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Four azoles' profile in the control of Septoria, yellow rust and brown rust in wheat across Europe

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ABSTRACT

Leaf diseases cause major yield losses in winter wheat every year across Europe. Septoria leaf blotch – STB (*Zymoseptoria tritici)* is the most serious leaf disease in Northern Europe, but also yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia triticina)* are known to cause major problems in some regions and seasons. Problems with fungicide resistance in the populations of *Z. tritici* have caused concerns for future control options. With the aim of investigating the differences in azole performances against STB, yellow rust and brown rust, 40 field trials were carried out during two seasons (2015 and 2016) in 10 different countries across Europe covering a diversity of climatic zones and agricultural practices. Four single triazoles (epoxiconazole, prothioconazole, tebuconazole and metconazole) and two mixtures of azoles (epoxiconazole + metconazole; prothioconazole + tebuconazole) were tested at full and half rates. Regarding control of yellow rust and brown rust similar control patterns were seen across Europe and solutions with epoxiconazole and tebuconazole provided between 80 and 100% control. In contrast lower levels of control and major variations in azole performances against STB were seen across Europe, with the better of the azoles varying significantly across the continent. Similarly, the CYP51 mutation frequencies varied greatly across Europe with a clear pattern of decreasing frequencies from west to east of all investigated mutations except I381V and A379G. Azoles were most effective against STB when used as mixtures of epoxiconazole + metconazole or prothioconazole + tebuconazole. This was especially clear in the western part of Europe with high frequencies of CYP51 mutations D134G, V136C and S524T. Effectiveness of all single azoles decreased from 2015 to 2016 except for tebuconazole and azole mixtures, with the latter showing an increased advantage. EC_{50} values for *Z.tritici* from the trial sites measured for the four azoles involved could to some extent support the control levels measured at the sites. Across all trials full rates of azole mixtures were best at increasing yields by up to 20%. Single azoles increased yields between 14 and 18%. Yellow rust gave rise to the highest yield increases.

Keywords: Triazoles, Europe, *Zymoseptoria tritici*, CYP51 mutations, EC_{50}, Rust.

Introduction

Every year severe attacks of leaf diseases in winter wheat give rise to significant and economically important losses (Oerke, 2006, Jørgensen et al., 2014). This leads to common use of fungicides in order to keep down the yield loss. Septoria leaf blotch (STB) caused by *Zymoseptoria tritici* is seen as the most serious leaf disease in Northern Europe (Fones & Gurr, 2015), but also yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia triticina*) are known to cause major problems in some regions and seasons (Jørgensen et al., 2014).

Four major modes of Action (MoA) of fungicides are available for management of leaf diseases in wheat: (1) quinone outside inhibitors (QoI), (2) sterol 14α-demethylation inhibitors (DMI), in this paper mentioned as azoles, (3) succinate dehydrogenase inhibitors (SDHI) and (4) multi-site inhibitors. Among these, target site-specific systemic fungicides such as DMIs and SDHI's are regarded as the most active (Fraaije et al., 2007).

The DMI fungicides have been authorized for control of leaf diseases since the late 1970s (Russell, 2005; Lucas et al. 2016). The DMIs consists of azoles, which again represents both triazoles, the triazolinthione deriviate prothioconazole and the imidazole prochloraz. Azoles are still regarded as the core group of fungicides for control of leaf diseases. Depending on weather, disease pressure and cultivars grown, fungicides, including triazoles are often applied 1-3 times per season. Due to this very common use, resistance to DMIs has evolved in several fungal plant pathogens (Russell, 2005). Since resistance to QoI fungicides developed, the azoles have been seen as the backbone of STB control (Fraaije et al., 2007) and in recent years major changes in the sensitivity of the populations have been observed across Europe (Dooley et al., 2016a, Stammler & Semar, 2011).

Resistance against DMIs, unlike most other target specific fungicides, has resulted not just from single mutations, but several resistance mechanisms have been found to be involved. Three main resistance mechanisms in agricultural fungi have been described for DMIs: mutations in the target enzyme CYP51 enzyme, overexpression of the target gene CYP51 and enhanced efflux activity reducing the accumulation of DMIs in the fungal cell. The increased resistance of *Z. tritici* towards DMIs has been associated with all three mechanisms (Cools & Fraaije, 2013). The large number of CYP51 mutations which have been discovered during the past 10-15 years in different combinations have been associated with the most significant changes in sensitivity. The different haplotypes of STB, which have been identified, are differently affected by different DMIs (Leroux et al., 2007, Cools & Fraaije, 2013).

The changes seen in control of STB have to some extent been shown to be influenced by specific CYP51 mutations. Furthermore, the patterns of decreasing field performances have been confirmed by rising EC_{50} values for several DMIs, especially tebuconazole and metconazole (Clark, 2006, Fraaije et al., 2007). The level of resistance is found to be highly influenced by the local risk of STB, intensity of control and the strategies and fungicides applied. In spite of major shifts occurring in the field effects of other DMIs, epoxiconazole and prothioconazole were until 2008 reported as being unaffected by mutations in the CYP51 gene (Stammler et al., 2008). However, recent studies have found the effectiveness of these two compounds to be decreasing as well (Cools & Fraaije, 2013, Kildea, 2016).

The very common CYP51 mutation I381V, which was initially seen to reduce DMI sensitivity broadly, was in particular seen to affect the field performances of tebuconazole (Leroux et al., 2007, Lucas et al., 2016). More recently the CYP51 mutation S524T has emerged in some western European regions conferring reduced efficacy of the most commonly used azoles, i.e. prothiconazole and epoxiconazole (Cools & Fraaije, 2013, Buitrago et al., 2014, Leroux & Walker, 2011).

In the current study the over all aim was to collect an updated dataset of the efficacy profile of the azoles for control of major wheat diseases across Europe. More specificly the aims were to: (1) Investigate the field performances of major azoles against the current Z. *tritici*, *P. striiformis* and *P. triticina* populations across Europe using both single azoles and azole mixtures. (2) Elucidate the interrelation of azole field performances, *in vitro* sensitivity of *Z. tritici* populations and CYP51 mutation frequencies. (3) Detect indications of developing trends across Europe. (4) Discuss the optimum available management strategies based on available data. The project is seen as a follow-up to a previous collaboration in the EuroWheat group – initiated by activities in the European Network of excellence - ENDURE (Jørgensen et al., 2014, Anon, 2009).

2. Materials and method

2.1 Field trial

The project was carried out over the growing seasons of 2015 and 2016 at different locations across Europe, covering different climate zones and agricultural practices. A total number of 26 and 14 trials were carried out in 2015 and 2016 respectively. The trials were carried out by local scientific organisations in Poland, Germany, France, Belgium, Hungary, Ireland, the UK, Lithuania, Latvia and Denmark. Standard procedures and assessment methods were applied using a randomized plot design with a minimum plot size of

10 m^2 and 3-4 replicates. Moderately susceptible to susceptible cultivars were chosen, which could provide good levels of attack aimed at having STB, yellow rust or brown rust as the main disease target. The fungicides were applied with local equipment varying from knapsack sprayers to self-propelled sprayers using low pressure and water volumes in the range of 150-250 l/ha. Spraying was carried out at flag leaf emergence at growth stage (BBCH GS) 37-39. In a few cases a cover spray of a multisite fungicide was also applied early in the season to keep down early levels of attack, no later than two weeks before the main treatments. Fungicides were provided by BASF and all products were tested at full and half rates as given in **Table 1.** Detailed trial information is given in supplementary section (**S-Tabel 1**).

