µL of culture medium (4 g of yeast extract, 4 g of malt extract, and 4 g of glucose in 1 L of deionized water; all obtained from Carl Roth) and 100 µL of spore solution with a dilution of 2.5 × 10^5 spores/mL (medium–spore solution) were transferred to each well containing the abovementioned fungicide dilutions. Each 96-well plate was also loaded

with medium–water controls containing the abovementioned fungicide concentrations in triplicate, but without spore solution consisting of 100 µL medium and 100 µL sterile deionized water. Finally, three microtiter plates were created for each fungicide and each location according to the three replications of field plots per location used for *Z. tritici* isolation. Microtiter plates were incubated at 22 °C on an orbital shaker at 120 rpm with an 8 h photoperiod. After 5 days, the optical density (absorbance) was measured at 595 nm with a shaking period of 5 s prior to measurement using a Multiskan FC microtitration plate photometer (Thermo Fisher Scientific, Schwerte, Germany).

2.3. Data Analysis

Optical densities of the six tested fungicide concentrations (medium–spore solution) were corrected for the medium, the absorbance of the microtiter plate, and the intrinsic colour of the fungicide (medium–water controls) for each concentration separately within the same plate. The corrected optical density data were then normalized by dividing by the optical density of the fungicide untreated control (medium–spore solution with fungicide concentration of 0 mg/L). For these relative optical density data, means and standard deviations (±SD) were calculated for each year of *Z. tritici* isolation and each concentration including both locations and the three replications per location, split for the different fungicides. Using the sigmoid regression model of the software package Sigmaplot 13.0 (Systat Software, Erkrath, Germany), optical density data were plotted against the several fungicide concentrations for each year of *Z. tritici* isolation in order to determine the fungicide concentration at which the optical densities of the spore cultures were half that of the cultures with no fungicide. Those fungicide concentrations reducing the in vitro fungal growth by 50% (EC_{50}) were determined for each fungicide and year of *Z. tritici* isolation as well as for each of the six replications originating from the two locations. From these values EC_{50} means (±SD) were calculated for each year of fungal isolation, split for the different fungicides.

2.4. Statistical Analysis

Statistical analysis was done for EC_{50} values by use of the statistical software R, version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria) [48]. The data evaluation started with the definition of an appropriate statistical mixed model [49,50]. The statistical model included the fungicide (tebuconazole, propiconazole, prothioconazole, mefentrifluconazole) and year of *Z. tritici* isolation (1999, 2009, 2014, and 2020), as well as their interaction term as fixed factors. Replications (plates), nested in location, were regarded as random factor. The residuals were assumed to be approximately normally distributed and to be heteroscedastic. These assumptions are based on a graphical residual analysis. Based on this model, a Pseudo R^2 was calculated [51] and an analysis of variance (ANOVA) was conducted, followed by multiple contrast tests [52] in order to compare the several years (2009, 2014, and 2020) versus 1999, split for the different fungicides.

3. Results

Changes in fungicide sensitivity of *Z. tritici* field populations isolated in 1999, 2009, 2014, and 2020 were tested for the four DMI fungicides tebuconazole, propiconazole, prothioconazole, and mefentrifluconazole. Averaged over all tested fungicides and years of *Z. tritici* isolation, ANOVA results showed that EC_{50} values were significantly affected by the interaction of fungicide and year ($p < 0.0001$; Table 1), indicating that the four tested DMIs expressed a different change in sensitivity of *Z. tritici* over time. Both single factors had a significant effect on EC_{50} values ($p < 0.0001$).

Table 1. Analysis of variance (ANOVA) for the effect of fungicide, year and their interaction on EC_{50} values (mg/L) of *Zymoseptoria tritici* field populations summarized for all tested demethylation inhibitors (DMIs; tebuconazole, propiconazole, prothioconazole, mefentrifluconazole) and years of *Z. tritici* isolation (1999, 2009, 2014, 2020). In each year, *Z. tritici* field populations were isolated from wheat crops (cultivar "Ritmo") at two locations in Northern Germany.

Effect	df	F	*p*
Fungicide	7	96.326	<0.0001
Year	3	118.116	<0.0001
Fungicide × Year	21	22.260	<0.0001

For the three DMI fungicides tebuconazole, propiconazole, and prothioconazole a shift towards decreasing sensitivity of *Z. tritici* field populations was observed over the last 21 years from 1999 to 2020 (Figure 1A–C and Figure 2A–C).

