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MEFENTRIFLUCONAZOLE – THE FIRST ISOPROPANOL-AZOLE FUNGICIDE FOR THE CONTROL OF *ZYMOSPETORIA TRITICI* INCLUDING FIELD ISOLATES WITH KNOWN COMPLEX CYP51 HAPLOTYPES

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ABSTRACT

Mefentrifluconazole is the first Isopropanol-azole which belongs to the triazoles chemical family. It shows outstanding biological performance and conforms to very high Global and European regulatory standards. Over many years, triazole-based fungicides have been the backbone of crop protection programmes on many economically important crops. Mefentrifluconazole, with its unique chemistry, exhibits a very broad level of efficacy against many fungal pest species in arable and speciality crops, turf and on seeds, including isolates which already showed a reduced sensitivity to older triazoles, such as *Zymoseptoria tritici*. This unique ability to control already "shifted" isolates of pathogens demonstrates the important role of Mefentrifluconazole in resistance management. The first market introductions of Mefentrifluconazole are expected in 2019.

Keywords: New active ingredient, Mefentrifluconazole, Mode of action, *Zymoseptoria tritici*, Resistance management.

RÉSUMÉ

MÉFENTRIFLUCONAZOLE – LE PREMIER FONGICIDE À BASE D'ISPOPROPANOL-AZOLE POUR LE CONTRÔLE DE *ZYMOSEPTORIA TRITICI* DONT DES SOUCHES PRÉSENTANT L'HAPLOTYPE CYP51

Le méfentrifluconazole est le premier isopropanol-azole appartenant à la famille chimique des triazoles. Il présente d'excellentes performances biologiques et répond à des normes réglementaires mondiales et européennes strictes. Depuis de nombreuses années, les fongicides de la famille des triazoles sont à la base de la protection de nombreuses cultures d'intérêt économique. Le méfentrifluconazole présente un niveau d'efficacité très élevé contre de nombreux pathogènes fongiques des grandes cultures et cultures spéciales, du gazon et des semences. Il contrôle aussi des souches ayant une sensibilité réduite aux triazoles plus anciennes, telles que celles de *Zymoseptoria tritici*. Cette capacité unique démontre le rôle important du méfentrifluconazole dans la gestion de la résistance. Les premières mises en marché sont attendues en 2019.

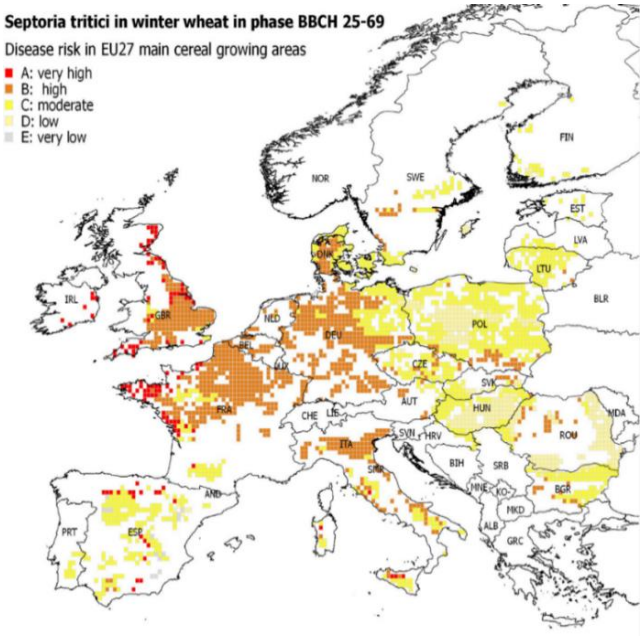
Mots-clés : Nouvelle substance active, Méfentrifluconazole, Mode d'action, *Zymoseptoria tritici*, Gestion de la résistance.

INTRODUCTION

Mefentrifluconazole is a fungicide which can be used to control a broad range of foliar diseases on a wide spectrum of crops including those on arable crops, speciality crops, such as pome and stone fruits, as well as those infecting vegetables. On cereal crops mefentrifluconazole can control many economically important pathogens including *Zymoseptoria tritici*, one of the most economically important cereal pathogen in Europe.