Per cent leaf area attacked by specific diseases was assessed at regular intervals after applications following EPPO guideline (1/26 (4) (OEPP/EPPO, 2014). Focus was put on assessments carried out 30-50 days after application (DAA) at growth stage (GS) 73-75. Data from full and half rate has been presented. In case of epoxiconazole also 66% of full rate has been included. Except for one trial all trials were carried through to harvest. Grain yields were measured for each plot and yields were adjusted to 85% dry matter. Grain samples from each plot were used for dry matter and thousand grain weight (TGW) assessments.

Table 1: Tested protocol across all sites. Fungicide doses (l/ha) and amount of active ingredient (g/ha) used per treatment. In bracket per cent of full rate (N) is stated.

Trt. No.	Product	l/ha	Active ingredient	g/ha (% N)
1	Untreated	-	-	-
2	Opus Max	1.5	Epoxiconazole (EPX)	125 (100%)
3		1		83 (66%)
4		0.75		62.5 (50%)
5	Proline 250 EC	0.8	Prothioconazole (PTH)	200 (100%)
6		0.4		100 (50%)
7	Caramba 90	1	Metconazole (MCA)	90 (100%)
8		0.5		45 (50%)
9	Folicur 250 EW	1	Tebuconazole (TCA)	250 (100%)
10		0.5		125 (50%)
11	Osiris	3	epoxiconazole + metconazole (EPX+MCA)	113 + 83 (182%)
12		1.5		56 + 41 (91%)
13	Prosaro 250 EC	1	tebuconazole + prothioconazole (TCA + PTH)	125 + 125 (112%)
14		0.5		63 + 63 (56%)

2.2 CYP51 mutation frequencies and EC50 values

Leaf samples of STB were collected at GS 65-75 from all sites and forwarded for to BASF and Epilogic for further characterization. CYP51 mutation profiling of local *Z. tritici* populations was carried out by pyrosequencing and qPCR by BASF (Stammler et al., 2012), and EC_{50} values of the four azoles were measured on single pycnidium isolates by EpiLogic in Munich using the common FRAC protocol (WWW.FRAC). Ten isolates were tested from most locations for EC_{50} asssessments. However in 2016 fewer isolates were tested from some sites due to problems with isolation of spores from poor samples resulting from bad weather conditions.

All data were collected locally by the subcontractors and forwarded to AU-Flakkebjerg for further analysis.

2.3 Statistical analysis

All data on yields were organized in ARM for statistical analysis. Individual trial data were subjected to analysis of variance, and treatment means were separated at the 95% probability level using Fishers LSD test. The LSD values presented in **Table 8** were calculated in ARM using the "function summary across trials". Disease assessments were looked at site by site and transferred to % control; following this the efficacy was ranked for each site individually and colour coded to highlight differences. Statistical analysis of % control data was carried out using RStudio version 1.0.136. LSD values presented in **Table 9** were calculated by Fishers LSD test on average values across trials. One trial (15380-15) contained only one replication per treatment, which represented average values of subsamples from four replications per treatment. Thus, in order to attain four replicates for this trial in the dataset, this value was repeated four times per treatment. In certain cases outliers were removed from specific trials in order to meet the assumptions of normal distribution and homogeneity of variance (see table 9). One dataset was asin square root transformed in order to meet the test assumptions. The presented LSD value was back transformed (see table 9). Statistical analysis of CYP51 mutation frequencies was not possible since only one sample from each trial was analysed.

3. Results

3.1. Field performances

Disease severities and treatment efficacy were highly variable across the 10 countries and 40 trials involved in the project. However, general trends regarding treatment effects were observed. Only trials with more than 5% attack in untreated plots were included in the efficacy evaluations. In 25 trials across the two seasons STB developed sufficiently for a ranking of product performances. Even so, some sites gave a very clear ranking while others showed a less clear ranking of the efficacy of the products. A summary of the efficacies is given for the three diseases in **Table 2**. For STB and yellow rust efficacy is given for both 1st and 2nd leaf, where 1st leaf represent a preventive effect and 2nd leaf typically represents a more curative control situation. In 2015, the overall best control of STB was provided by epoxiconazole or prothioconazole used alone or the co-formulations epoxiconazole + metconazole and tebuconazole + prothioconazole. This pattern was confirmed in 2016. However, in 2016 the co-formulations had gained an edge over epoxiconazole and prothioconazole used alone.

Looking at individual trial data, products performed very diversely (**Table 3 & 4**). Metconazole gave better curative control of STB in France and Ireland providing high control (70-90%) compared with other countries (30-70%). The opposite was true of the curative control of STB by prothioconazole and epoxiconazole; the efficacy of these two actives was relatively weak (40-60%) compared with higher efficacy (60-90%) in most other trials. The same tendency was seen in both seasons although it was most pronounced in 2015. Furthermore, tebuconazole performed very well in Ireland and Belgium (ca. 70–80%), and to some extent in one French location and one British location (ca. 70 %), whereas this active performed poorly in all other countries (ca. 50%). Poland stood out due to the high control effects of all azoles against STB on leaf 1 (80-96%) except Folicur (58-72 %). In 2016, exceptionally high curative control was also achieved in Poland by all azoles (80-95%) except tebuconazole, and a similar trend was seen for Lativa and Hungary.

The preventive control of single azoles was more effective than curative control on average (ca. 10% difference). However, this difference was less pronounced regarding mixed azoles. Generally, decreased control effects of azoles against STB were seen from 2015 to 2016, except for tebuconazole and the mixture prothioconazole + tebuconazole (**Table 2, Figure 1**). As an average of all assessments a clear reduction in per cent control of STB was seen for most products when comparing full and half rates (**Table 2**).

Much less variation in field performances of azoles across locations was found against yellow rust and brown rust (Suplementary data). Furthermore, the products were generally much more effective in their control of yellow rust (ca. 80-90%) compared to STB (ca. 60-70%) in both 2015 and 2016. In 2016 the control of yellow rust was close to 100% in most cases. Control effects were especially high for epoxiconazole and tebuconazole but also for the two azole mixtures. Metconazole was the weakest product for control of yellow rust. The most effective treatments against brown rust were epoxiconazole and the mixture epoxiconazole+metconazole (>80%), whereas the control from prothioconazole was clearly inferior (ca. 50%).