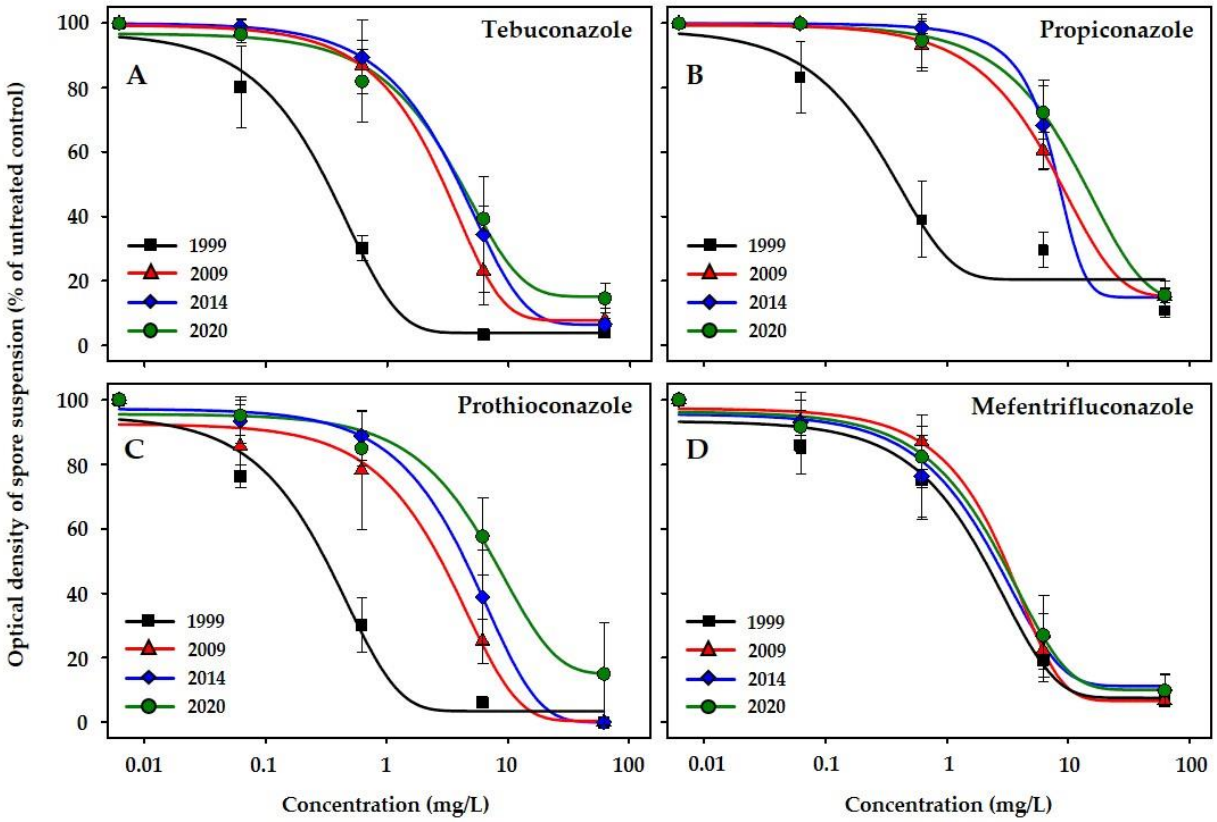

Figure 1. Relationship between fungicide concentration (mg/L) of tebuconazole (**A**), propiconazole (**B**), prothioconazole (**C**), mefentrifluconazole (**D**), and optical density of spore suspension of *Zymoseptoria tritici* field populations isolated from wheat crops (cultivar "Ritmo") at two locations in northern Germany between 1999 and 2020 (isolation years 1999, 2009, 2014, and 2020) using microtiter assays. Optical density data (mean ±SD) of the several fungicide concentrations are expressed relative to the optical density of the fungicide untreated control. Means (±SD) were calculated for each year of *Z. tritici* isolation and each concentration including both locations and three replications per location.