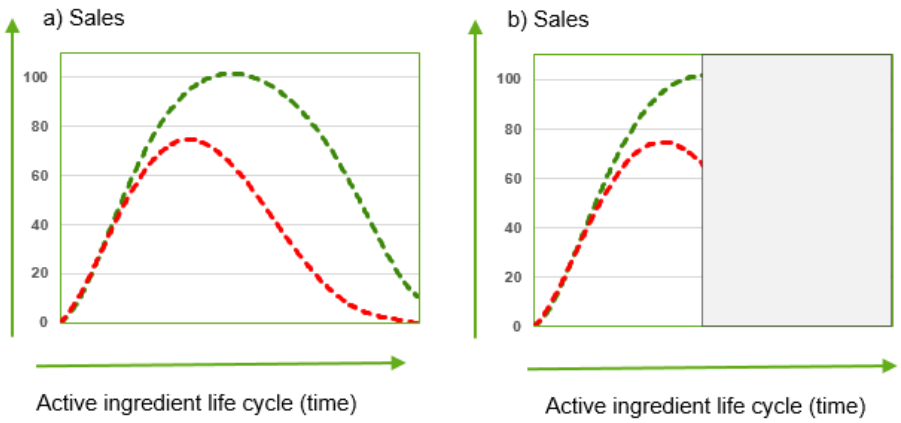
The ascomycete fungus, *Z. tritici* causes Septoria leaf blotch, a foliar disease of wheat which is found in most temperate wheat-growing areas worldwide. It can be found in most wheat crops in Europe, but disease severity is greatest in areas with mild and wet climates as shown in the predisposition map (Figure 1). Estimates of yield loss due to *Z. tritici* vary greatly but in an untreated situation, losses can be as high as 30-40% (Leadbeater, West & Pichon 2000).

Figure 1: *Zymoseptoria tritici* predisposition map for the EU 27 based on wheat crop biomass and epidemiology data
Carte du risque maladie de *Zymoseptoria tritici* pour l'UE 27 basée sur des données épidémiologiques et de biomasse en culture de blé



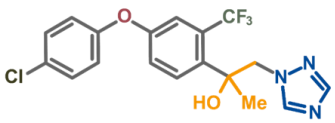
The number of active ingredients available on the market to control *Z. tritici* are limited both due to the loss of some actives from the market resulting from the current EU legislation (EC 1107/2009, Bryson *et al*, 2016) and the occurrence of resistance of the pathogen to some modes of action such as the QoIs where the spread of the G143A mutation was widespread and rapid in the main wheat growing areas of western Europe (Lucas, 1998). As a result, crop protection companies must evaluate both the regulatory and resistance risk of any new active ingredient before bringing it to the market. Figure 2 shows the potential impact on the return on investment for a company resulting from regulation and/or resistance (Bryson & Brix, 2018). The costs of development of a new fungicide are now very high both in terms of expenditure and resource (Philips McDougall, 2013, Bryson & Brix, 2018), however, with the European submission of the new active ingredient mefentrifluconazole, BASF has shown itself to be focused on the future development of agricultural solutions within Europe.

Figure 2. Return on investment from the launch of an active ingredient; green dotted line: life cycle; (a) red line: impact of pathogen resistance on sales; (b) impact of both the resistance & the loss of the active ingredient (loss of approval – Directive EC 1107/2009 – grey box).
Retour sur investissement après la mise en marché d'un produit ; courbe verte : cycle de vie du produit ; (a) courbe rouge : impact de la résistance sur les ventes ; (b) impact résistance + retrait de la substance active (Directive EC 1107/2009 – rectangle gris)



Based on the fungicide mode of action classification of the Fungicide Resistance Action Committee (FRAC), mefentrifluconazole is a fungicide belonging to the group of the sterol biosynthesis inhibitors (SBI, mode of action class G). Within the SBIs, it belongs to the sub group of demethylation inhibitor (DMI, G1) and the chemical group of triazoles. The primary mode of action of DMIs is the blocking of ergosterol biosynthesis through inhibition of cytochrome P450 sterol 14α-demethylase (*CYP*51). The depletion of ergosterol and accumulation of non-functional 14α-methyl sterols results in inhibition of growth and cell membrane disruption. The key attributes of the mefentrifluconazole are given in Table 1.