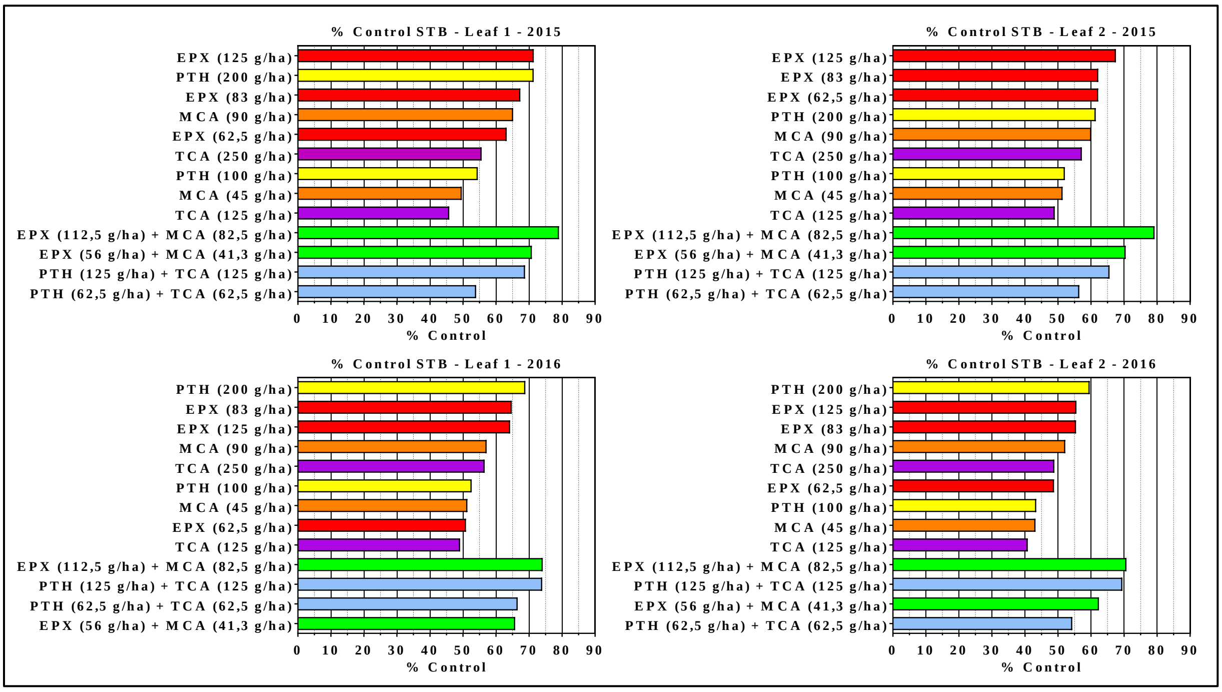


Figure 1. . Summary of average STB control from full and half rate of azoles assessed on flag leaves and 2nd leaves in 21 trials carried out in 2015 and 20 trials of 2016. Control effects are summarized as average percentage reduction of attack relative to untreated plots.

Table 2. Average per cent control of septoria tritici blotch (STB), yellow rust (YR) and brown rust (BR) on flag leaves and 2nd leaves in 2015 and 2016. Fisher-LSD values were calculated on avereage values across trials without untreated. Raw data are presented without outliers.

% Control				Untr.	EPX			PTH		MCA		TCA		EPX + MCA		TCA + PTH			
Disease	Year	Trials	Leaf	-	125 g/ha	83 g/ha	62,5 g/ha	200 g/ha	100 g/ha	90 g/ha	45 g/ha	250 g/ha	125 g/ha	112.5 + 82.5 g/ha	56 + 41,3 g/ha	125 + 125 g/ha	62,5 + 62,5 g/ha	LSD-untr.	
STB	2015	8	1	24	71	67	63	71	54	65	50	56	46	79	71	69	54	6,9	
		13	2	46	68	62	62	61	52	60	51	57	49	79	70	66	56	6,3	*
	2016	10	1	38	64	65	51	69	53	57	51	57	49	74	66	74	67	8,3	
		10	2	54	56	55	49	60	43	52	43	49	41	71	62	69	54	8,5	
YR	2015	9	1	19	92	82	85	80	72	64	59	89	82	84	83	91	90	7,8	
	2016	4		14	100	99	99	98	94	84	89	94	95	98	91	99	97	15,7	**
BR	2015	5	1	31	83	78	77	54	60	80	70	72	58	86	85	71	64	10,0	***
	2016	1		6	99	97	85	84	91	77	58	53	45	96	97	89	81	9,8	

* One trial (15, 2015) contains only one replicate per treatment. **Data on YR control on 1st leaf, 2016 were asin square root transformed, but did not meet the assumptions of normal distribution and homogeneous variance. The HSD value is presented in this case as extrapolated from Tukey's range test. ***Three ouliers were removed from data on BR control of 2015 (trials 8 and 9).

Table 3. Summary of STB control from full rate of azoles assessed on flag leaves in 18 trials carried out in 2015 and 2016. Control effects are summarized as percentage reduction of attack relative to untreated plots. The column "untr." represents per cent attacks in untreated plots. Colours signify ranking of treatment effects within each trial. Green: best/high effect. Yellow: medium effect. Orange: low effect. DAA=Days after application and GS=Growth stage at assessment.

% Control - *Z. tritici* Leaf 1			**-**	**1,5 l/ha**	**1 l/ha**	**0,8 l/ha**	**1 l/ha**	**1 l/ha**	**3 l/ha**	**1,5 l/ha**	**1 l/ha**
Year-Trial-Country	**GS**	**DAA**	**Untr.**	**EPX**		**PTH**	**MCA**	**TCA**	**EPX +MCA**		**PTH+TCA**
15-2-DNK	75	47	35,0	76	74	86	56	53	84	77	75
15-3-DNK	75	47	13,5	57	56	72	56	46	70	52	48
15-4-DNK	75	43	19,3	65	56	62	48	45	59	52	49
15-8-POL	75	46	9,7	85	83	79	79	72	83	80	80
15-10-FRA	75	41	25,9	82	69	85	84	69	95	78	84
15-14-DEU	75	31	13,3	68	68	67	68	47	70	61	68
15-22-IRL	85	42	54,2	80	70	72	90	89	93	88	93
15-25-HUN	75	39	17,5	64	61	50	43	29	80	80	57
16-1-DNK	75	42	20,8	83	81	76	67	57	91	82	80
16-2-DNK	75	42	84,0	80	76	70	56	52	90	84	86
16-3-FRA	75	42	90,6	7	10	8	9	17	26	8	19
16-4-FRA	71	46	29,6	59	72	71	67	73	76	64	76
16-5-SCT	57	30	4,3	65	76	71	41	65	76	76	82
16-7-GRB	76	42	14,0	63	61	56	53	70	67	65	75
16-9-DEU	73	34	23,7	36	34	61	36	32	46	36	57
16-10-POL	76	37	12,5	94	94	94	90	58	96	92	93
16-12-IRL	80	62	83,9	65	71	71	83	89	87	82	74
16-13-LVA	75	49	15,6	78	59	86	65	46	87	59	84
Avr. 2015			23,5	71	67	71	65	56	79	71	69
Avr. 2016			38,0	64	65	69	57	57	74	66	74

Table 4. Summary of STB control from full rate of azoles assessed on 2nd leaves in 23 trials carried out in 2015 and 2016. Control effects are summarized as percentage reduction of attack relative to untreated plots. The column "untr." represents per cent attacks in untreated plots. Colours signify ranking of treatment effects within each trial. Green: best/high effect. Yellow: medium effect. Orange: low effect. DAA=Days after application and GS=Growth stage at assessment.