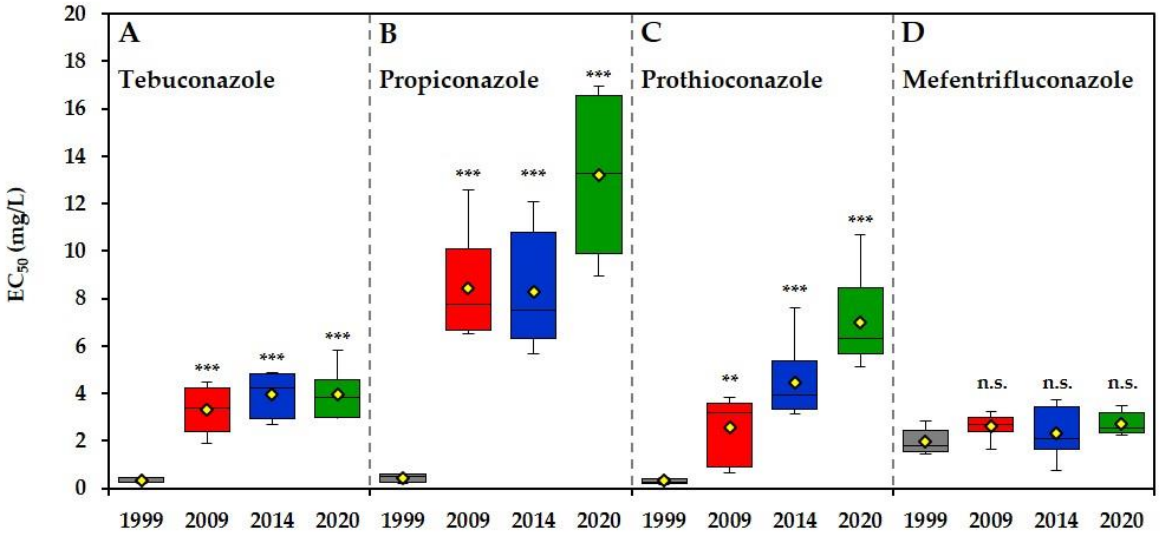

Figure 2. Changes in fungicide sensitivity (EC_{50}; mg/L) of *Zymoseptoria tritici* field populations isolated from wheat crops (cultivar "Ritmo") at two locations in northern Germany between 1999 and 2020 (isolation years 1999, 2009, 2014, and 2020) to the demethylation inhibitors (DMIs) tebuconazole (**A**), propiconazole (**B**), prothioconazole (**C**), and mefentrifluconazole (**D**) using microtiter assays. EC_{50} values were determined as the fungicide concentration reducing fungal growth by 50%. Boxplots and means (yellow rhombus) of EC_{50} values were calculated from EC_{50} values of both locations and three replications per location. Five statistics are represented in each boxplot from bottom to top: the smallest observation, lower quartile, median, upper quartile, and largest observation. Multiple contrast tests were conducted in order to compare EC_{50} values of the several years (2009, 2014, and 2020) versus 1999, split for the different fungicides. Statistical significance: ** = $p \leq 0.01$; *** = $p \leq 0.001$. n.s. = not significant ($p > 0.05$).

The relationships between fungicide concentrations of tebuconazole, propiconazole, and prothioconazole, and optical densities of spore suspensions of *Z. tritici* were not stable between the four tested years 1999, 2009, 2014, and 2020 (Figure 1A–C). The main shift in sensitivity of *Z. tritici* towards tebuconazole (Figure 1A), propiconazole (Figure 1B), and prothioconazole (Figure 1C) was determined between 1999 and 2009. In this period the EC_{50} values increased significantly from 0.329 to 3.306 mg/L for tebuconazole (Figure 2A), 0.457 to 8.435 mg/L for propiconazole (Figure 2B), and 0.311 to 2.571 mg/L for prothioconazole (Figure 2C). For tebuconazole the sensitivity of *Z. tritici* remained stable since 2009 (Figures 1A and 2A), whereas for propiconazole a further shift was detected between 2014 and 2020 with EC_{50} values increasing from 8.281 to 13.190 mg/L (Figures 1B and 2B). A continuously decreasing sensitivity of *Z. tritici* was observed for prothioconazole since 2009 (Figures 1C and 2C); EC_{50} values increased from 2.571 mg/L in 2009 to 4.455 mg/L in 2014, and to 7.005 mg/L in 2020 (Figure 2B). From 1999 to 2020 the EC_{50} values increased by 3.615 mg/L for tebuconazole, 12.733 mg/L for propiconazole, and 6.694 mg/L for prothioconazole (Figure 2A–C).

In contrast to the abovementioned older triazoles, the efficacy of mefentrifluconazole in reducing the in vitro fungal growth of *Z. tritici* remained unchanged for the four tested years of *Z. tritici* isolation (Figures 1D and 2D). A stable relationship between fungicide concentration and optical density of spore suspension was observed between 1999, 2009, 2014, and 2020, respectively (Figure 1D). Compared to 1999 (1.956 mg/L), insignificant differences in EC_{50} values were determined for the remaining years 2009 (2.642 mg/L), 2014 (2.337 mg/L), and 2020 (2.716 mg/L) (Figure 2D), demonstrating a stable sensitivity of *Z. tritici* towards mefentrifluconazole.