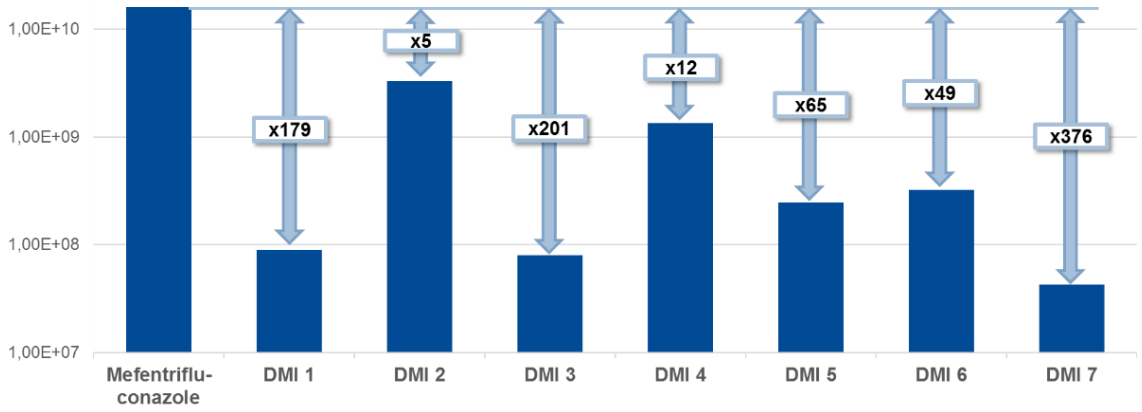
Table 1. Summary information on the active substance mefentrifluconazole
Informations relatives à la substance active méfentrifluconazole

Active ingredient	Mefentrifluconazole
CAS number	1417782-03-6
IUPAC name	2-[4-(4-chlorophenoxy)-2-(trifluoromethyl)phenyl]-1-(1H-1,2,4-triazol-1-yl)propan-2-ol
Molecular weight	397.8 g/mol
Structural formulation	$C_{18}H_{15}ClF_3N_3O_2$
Chemical groups	Triazoles / Isopropanol-azoles (proposed)
Mode of action	Blocking of ergosterol biosynthesis demethylation inhibiton (DMI)
Plant translocation	Systemic
Biological action	Fungicide with preventative and curative properties
Resistance group	G1/DMI-fungicides (SBI: class I)
Chemical structure	

MODE OF ACTION

As previously stated, mefentrifluconazole belongs to the sub group of DMIs and is in the chemical group of triazoles, however it is the first isopropanol azole. The isopropanol azole is unique in that the triazole 'head' sits on the 'neck' of a slim isopropanol linker. This chemical constellation ensures a high degree of structural flexibility that is unique among the DMI linkers. This slim isopropanol linker requires less energy to adjust its conformation compared to conventional DMIs. When mefentrifluconazole approaches the active site of its target enzyme, the flexible linker allows it to form a hook, which fits into the enzyme's binding pocket, resulting in strong inhibition of enzyme activity. This might explain the high intrinsic activity of mefentrifluconazole on the target enzyme, which has been shown in studies with the *CYP*51 of *Z. tritici* in comparison with other DMIs. Figure 2 shows the binding constant of the cytochrome P450 sterol 14α-demethylase *(CYP*51*)* of a wild type strain from the field. The binding constant is calculated by determining the strength of the association which is related to the affinity of the compound to its target. In this case the higher the binding constant the higher the intrinsic activity. In Figure 2 different commercially available DMIs have different binding constants but none at the level of mefentrifluconazole.

Figure 2. Binding constant of mefentrifluconazole and different DMIs $[mol/l]^{-1}$ on the cytochrome P450 sterol 14α-demethylase (CYP51).
Constante d'association du méfentrifluconazole et d'autres IDMs sur l'enzyme 14α-déméthylase du cytochrome P450 (CYP51)



Mefentrifluconazole is active against different fungal stages both on the plant surface and in the plant tissue. After application to the plant, the active ingredient is rapidly taken up via the leaf and slowly but consistently translocated apically via the transpiration flow. The limited translocation leads to a formation of inner-leaf reservoirs which allow a well-balanced, long lasting systemic activity. As a result, mefentrifluconazole can control fungal stages which have already become established in deeper tissue layers of the plant (curative activity). Furthermore, mefentrifluconazole shows impressive long-lasting activity, as most of leaf deposits are well-protected in the inner leaf. Since the vapour pressure of mefentrifluconazole is very low, a gas phase activity was not observed.