% Control - *Z. tritici* Leaf 2			-	1,5 l/ha	1 l/ha	0,8 l/ha	1 l/ha	1 l/ha	3 l/ha	1,5 l/ha	1 l/ha
Year-Trial-Country	**GS**	**DAA**	**Untr.**	**EPX**		**PTH**	**MCA**	**TCA**	**EPX +MCA**		**PTH+TCA**
15-2-DNK	75	47	72,5	76	72	79	62	55	83	76	75
15-3-DNK	75	46	58,8	60	59	52	45	43	60	51	53
15-4-DNK	75	43	40,0	75	61	63	47	47	71	58	56
15-6-POL	75	58	5,3	45	35	59	62	62	69	34	50
15-8-POL	75	46	17,5	90	87	63	56	62	91	84	65
15-10-FRA	75	41	79,7	58	57	48	69	57	81	64	72
15-15-DEU	75	37	30,0	80	33	93	77	50	87	63	73
15-20-GBR	72	40	55,0	83	83	82	83	75	73	89	67
15-22-IRL	85	42	74,9	60	25	38	84	69	86	81	77
15-23-BEL	87	50	35,5	28	46	63	46	72	85	77	74
15-24-BEL	70	42	28,3	56	38	70	57	58	64	51	66
15-25-HUN	75	39	45,0	83	83	56	47	11	89	83	58
15-26-HUN	75	39	50,0	72	72	60	67	70	87	82	75
16-1-DNK	75	42	42,0	88	87	79	68	57	92	86	81
16-2-DNK	75	42	97,2	23	21	21	15	18	65	39	51
16-3-FRA	75	42	94,4	10	9	15	28	31	50	15	35
16-4-FRA	71	46	94,8	39	39	43	49	42	70	43	53
16-5-SCT	67	30	6,5	23	38	46	4	23	27	54	65
16-7-GRB	76	42	47,3	52	36	34	42	44	63	47	58
16-9-DEU	73	34	49,4	62	50	68	50	37	73	61	72
16-10-POL	74	29	14,9	95	87	95	82	52	95	85	84
16-12-IRL	80	62	87,8	48	64	69	78	81	70	79	77
16-13-LVA	69	29	9,1	86	87	90	91	82	91	87	96
Avr. 2015			45,6	68	62	61	60	57	79	70	66
Avr. 2016			54,4	56	55	60	52	49	71	62	69

3.2 Yields

Yield levels and yield increases varied greatly across locations where most sites still provided yields above 7 tonnes per ha in untreated plots. Most trials gave positive and significant yield increases from treatments (**Table 4**). Higher yield increases were achieved by treatments in trials dominated by yellow rust (13-42%), than those dominated by STB (7-17%) or brown rust (5-18%).

Overall, full rates of prothioconazole and epoxiconazole as well as the mixtures tebuconazole + prothioconazole and epoxiconazole + metconazole gave the highest yield increases of 17-20%, whereas metconazole and tebuconazole treatments resulted in the lowest yield increases of 14% and 16% respectively. In 2015 prothioconazole and epoxiconazole yielded similarly to the azole mixtures, however in 2016 all single azoles provided lower and similar yield increases of around ca. 10% in STB dominated trials, whereas the azole mixtures epoxiconazole+metconazole and prothioconazole+tebuconazole both out performed single azoles giving yield increases of ca.15%.

In line with variations seen for efficacy also yield data from STB dominated trials varied significantly. The Irish trials and one French trial had relatively high yield increases from tebuconazole, whereas this active gave among the lowest yield increases at the other locations. Denmark and Germany had similar yield responses from treatments with epoxiconazole and prothioconazole in line with the azole mixtures. Poland and Hungary were distinguished by the fact that the single azoles epoxiconazole and/or prothioconazole gave higher yield increases than the mixtures in 2015. In 2016, however the picture was less varied among locations, here nearly all locations had clearly higher increases from azole mixtures than from single azoles.

Yield increases in the 10 yellow rust dominated trials (7 trials in 2015 and 3 trials in 2016) were clearly higher than in other trials reflecting a general excellent control from azoles against this disease. In 2015 and 2016 epoxinazole increased yields by 39% and 19% respectively, which was comparable to the yield increases from the two azole mixtures, which gave increases between 22 and 42% in the two seasons.

Yield increases from the five trials dominated by brown rust provided quite similar yield responses with the exception of prothioconazole, which provided relatively poor brown rust control. Together with the azole mixture epoxiconazole + metconazole, epoxiconazole provided the best yield responses reflecting the best control.

Table 5. Average yield and yield increase (dt/ha) of septoria tritici blotch (STB), yellow rust (YR) and brown rust (BR) dominated trials. Average and relative yields of 26 trials of 2015, 13 trials of 2016 and 39 trials from 2015-2016 are presented. Fisher-LSD values were calculated without untreated.

Yield, 2015-2016				Untr.	EPX			PTH		MCA		TCA		EPX+MCA		TCA+PTH		
Disease		Year	Trials	dt/ha	125 g/ha	83 g/ha	62,5 g/ha	200 g/ha	100 g/ha	90 g/ha	45 g/ha	250 g/ha	125 g/ha	112.5 + 82.5 g/ha	56 + 41,3 g/ha	125 + 125 g/ha	62,5 + 62,5 g/ha	LSD - untr.
STB	(dt/ha)	2015	15	83,6	+ 9,2	+ 7,6	+ 7,1	+ 10,1	+ 7,6	+ 7,3	+ 5,8	+ 7,0	+ 5,2	+ 9,7	+ 8,7	+ 10,4	+ 8,0	1,3
		2016	9	70,1	+ 7,3	+ 7,2	+ 5,0	+ 7,3	+ 5,4	+ 6,5	+ 4,9	+ 6,8	+ 5,6	+ 11,5	+ 8,9	+ 10,0	+ 6,3	1,3
YR	(dt/ha)	2015	7	74,0	+ 23,6	+ 22,5	+ 21,6	+ 19,6	+ 17,1	+ 16,8	+ 14,7	+ 21,9	+ 18,9	+ 21,8	+ 20,2	+ 25,5	+ 20,1	2,8
		2016	3	73,1	+ 13,7	+ 13,0	+ 12,8	+ 13,2	+ 10,4	+ 11,8	+ 9,3	+ 12,7	+ 9,6	+ 15,8	+ 14,7	+ 16,4	+ 13,2	2,3
BR	(dt/ha)	2015	4	83,8	+ 13,1	+ 12,0	+ 9,6	+ 5,9	+ 4,9	+ 9,1	+ 6,5	+ 9,2	+ 9,7	+ 12,5	+ 10,1	+ 8,3	+ 6,3	3,6
		2016	1	66,5	+ 4,3	+ 3,8	+ 3,5	+ 4,7	+ 4,5	+ 4,8	+ 3,9	+ 3,4	+ 1,8	+ 5,8	+ 5,0	+ 4,8	+ 4,5	3,0
all trials	(dt/ha)	2015	26	81,0	+ 13,7	+ 12,3	+ 11,4	+ 12,1	+ 9,8	+ 10,2	+ 8,3	+ 11,4	+ 9,7	+ 13,5	+ 12,1	+ 14,2	+ 11,1	1,3
	(rel. Y)			100,0	119,1	117,7	116,8	117,5	114,0	114,7	112,0	116,2	114,1	118,9	117,3	120,2	116,0	1,8
all trials	(dt/ha)	2016	13	70,6	+ 8,6	+ 8,3	+ 6,7	+ 8,5	+ 6,5	+ 7,6	+ 5,9	+ 8,0	+ 6,2	+ 12,1	+ 9,9	+ 11,1	+ 7,9	1,1
	(rel. Y)			100,0	114,8	113,9	111,7	114,7	111,2	113,0	110,2	114,4	111,2	120,7	116,6	118,6	113,7	1,8
all trials	(dt/ha)	2015-2016	39	77,6	+ 12,2	+ 11,0	+ 9,9	+ 11,0	+ 8,8	+ 9,3	+ 7,5	+ 10,5	+ 8,6	+ 13,1	+ 11,4	+ 13,2	+ 10,1	0,9
	(rel. Y)			100,0	117,7	116,4	115,1	116,6	113,1	114,2	111,4	115,6	113,1	119,5	117,1	119,7	115,2	1,3

3.3 Mutation frequencies and EC_{50} values in populations of *Z. tritici*

The analyses of the different *Z.tritici* populations revealed variable distributions of CYP51 mutations. Out of the 6 investigated CYP51 mutations, V381V was the most predominat mutation detected in about 90% of all investigated populations (**Table 6**). In 2015, the least frequently detected mutations were V136C and S524T. By 2016, the frequency of both V136C and S524T had increased on average, while the frequency of A379G had decreased on average.