4. Discussion

Septoria tritici blotch (STB), caused by the wheat pathogen *Z. tritici*, is mainly controlled by the intensive use of fungicides during crop growth, whereby triazoles have been used for STB control for more than 30 years. However, the frequent use of fungicides in

disease management gives rise to selection of fungicide resistance. In fact, in recent years a gradual loss of sensitivity of *Z. tritici* field populations to several active ingredients of the triazole group has been documented in many wheat growing areas [11,13,16,18,20]. Although triazoles have suffered from a general loss of sensitivity due to the selection and spread of less triazole sensitive strains within the *Z. tritici* population, the efficacy of the new isopropanol triazole mefentrifluconazole is to be demonstrated. In our present study, the trend in sensitivity development of *Z. tritici* towards older triazoles (tebuconazole, prothioconazole, and propiconazole) and mefentrifluconazole was investigated in microtiter assays using *Z. tritici* field populations isolated from the same reference locations and a uniform cultivar between 1999 and 2020 in northern Germany in order to check whether the general loss of sensitivity of *Z. tritici* to triazoles also applies to mefentrifluconazole.

The triazoles belong to the sterol biosynthesis inhibitors (SBIs). They act in the sterol biosynthesis of the fungal metabolism. In the metabolic process of sterol biosynthesis, the conversion of lanosterol to ergosterol takes place. Based on different targets, the SBIs can be divided into different classes [53]. The most important class of SBIs are the demethylation inhibitors (DMIs). Their representatives are the nitrogen heterocycles, the so-called azoles. The azoles are subdivided in triazoles, imidazoles, and pyrimidines. Triazoles got their name due to their chemical structure, which consists of a five-membered ring system with three nitrogen atoms. Triazoles are among the azole group most commonly used in disease control of STB [24]. Therefore, the omission of this important group of active ingredients due to resistance development would be dramatic for practical agriculture.

DMIs act by inhibiting the enzyme C_{14}-demethylase, which is encoded by the CYP51 gene [53]. This enzyme is required for the biosynthesis of ergosterol. Due to the inhibition of the C_{14}-demethylase, the conversion of lanosterol to ergosterol is prevented at position C_{14} of lanosterol. An interaction occurs between the uncharged N atom of the azole ring and the central Fe^{3+} of the haem group by binding to the active side of the cysteine pocket of the CYP51 enzyme [54–56]. This leads to an accumulation of methylsterols in the fungal cell, which are toxic for the cell and lead to an undersupply of ergosterol. Ergosterol is an essential component of the fungal cell membrane and is required for the growth of fungal structures. A decrease in the concentration of ergosterol leads to a restructuring of the membrane, resulting in increased permeability and susceptibility of the fungal cell [57–59].

Several mechanisms mediate reduced sensitivity of *Z. tritici* to DMIs [25]. The molecular mechanisms of fungal resistance to DMI fungicides are mostly known [60]. In addition to alterations in the CYP51 gene leading to amino acid changes of the CYP51 enzyme [20–23], there are two further molecular mechanisms, which can contribute to the decline in sensitivity towards DMIs. One is the overexpression of the target gene CYP51, the other one is the enhanced efflux reducing the accumulation of DMIs in the fungal cell [20,22,25–28]. To date, all of these mechanisms have conferred a gradual loss of sensitivity of *Z. tritici* field populations towards DMIs. However, higher doses are now required to achieve effective disease control due to the reduced field efficacy of DMIs in controlling STB [10,17,32].

However, the amino acid substitutions in the CYP51 enzyme, which directly affect the structure of the active site of the enzyme, are by far most important for decreasing DMI sensitivities in European *Z. tritici* populations [20,21,33]. Using a model of the CYP51 enzyme, Cools et al. [21] described that amino acid substitutions in the CYP51 enzyme cause alterations of the binding site leading to a reduced interaction between DMIs and their binding site within the CYP51 enzyme, resulting in a reduced sensitivity of the fungus towards these active ingredients. As a result, an increased amount of active ingredient is required at the binding site to increase the binding strength of the active ingredient by increasing their concentration [61]. Several alterations in the CYP51 enzyme gene have been extensively determined in recent years by comparing the amino acid sequences of the *Z. tritici* wild type with isolates found after long-term DMI use [26].

The CYP51 enzyme is a functional protein composed of many different amino acids. These amino acids can have completely different side chains and thus completely differ-

ent chemical properties (acidic or basic, hydrophilic or hydrophobic). The size, shape, and position result in the specific chemical behaviour of the enzyme [62].