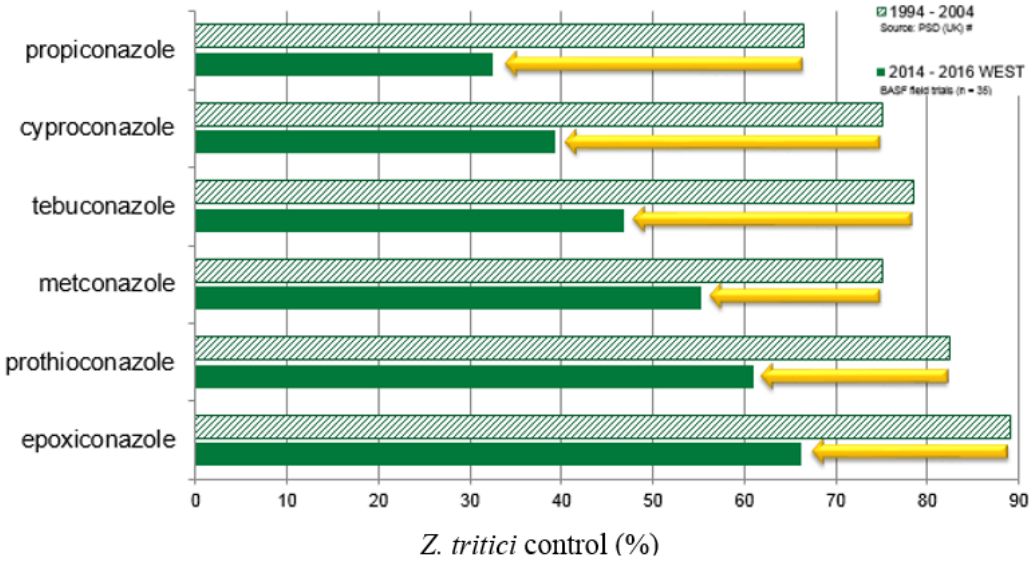
SENSITIVITY STATUS OF *ZYMOSEPTORIA TRITICI* IN THE EUROPEAN MARKET

Z. tritici is considered a medium risk pathogen in the FRAC Pathogen Risk List (Dec. 2013) (www.frac.info). However, resistance risk is not just defined by the life cycle of the pathogen but also by the agronomic environment in which the host plant, in this case wheat, is produced. Wheat production in Europe fulfils many agronomic risk factors, which increase the risk of pathogen resistance (EPPO

PP1/13 (1) (2015), Russell, 2005) with Western European countries including Ireland, UK, NL, Belgium, Germany and France having the highest intensity of wheat production and fungicide use and thereby highest resistance risk.

In Western Europe QoI resistance due to the G143A mutation has been widespread since 2004. The level of control of *Z. tritici* by the SDHIs is still at a high level however, there are now early signs of an emerging resistant population in some of the key Western European countries including Ireland, the NLs and the UK (Rehfus *et al*., 2017). In addition, the sensitivity of the *Z. tritici* populations to DMIs presently on the market has been reducing over time as seen in Figure 3.

Figure 3. Comparison of the % control of 35 *Z. trit*ici isolates of 6 commercially available DMIs in the West of Europe (FR, DE, UK, IE) based on historic data 1994 - 2004 from PSD (PSD project report PS 2711/CSA 7236 – 2007) (Clark, 2006) and 2015 – 2016 BASF internal trial data
Comparaison de l'efficacité de 6 IDMs pour le contrôle de 35 souches de *Z. tritici* en Europe Occidentale (FR, DE, UK, IE), basée sur des données de 1994 à 2004 et de 2015 à 2016 (données BASF)