V136C was detected with a frequency of 0-34% in 2015 and 0-45% in 2016, with the highest frequency in the Central UK in 2015 and in Southern France in 2016. In both years, low frequencies of S524T (below 10%) were detected in all countries except the UK (ca. 30%) and Ireland (ca. 50%).

Frequencies of mutation A379G were around 10-30% at all locations except Belgium (0%), the Central UK (0%) and Hungary, where frequencies were around twice as high as in other locations in 2015. In 2016, the frequency of this mutation in Hungary was similar to that of other locations. The two mutations D134G and V136A were detected at comparable frequencies in the medium range at most locations in 2015. The exceptions were South Poland and Hungary with 0%. Belgium was also an exception, since high frequencies of above 60 % were detected for both mutations. Ireland also had high frequency of V136A (73%). In 2016, Latvia and Hungary had exceptionally low frequencies of these two mutations, while northern France had high frequencies (above 60%) and Ireland, one Danish location and two British locations had high frequencies of V136A (above 50%). A clear division could be seen across Europe from west to east regarding all mutation frequencies except for those of I381V, which was highly prolific at all locations (**Figure 2 and 3**). Frequencies of other mutations decreased from west to east except those of A379G for which the opposite was true. Furthermore, the data indicated that average frequencies of all mutations except A379G had increased during the trial period. Specific maps on CYP51 mutations can be found on **WWW.eurowheat.au.dk** and examples from 2 mutations are given in **Figure 3**.

EC_{50} values for the four azoles showed similarly major variation across the different localities. In both 2015 and 2016 Ireland and the UK had relatively high values for all 4 azoles although Ireland had moderate EC_{50} values for tebuconazole in 2015 and also low values in 2016, which is in accordance with the relatively better performances from this product. In the northern part of France data from both 2015 and 2016 showed good sensitivity to both metconazole and tebuconazole. This was less pronounced in Southern France when assessed in 2016. Belgium similarly stood out by having the lowest EC_{50} values for both metconazole and tebuconazole among all the locations in 2015.

Hungary had low EC_{50} values for all azoles in both seasons, with tebuconazole having the highest value in line with results also found in Latvia in 2016. In Poland a similar pattern was revealed, but *Z. tritici* populations were generally about half as sensitive here as in Hungary. Denmark and Germany had overall similarly intermediate EC_{50} values in 2015. No data exist from Germany in 2016, as the pycnidia were empty for spores when incubated. In 2016 Irish and UK isolates had EC_{50} values beyond one for epoxiconazole, and also for prothioconazole the values were at the higher scale.

Table 6. Frequency of CYP51 mutations (%) in 2015 and 2016 based on leaf samples from untreated plots collected at GS 65-75 and EC_{50} values for 4 main azoles. Green: no mutation/low EC50. Yellow: low frequency/medium EC_{50}. Orange: Medium frequency/medium to high EC50. Red: High frequency/high EC_{50}. NA= no data available.

		Frequencies of mutations (%) in 2015						EC_{50} (mg/l)			
Country	**Trial**	**I381V**	**V136A**	**D134G**	**A379G**	**V136C**	**S524T**	**EPX**	**MCA**	**TCA**	**PTH-desthio**
DNK, E	2	91	28	17	30	0	2	0,15	0,17	4,67	0,03
DNK, E	3	89	43	37	14	21	1	0,28	0,13	2,11	0,04
DNK, S	4	95	52	47	19	0	1	0,29	0,13	1,26	0,07
DEU, N	12	98	24	22	16	18	8	0,45	0,26	2,84	0,09
DEU, S	14	98	29	22	34	0	8	0,21	0,17	3,86	0,03
FRA, M	10	89	47	40	10	0	3	0,16	0,07	1,76	0,04
GRB, N	19	100	48	33	16	14	34	0,99	0,41	2,74	0,23
GRB, S	21	97	35	15	14	20	30	0,55	0,53	5,97	0,1
GRB, M	16	NA	NA	NA	NA	NA	NA	0,66	0,53	4,75	0,14
GRB, M	20	100	38	33	0	34	29	0,57	0,39	5,43	0,11
IRL, E	22	88	73	33	27	22	51	0,82	0,46	2,37	0,18
BEL, W	24	94	64	62	0	28	6	0,31	0,1	0,37	0,09
POL, MW	6	96	44	39	28	22	4	NA	NA	NA	NA
POL, S	8	94	10	0	13	11	2	0,13	0,08	3,84	0,02
HUN, SE	25	76	0	0	50	0	0	0,05	0,05	1,61	0,01
HUN, SE	26	95	0	0	73	0	0	0,05	0,06	2,82	0,01
Avr.		93,3	35,7	26,7	22,9	12,7	11,9	0,4	0,2	3,1	0,1

		Frequencies of mutations (%) in 2016						EC_{50} (mg/l)			
Country	**Trial**	**I381V**	**V136A**	**D134G**	**A379G**	**V136C**	**S524T**	**EPX**	**MCA**	**TCA**	**PTH-desthio**
DNK, E	1	92	33	21	33	12	4	0,76	0,85	4,58	0,01
DNK, E	2	92	54	49	19	25	5	0,29	0,21	2,71	0,08
FRA, N	3	91	68	66	0	30	3	0,34	0,10	0,61	0,09
FRA, S	4	78	38	15	28	45	9	0,16	0,14	3,29	0,05
DEU, SE	9	94	37	28	20	16	7	NA	NA	NA	NA
GRB, E	6	100	46	39	17	31	25	1,01	0,79	4,63	0,31
GRB, N	5	95	52	25	14	28	45	1,01	0,68	7,71	0,13
GRB, M	7	99	57	47	22	26	20	0,96	0,64	4,20	0,16
IRL, E	12	95	86	33	14	14	56	1,17	0,60	0,99	0,26
POL, S	10/11	94	20	15	14	15	3	0,65	0,15	3,50	0,17
LVA, M	13	99	11	0	21	0	0	0,10	0,09	4,23	0,02
HUN, MN	14	95	0	0	34	0	0	0,08	0,08	1,25	0,01
Avr.		93,7	41,8	28,2	19,7	20,2	14,8	0,6	0,4	3,4	0,1

Figure 2. Mutation frequencies across Europe (%) in 2015 and 2016. Frequencies in the interval 0-5 % are green, 6-20 % yellow, 21-50 % orange and 51-100 % red. Data from both years are included for comparable trial locations. The year is indicated at the side of individual heat maps. Danish data is presented as average frequencies in three trials in 2015 and two trials in 2016. Data from Hungary represents average frequencies of two trials in 2015 ad one trial in 2016.