The binding and conversion of the substrate by the enzyme takes place in the so-called active site. The active site gets its properties from the amino acids of which it consists. This is a special spatial structure, which is formed by the folding of polypeptide chains. The catalytic reaction in the metabolic process also takes place in this active site [63]. As a rule, only the spatial structure of the substrate to be converted matches the spatial structure of the active site of the enzyme. As a result, very specific enzymes are required for each metabolic pathway, and often they only fit there.

Due to the high specificity of the active site, the enzyme can only bind a certain target molecule, the substrate, and support the chemical reaction [62]. Due to this specificity, however, every mutation in the CYP51 gene and therefore every change in the amino acid sequence of the enzyme has considerable consequences for the substrate, and thus for the metabolism of the fungus itself. At the same time, it also has consequences for the fungicidal active ingredient that is supposed to bind at the same active site. The active ingredient acts here as a substrate substitute and blocks the active site of the enzyme for further catalytic reactions. Due to the high binding affinity of azoles to CYP51 enzymes in the fungal organism, they are considered as effective active ingredients in disease management of fungal diseases in many crops. However, this effectiveness of azoles has been significantly reduced due to various mutations in the CYP51 gene of *Z. tritici* [20–23].

Unfortunately, positive cross-resistance relationships were found between several triazoles [16,20,22,24,26,30]. Hence, the loss of sensitivity to a single active ingredient of this group affects also the sensitivity of most remaining triazoles. In our study, a reduced sensitivity was also observed for the older triazoles, namely, tebuconazole, prothioconazole, and propiconazole. In contrast, mefentrifluconazole, the first active ingredient of the isopropanol subclass, apparently occupies a special position within the triazole group. In fact, the investigated older triazoles have suffered from a loss of sensitivity of *Z. tritici* field populations between 1999 and 2020, whereas the efficacy of mefentrifluconazole with its unique isopropanol unit in reducing the fungal growth by 50% (EC_{50}) was not affected. Furthermore, the variance of efficacy was extraordinarily low compared to the older triazoles. This indicates that high efficacy levels can be expected regardless of location and population. The high efficacy in the field was also confirmed by field studies of Jørgensen et al. [38] in comparison to older triazoles commonly used in STB control. According to Strobel et al. [39], the efficacy of various triazoles against highly adapted strains of *Z. tritici* (glasshouse trials) was poor, whereas mefentrifluconazole was able to show full control. Thus, it could be demonstrated that mefentrifluconazole has a unique selling point in its behaviour towards less sensitive populations in the field.

This is remarkable, since it appears that the novel flexible isopropanol unit, which is located in front of the ring structure of the triazole [39], copes much better with alterations of the active site of the CYP51 enzyme caused by mutations in the CYP51 gene. Due to this isopropanol unit, a high degree of structural flexibility of the molecule is possible, which influences the binding at the active site of the enzyme. Especially in populations with mutations in the CYP51 gene and therefore altered spatial structures of the binding pocket of the target enzyme, positive effects result. It seems that significantly higher binding forces act at the active site of the CYP51 enzyme, which in turn leads to an improved efficacy in less sensitive populations.

5. Conclusions

Mefentrifluconazole is a new triazole with a novel isopropanol unit. In contrast to older triazoles, the mefentrifluconazole molecule is more flexible in its structure and is able to bind even if the active site of the molecular target (CYP51 enzyme) is altered. Due to this isopropanol unit, no changes in sensitivity of *Z. tritici* field populations was observed between 1999 and 2020, whereas a significant shift towards decreasing sensitivity was determined for the older triazole fungicides. These results are of great importance for

the sustainable use of triazoles and can secure this important fungicide group for further disease management of STB in a long-term.

Two important approaches can be derived from our presented results. Firstly, our findings open up new aspects in the long-term evaluation of several groups of active ingredients. Due to innovations in the chemical structure also older groups of active ingredients can be reactivated, which have previously suffered from a loss of sensitivity. This approach is very encouraging, as the preservation of as many groups of active ingredients as possible with different modes of action in the fungal metabolism is the basic prerequisite for sustainable resistance management. The higher the diversity of active ingredients with different modes of action available for disease control, the more difficult is the adaptation of the various pathogens to these fungicidal active ingredients. However, resistance management means not just managing active ingredients. Moreover, agronomic practices (e.g., sowing date, cultivar susceptibility, crop rotation, and tillage) must be included in anti-resistance strategies. Strengthening and maintaining one group of active ingredients simultaneously reduces the risk of the development and spread of resistance to other groups of active ingredients. Furthermore, flexible units as the isopropanol unit could represent the answer to further resistance development caused by selective alterations within the target enzymes.

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