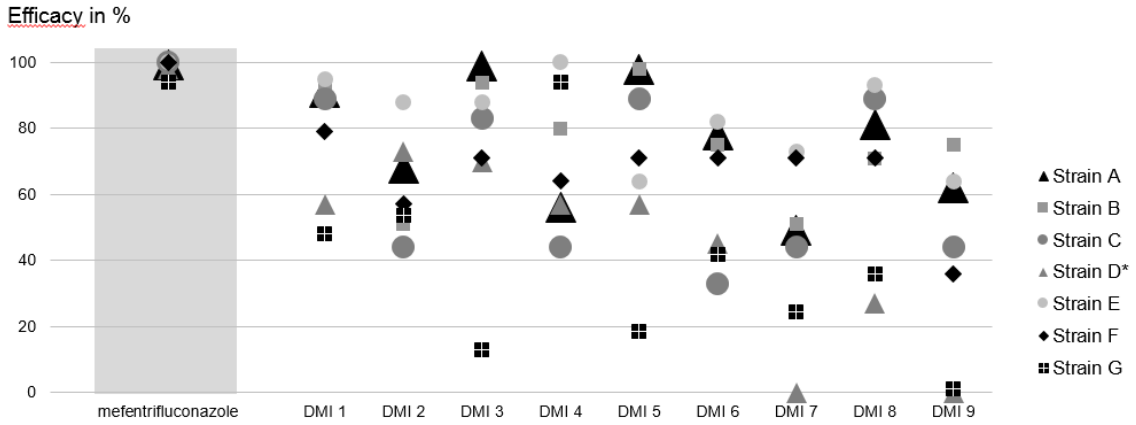
Three major mechanisms are associated with changes in DMI-sensitivity in *Z. tritici*, in order of importance they are: mutations in the target gene (CYP51) (Leroux *et al.* 2006, Stammler *et al.* 2008), overexpression of the target protein (Cools *et al.* 2012) and reduced intracellular accumulation of DMIs by overexpression of efflux-pumps (Leroux and Walker 2011), also sometimes referred to as Multi Drug Resistance (MDR).

Various mutations in the target gene have different effects on different DMIs (Fraaije *et al.* 2007, Stammler *et al.* 2008). Target gene mutations might be combined and accumulate and can result in higher levels of resistance (Cools and Fraaije 2013). The number and complexity of *CYP*51 haplotypes in "shifted strains" is now at a high level and is described by Huff *et al*., 2018. In addition, target site overexpression and/or enhanced efflux can also be found simultaneously in isolates (Stammler & Semar 2011, Cools and Fraaije 2013, Strobel *et al.* 2014). The accumulation of different resistance mechanisms results in a quantitative (directional) type of resistance and changes in the sensitivity of a population are gradual.

CONTROL OF SHIFTED STRAINS BY MEFENTRIFLUCONAZOLE

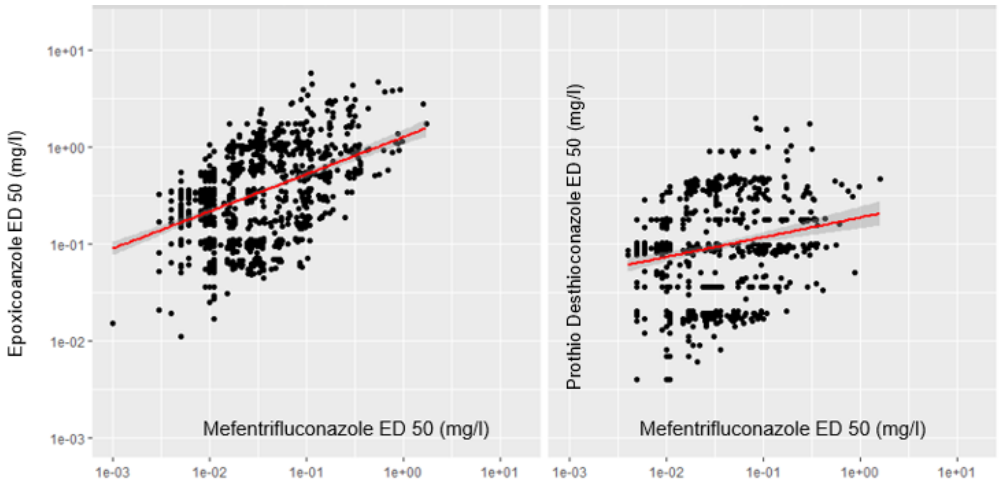
Isolates belonging to different *CYP*51-haplotypes have been shown to vary in their sensitivity response to different DMIs, those with complex haplotypes are often referred to as "shifted strains", although the correlation of sensitivity between various DMIs can be low or even negative (Stammler & Semar 2011). This is confirmed by frequency analyses of *CYP*51-haplotypes in the field after various DMI applications, which showed that DMIs select *CYP*51-haplotypes differently (Fraaije *et al.* 2007, Stammler *et al.* 2008). Greenhouse experiments were set up to assess the activity of mefentrifluconazole on several strains of *Z. tritici*. These strains were isolated from field samples across Western Europe as part of the BASF resistance monitoring program (2012- 2016). Fungicides were applied one day preventative at 14-day-old seedlings. Then, plants were spray-inoculated with spore suspensions (2.5 x 10^6 spores/mL). Diseased leaf area containing pycnidia was assessed 21 days after inoculation and fungicide efficacy was calculated. Figure 4 shows the level of efficacy (% control of *Z. tritici* on greenhouse grown plants) of a range of commercially available DMIs compared with mefentrifluconazole against several "shifted" strains; where strains A – G are isolates with different *CYP*51 haplotypes (strain D has an additional efflux phenotype). It can be clearly seen that unlike the range of presently commercially available DMIs mefentrifluconazole was able to control all of the "shifted" strains giving 100 % efficacy.