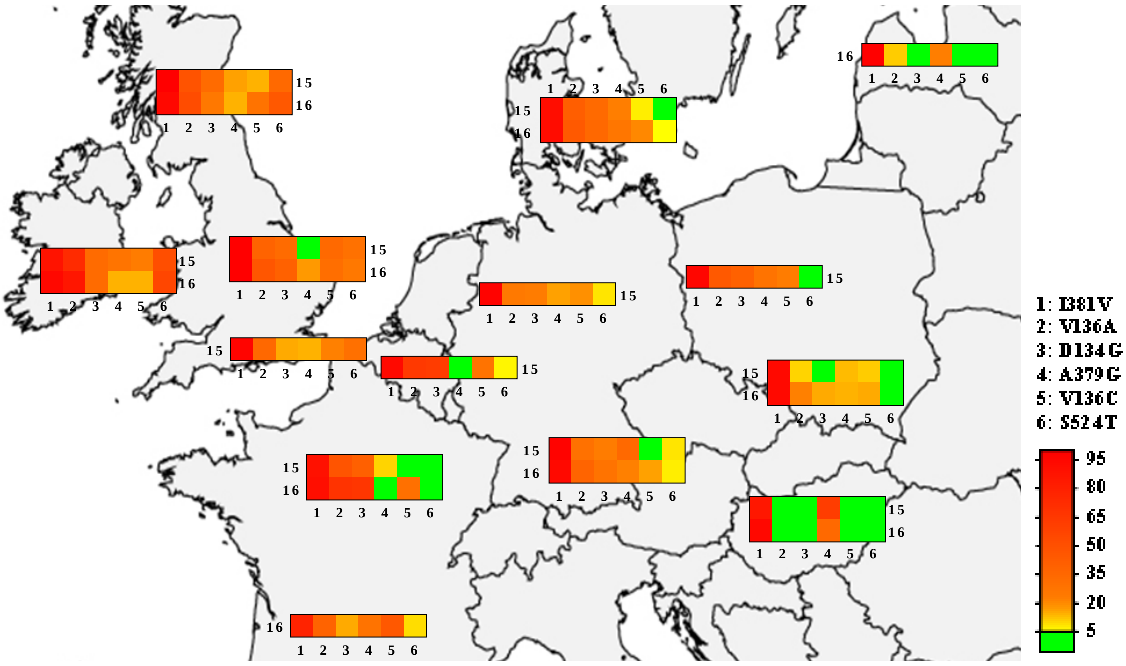
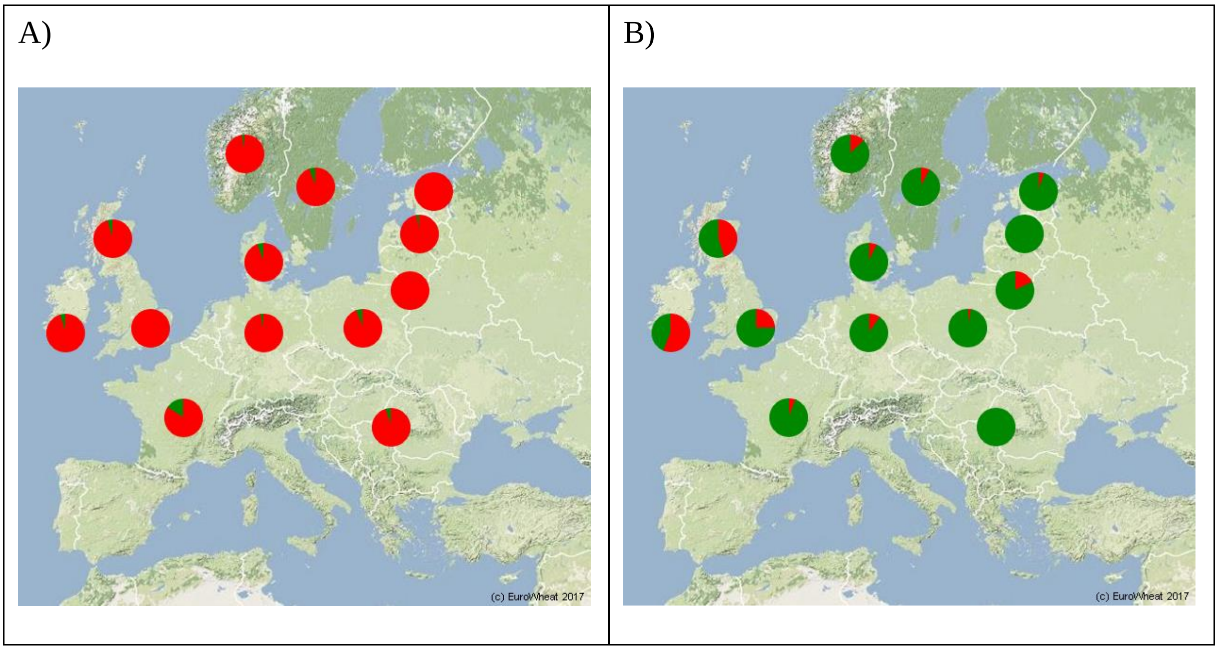


Figure 3. Frequency of two CYP51 mutations (%) A) I381V B) S524T in 2016. Data is based on leaf samples from untreated plots collected at GS 65-75. Examples of data presented on the **www.EUROWHEAT.AU.DK** platform.



4. Discussion

The DMI group of fungicides has been authorized for control of leaf diseases since the late 1970s, and these fungicides are still regarded as the most important option for control of leaf diseases of wheat. DMIs provide significant control of three of the most damaging diseases in wheat; yellow rust, brown rust and STB. Today most control strategies in Europe still rely upon the continued effectiveness of DMIs, which still account for approximately 50% of the fungicide input in European wheat production (McDougall, 2015). In the present study the field performances of four of the most used azoles were tested across Europe for their field efficacy. Data collected from 26 trials carried out in 2015 and 2016 confirmed that azoles tested at full rates still provide significant effects (typically 50-70%) on STB, but major variations in field performances were found across Europe partly related to changes having occurred in the sensitivity of *Z.tritici*. Reduction in field control from single azoles have been seen in recent years (AHDB, 2016, Kildea, 2016), and a similar trend was seen looking at data from 2015 and 2016 in this project. Mixtures of azoles proved to provide better and more stable control across all countries. Part of the better control from mixtures can be seen as a dose effect particularly from the full rate of epoxiconazole + metconazole, which contain 184% active in total, but even at equivalent rates the mixtures out performed the performace of the single azoles. A dose effect was seen for all tested solutions when full and half rates were compared, and differences were in most situations significantly different. Similarly a lower control was seen on the 2nd leaf representing a more curative control compared with control on 1st leaf representing a preventive control. These later results are also in accordance with results from other investigations from the UK and Ireland (ADHB, 2016).

The performances of azoles against STB varied significantly across Europe. Variability was also identified in patterns of CYP51 mutation profiles and in isolate sensitivity to azoles measured as EC_{50} values in *in vitro* tests. Overall, epoxiconazole and prothioconazole together with the co-formulations gave the best control of STB, with the co-formulations showing higher control (5-15 % point better) than the two single azoles in 2016. The overall effect of metconazole and tebuconazole was seen as inferior against STB, allthough not consistent for all countries.

A clear pattern could be seen across Europe of increasing mutation frequencies from North/-West to South/-East, with the two exceptions of I381V and A379G. Brunner et al. (Brunner et al., 2008) proposed that resistance inducing CYP51 mutations emerged locally perhaps in the UK or Denmark, from where it spread eastward due to the prevailing wind direction from west to east. The gradient across Europe could support this diverse pattern of CYP51 mutations; however it can not be ruled out that the variable CYP51 profiles of *Z. tritici* populations across Europe could also be a result of variation in disease intensity and the intensity and diversity of fungicide use patterns.

Results from Germany and Denmark having high to moderate field effects were in line with this overall average pattern from the whole investigation. Furthermore these countries/locations had quite similar mutation frequency profiles and intermediate EC_{50} values. Ireland and the UK had unique profiles with high frequencies of S524T and the highest EC_{50} values for all four azoles. This confirms other findings in which the mutation S524T in combination with several other mutations (V137F or V136A) has been found to reduce the sensitivity to commonly used azoles like prothioconazole and epoxiconazole (Leroux et al., 2007, Fraaije et al., 2007, Leroux & Walker, 2011).