Figure 4. Efficacy of various DMI compounds against highly adapted strains of *Z. tritici* in the glasshouse (inoculation one day after application; 33% of the registered dose rate). *Strain D = Phenotype with increased efflux (also referred to as MDR)
Efficacité de plusieurs IDMs pour contrôler des souches résistantes de *Z. tritici* testées en serre (inoculation 1 jour après traitement à 33% de la dose réglementaire). *Souche D = phénotype avec efflux accru (souches dites MDR)



For mefentrifluconazole, this low correlation of sensitivity between DMIs is even more pronounced compared with other DMIs already on the market. This is described in Figure 5, where sensitivity correlations of mefentrifluconazole and epoxiconazole and desthio-prothioconazole are poor with R^2 values of 0.181 and 0.026, respectively (prothioconazole-desthio was used instead of prothioconazole due to its' recognised role in disease control (Parker *et al.* 2013)). The low correlation coefficients (R^2) indicate a low correlation with the sensitivity to other DMIs.

Figure 5. Correlation of the mefentrifluconazole sensitivity of *Z. tritici* compared with epoxiconazole and desthio-prothioconazole by microtiter assays (BASF unpublished data) - R^2 are 0.181 and 0.026, respectively.
Corrélation entre la sensibilité de *Z. tritici* au méfentrifluconazole et celle à l'epoxiconazole (gauche – R^2=0.181) ou au desthio-prothioconazole (droite – R^2=0.026) – données BASF non publiées



HYPOTHESIS WHY MEFENTRIFLUCONAZOLE PROVIDES HIGH EFFICACY ON DMI SHIFTED STRAINS

Mutations in the *CYP*51 gene cause alterations of the binding site resulting in a widening of the binding pocket which affects the binding of conventional DMIs. In contrast, the mefentrifluconazole molecule is more flexible in its structure compared with other DMIs and is therefore able to bind even if the binding pocket shape is altered. This flexibility comes from the fact that the triazole 'head' sits on the 'neck' of a slim isopropanol linker. This chemical constellation ensures a high degree of structural flexibility that is unique among the DMIs (Figure 6). This slim linker requires less energy to adjust compared to conventional DMIs. When mefentrifluconazole approaches the active site of the target enzyme C14-demethylase (*CYP*51), the flexible linker allows it to easily form a "hook", which fits perfectly into the enzyme's binding pocket, resulting in strong inhibition of enzyme activity. It easily adapts to different shapes and sizes of binding pockets caused by various target site mutations (Figure 7).