Unlike in other locations, metconazole gave high control effects in France, Ireland and Belgium. In the case of France and Belgium these findings were supported by low EC_{50} values for this active, but the same could not be said for Ireland.

Belgium had high proportions of D134G and good performance from tebuconazole, which confirms other findings where haplotypes carrying D134G have been found to be more sensitive to tebuconazole. One of the French sites similarly had very low EC_{50} for tebuconazole, which again supports the relatively good control from tebuconazole in the French trials.

Hungary differed distinctly from all other locations as this country only had few mutations (I381V and A379C) and low EC_{50} values for all four azoles indicating a more sensitive population, which again reflects a less intensive use of azoles in this country. However, control effects of azoles against STB were not as high at this location as could be expected from the mutation profile and low EC_{50} values (29-64% for the single azoles in 2015). Ireland represented another example of low correlation between mutation freq uencies/EC_{50} values and field performances of azoles. Here most azoles had quite high control effects against STB (65-90% for the single azoles) in spite of the high frequencies of all six investigated mutations and high EC_{50} values of azoles, with the exception of tebuconazole in 2016. Similar examples of poor links between field effects, mutation frequencies and *in vitro* sensitivity of local *Z. tritici* populations were seen in other studies (Stammler et al., 2008). These findings suggest that other factors such as timing of applications and weather conditions might under certain circumstances also be very important for the level of STB control achieved (Strobel et al., 2016)

Over the past 15 years a significant number of mutations in the CYP51 gene, which confer resistance against DMIs, have emerged and been documented (Cools & Fraaije, 2013). The mutations in the *Z. tritici* populations occur in combinations and the mutations described in this paper reflect the overall dominance of certain mutations but do not indicate how specific haplotypes are composed. Homology studies (Mullins JGL et al., 2011) and heterologous expression of mutated *Z. tritici CYP51* genes (Cools et al., 2010) have verified that it is often specific combinations of alterations, which play a role on the sensitivity of specific DMIs, rather than the individual alterations. This again can explain that there is not always a clear link at specific sites between the CYP51 genes occuring and the efficacy seen from specific azoles. Even so several of the specific CYP51 genotypes are known to have variable impacts on particular DMIs (Cools & Fraaije, 2013, Leroux & Walker, 2011), for example, tebuconazole positively selects for the I381V mutation but selects negatively for the V136A mutation.

The Western European population of *Z. tritici* does now widely contain the CYP51 mutations V136A and D134G, which have been selected following widespread use of epoxiconazole and prothioconazole. Isolates with these mutations remain sensitive to difenoconazole and tebuconazole (Leroux & Walker, 2011). Studies have shown that different cross resistance patterns exist for several DMIs; one group consists of cyproconazole, epoxiconazole and prothioconazole and another group consists of difenoconazole, tebuconazole and metconazole (Buitrago et al., 2014). These findings point towards the direction of applying differentially selecting azoles as possible anti-resistance tactics, while maintaining STB control at an acceptable level (Cools & Fraaije, 2008). The control benefit from using azole mixtures has also been documented in this investigation, where mixtures particularly in 2016 outperformed single azoles.

The data in this study indicate a trend of decreasing performances against STB from all single azoles going from 2015 to 2016, except for tebuconazole where performance seems to have improved compared with historic data (Clark, 2006). The performances of single azoles decreased more than those of the mixtures. The two included azole mixtures gave more stable STB control assessed both curatively and preventatively, which also led to higher yield increases than the individual azoles used alone. This was seen in both years but was more pronounced in 2016 than in 2015. During this period, an overall shift towards higher mutation frequencies was also seen. In 2016 the UK and Irish locations also reached EC_{50} values for epoxicoanzole above one ppm, whereas locations in Hungary and Latvia still showed very low EC_{50} values (0.01 ppm). Since mutation frequencies increased generally, it is most likely that mixed azoles had an increased advantage as a result of their broader control of the different haplotypes. A study by Heick et al. (Heick et al., 2017) similarly showed that frequencies of CYP51 mutations D134G, V136A/C and 524T increased in Danish and Swedish *Z. tritici* populations from 2015 to 2016. It was also shown in this study, that mixtures of azoles provide an important measure which can help to reduce the selection for specific CYP51 mutations in the *Z. tritici* populations (Heick et al. 2017). Furthermore, an Irish study (Dooley et al., 2016b) also found that the mixture epoxiconazole + metconazole more effectively controlled STB than any of the azoles used alone. One drawback could be that azole mixtures will increase the selection for new combined CYP51 mutations, which can be hard to control from all known azoles currently on the market. In fact isolates carrying combinations of alterations conferring lab resistance to all the most widely used azoles have emerged (Cools et al., 2011), raising concerns that combining azoles might not be a sustainable development. However, data is so far inconclusive and further work in this area need to be done. It is also important to note that in commercial situations azoles and azole mixtures will generally be used in combination with different MoA for the control of a range of diseases and for resistance management purposes.

Yellow rust and brown rust were the main diseases in 10 trials in 2015 and 5 trials in 2016. Relatively little variation across countries was seen regarding the performances of azoles against these diseases compared with variation in control of STB. This very likely reflects that no major changes in sensitivity to rust diseases to DMIs have been detected over the years. In accordance with earlier investigation DMIs are well known for their good control of rust diseases even when applied at low rates (Jørgensen & Nielsen, 1994). Epoxiconazole and tebuconazole gave consistently high control of both rust diseases. Prothioconazole was slightly inferior while metconazole generally provided least control. Overall, the highest yield responses were measured in trials with significant attacks of yellow rust. The generally high control of rust diseases is in line with other studies showing that only minor levels of resistance to DMI have developed in the rust fungi; levels which are only seen to have no or limited effect on field performances (Stammler et al., 2009). Although increasing problems with yellow rust been seen in recent years the availability of wheat cultivars with high levels of resistance against yellow rust and brown rust still plays a major role in the low prevalence and severity of these diseases (Singh et al., 2016, Hovmøller et al., 2016). This generally helps to reduce the need to spray against these diseases. Although fungicides resistance development can not be ruled out in rust populations (Oliver, 2014), so far a relatively lower selection pressure in the rust populations has helped to maintain a high proportion of azole-sensitivity in the rust populations.

In summary the presented data confirm that azoles still play an important role in disease management in wheat; this includes control of both rust disease and STB. Although cross resistance is described for this group of fungicides, the data presented verify a major variation in the efficacy profile of single azoles for control of STB across Europe. The trial results showed a clear benefit from mixing DMIs as a means of stabilizing STB control. The future control of STB relies heavily on having a selection of azoles available to apply azole mixtures, but azoles are also important as mixing partners for other fungicides with different modes of action, like SDHIs. Both of these two mixing strategies are important in order to achieve good and reliable disease control as well as options for applying an anti-resistance strategy. Although fungicides are essential for disease management; a sustainable control strategy also relies on farmers growing the most resistant cultivars to minimize the need for chemical control.

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REFERENCES

Ahdb, 2016. Wheat disease guide. In: Ahdb, ed. **WWW.ADHB:** ADHB.

Anon, 2009. ENDURE In. **http://www.endure-network.eu/what_is_endure**. (2009.)

Brunner PC, Stefanato FL, Mcdonald BA, 2008. Evolution of the CYP51 gene in Mycosphaerella graminicola: evidence for intragenic recombination and selective replacement. *Molecular Plant Pathology* **9**, 305-16.