Figure 6. Mefentrifluconazole in the A) "unbound" and B) "hook" conformations
Conformations linéaire (A) et en forme de crochet (B) du méfentrifluconazole

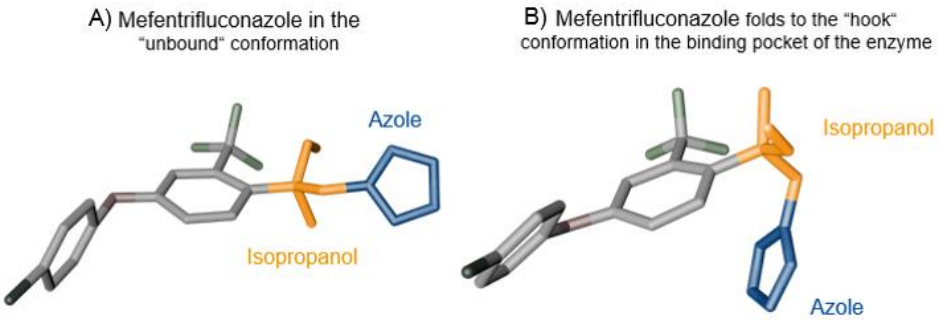
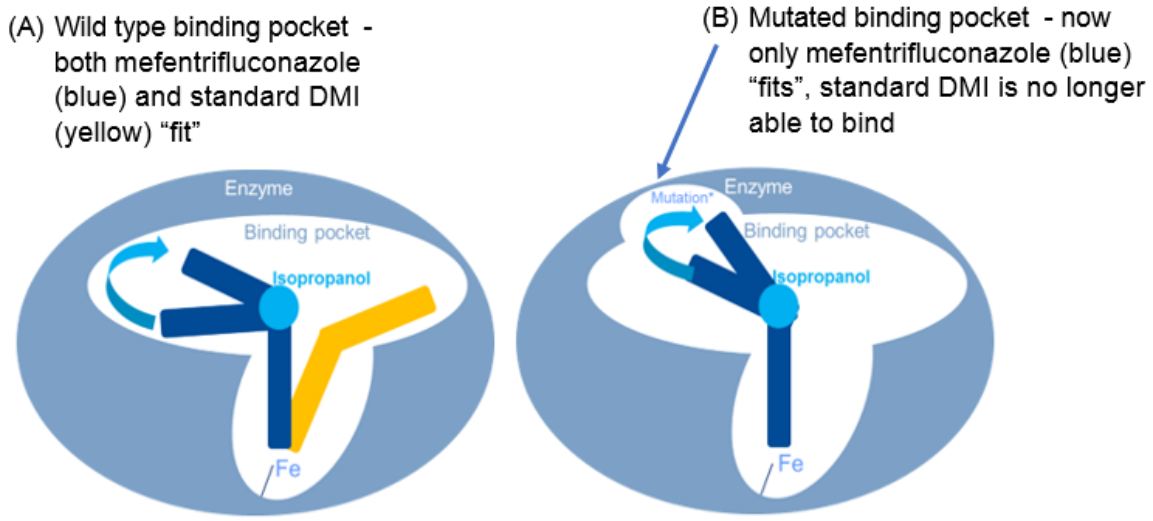


Figure 7. Adaptation of mefentrifluconazole in a wildtype (A) and a mutated binding pocket (B) schematic. Blue: mefentrifluconazole, yellow: other DMI. The heme iron (Fe) of the cytochrome P450 is the major binding partner for the triazole ring of DMI fungicides.
Mode de liaison du méfentrifluconazole au niveau du site actif d'une enzyme sensible (A) et résistante (B). Bleu : méfentrifluconazole, jaune : autres IDMs. L'hème du cytochrome P450 est le partenaire de fixation majeur pour les triazoles.

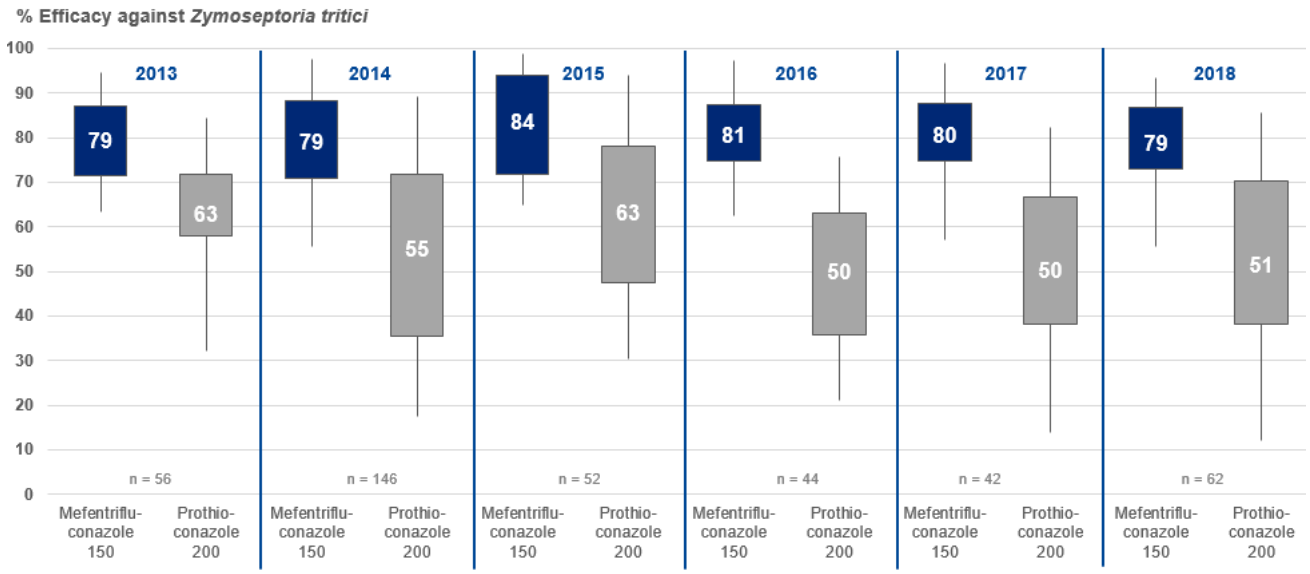


CONTROL OF *Z. TRITICI* ON THE FIELD WITH MEFENTRIFLUCONAZOLE

As previously mentioned and shown in Figure 3, the efficacy of DMI fungicides currently on the market against *Z. tritici* has eroded over time. This is due to the increasing frequency and complexity of the *CYP*51 haplotypes found in the *Z. tritici* population across the main wheat growing areas of Europe (Huff *et al*, 2018). With the already existing QoI resistance across Western Europe and the slowly emerging SDHI resistance starting in the far west of Europe (Ireland and UK), effective control of *Z. tritici* remains a challenge with the current active ingredients on the market (Rehfus et al., 2017).

European field trials were conducted for 5 years. 1 to 2 applications of fungicides were carried out at BBCH 31-65 and the level of *Z. tritici* control was assessed at 20-60 days after the last application. As can be seen in Figure 8, the level of control of *Z. tritici* achieved using mefentrifluconazole is far higher compared with that coming from one of the market leading DMIs, prothioconazole. Across the 5 years where field trials were carried out, the level of control from mefentrifluconazole was between 16 – 31% higher than that achieved with prothioconazole. In addition, disease control was also more consistent with a much lower level of variability seen when using mefentrifluconazole (Figure 8) probably because of the varying level of *CYP*51 haplotypes being poorly controlled by the prothioconazole.

Figure 8. Comparison of the efficacy of mefentrifluconazole and prothioconazole against *Z. tritici* in field trials across Europe from 2013 – 2018
Comparaison de l'efficacité du méfentrifluconazole et du prothioconazole contre *Z. tritici* dans des essais expérimentaux européens de 2013 à 2018 (1-2 applications au stade BBCH 31-65 et notations 20-60 jours après le dernier traitement)



CONCLUSION

Despite the severe challenges of bringing a new active ingredient to the market BASF has developed an effective new fungicide for the control of the leading wheat pathogen in Europe, *Z. tritici*. Mefentrifluconazole, although a DMI, is the first isopropanol azole which means that the triazole 'head' sits on the 'neck' of a slim isopropanol linker. This novel constellation increases the binding efficiency of mefentrifluconazole ensuring a high level of pathogen control even of highly shifted isolates with complex *CYP*51 haplotypes. With the continual threat and emergence of the pathogen population towards the SDHIs mefentrifluconazole is likely to play a pivotal role in disease control for the foreseeable future.

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