Buitrago C, Frey R, Wullschleger J, Sierotzki H. An update on the genetic changes in the CYP51 gene of Mycosphaerella graminicola and their relationship to DMI fungicides sensitivity. In: Dehne Hw DH, Fraaije B, Et Al.,, ed. *Proceedings of the Proceedings of the Modern Fungicdes and Antifungal Compounds 2014*. Reinhardsbrunn, Germany: Deutsche: Phytomedizinische Gesellschaft, Braunschweig, 103-10.

Clark B, 2006. Fungicide resistance: are we winning the battle but losing the war. *Aspects of Applied Biology* **78**, 127-32.

Cools H, Fraaije B, 2008. Are azole fungicides losing ground against Septoria wheat disease? Resistance mechanisms in Mycosphaerella graminicola. . *Pest Management Science* **64**, 681-4.

Cools H, Parker J, Kelly D, Lucas J, Fraaije B, Kelly S, 2010. Heterologous expression of mutated eburicol 14α-Demethylase (CYP51) proteins of Mycosphaerella graminicola to assess effects on azole fungicide sensitivity and intrinsic protein function. *Applied and Environmental Microbiology* **76**, 2866-72.

Cools HJ, Fraaije BA, 2013. Update on mechanisms of azole resistance in *Mycosphaerella graminicola* and implications for future control. *Pest Management Science* **69**, 150-5.

Cools HJ, Mullins JGL, Fraaije BA, *et al.*, 2011. Impact of Recently Emerged Sterol 14 alpha-Demethylase (CYP51) Variants of *Mycosphaerella graminicola* on Azole Fungicide Sensitivity. *Applied and Environmental Microbiology* **77**, 3830-7.

Dooley H, Shaw MW, Spink J, Kildea S, 2016a. Effect of azole fungicide mixtures, alternations and dose on azole sensitivity in the wheat pathogen Zymoseptoria tritici. *Plant Pathology* **65**, 124-36.

Dooley H, Shaw MW, Spink J, Kildea S, 2016b. The effect of succinate dehydrogenase inhibitor/azole mixtures on selection of Zymoseptoria tritici isolates with reduced sensitivity. *Pest Management Science* **72**, 1150-9.

Fones H, Gurr S, 2015. The impact of Septoria tritici Blotch disease on wheat: An EU perspective. *Fungal Genetics and Biology* **79**, 3-7.

Fraaije BA, Cools HJ, Kim SH, Motteram J, Clark WS, Lucas JA, 2007. A novel substitution I381V in the sterol 14 alpha-demethylase (CYP51) of Mycosphaerella graminicola is differentially selected by azole fungicides. *Molecular Plant Pathology* **8**, 245-54.

Heick TM, Justesen AF, Jørgensen LN, 2017. Fungicide spray strategies avoiding resistance development in winter wheat pathogen Zymoseptoria tritici, Crop Protection – minor revision, jan 2017. *Crop Protection – minor revision, jan 2017.*

Hovmøller MS, Walter S, Bayles RA, *et al.*, 2016. Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region. *Plant Pathology* **65**, 402-11.

Jørgensen LN, Hovmøller MS, Hansen JG, *et al.*, 2014. IPM Strategies and Their Dilemmas Including an Introduction to **www.eurowheat.org**. *Journal of Integrative Agriculture* **13**, 265-81.

Jørgensen LN, Nielsen BJ, 1994. Control of Yellow rust (Puccinia striiformis) by ergosterol inhibitors at full and reduced dosages. *Crop Protection* **13**, 323-30.

Kildea S, 2016. Wheat disease control and resistance isssues. In. *National Tillage Conference 2016.* Oak Park, Crop Research, Carlow: Teagasc, 39.

Leroux P, Albertini C, Gautier A, Gredt M, Walker AS, 2007. Mutations in the CYP51 gene correlated with changes in sensitivity to sterol 14 alpha-demethylation inhibitors in field isolates of Mycosphaerelia graminicola. *Pest Management Science* **63**, 688-98.

Leroux P, Walker AS, 2011. Multiple mechanisms account for resistance to sterol 14 alpha-demethylation inhibitors in field isolates of *Mycosphaerella graminicola*. *Pest Management Science* **67**, 44-59.

John A Lucas, Nichola J Hawkins, Bart A Fraaije (2015) The evolution of fungicide resistance. *Advances in Applied Microbiology 2015, 90: 29-92*
Mcdougall P, 2015. Fungicide market data. In.: Agribusiness Intelligence, Division of Informa PLC
Mullins Jgl, Parker Je, Cools Hj EA, 2011. Molecular Modelling of the Emergence of Azole Resistance in Mycosphaerella graminicola. PLoS One 6, e20973. *PLoS One 6* **e20973.**
Oepp/Eppo, 2014. Foliar and ear diseases on cereals. *EPPO Bulletin*, **44**.
Oerke EC, 2006. Crop losses to pests. *Journal of Agricultural Science* **144**, 31-43.
Oliver RP, 2014. A reassessment of the risk of rust fungi developing resistance to fungicides. *Pest Management Science* **70**, 1641-5.
Russell PE, 2005. A century of fungicide evolution. *Journal of Agricultural Science* **143**, 11-25.
Singh RP, Singh PK, Rutkoski J, *et al.*, 2016. Disease Impact on Wheat Yield Potential and Prospects of Genetic Control. *Annu Rev Phytopathol* **54**, 303-22.
Stammler G, Carstensen M, Koch A, Semar M, Strobel D, Schlehuber S, 2008. Frequency of different CYP51-haplotypes of Mycosphaerella graminicola and their impact on epoxiconazole-sensitivity and -field efficacy. *Crop Protection* **27**, 1448-56.
Stammler G, Cordero J, Koch A, Semar M, Schlehuber S, 2009. Role of the Y134F mutation in cyp51 and overexpression of cyp51 in the sensitivity response of Puccinia triticina to epoxiconazole. *Crop Protection* **28**, 891-7.
Stammler G, Semar M, 2011. Sensitivity of *Mycosphaerella graminicola* (anamorph: *Septoria tritici*) to DMI fungicides across Europe and impact on field performance. *EPPO Bulletin,* **48**, 149-55.
Stammler G, Taher K, Koch A, *et al.*, 2012. Sensitivity of Mycosphaerella graminicola isolates from Tunisia to epoxiconazole and pyraclostrobin. *Crop Protection* **34**, 32-6.

Stroebel, D. Bryson, R., Roth, J. Stammler, G. 2017. Field performance of DMI fungicides against Zymoseptoria tritici across Europe - Compromized by Further Sensitivity Shifts? In: Deising HB, Fraaije, B. Mehl, A; Oerke, EC; Sierotzki, H. Stammler, G (Eds.) "Modern Fungicides and Antifungal compounds" Vol VIII, pp 249-254. Deutsche Phytomedizinisxhe Gesellschaft, Braunsweig,

HIGHLIGHTS

- Azoles perform diversely against Septoria tritici blotch across Europe.
- Wester European countries tend to have higher CYP51 mutation frequencies, higher EC_{50} values, and lower field performances of azoles than Easter European countries.
- Azole mixtures are more effective against Septoria tritici bloch than azoles used alone.
- CYP51 mutation frequencies and EC50 values have increased and triazole performances have decreased across Europe on average from 2015 to 2016.
-Azoles provide high control of yellow rust and brown rust. Metconazole is inferior on yellow rust and prothioconazole on brown rust.
-Best azoles increase yields by 17-20% from a single treatment.

Supplementary data:

Table S2: Summary of yellow rust control from full rate of azoles assessed on 1st leaf in 13 trials carried out in 2015 and 2016. Control effects are summarized as percentage reduction of attack relative to untreated plots. The column "untr." represents attacks in untreated plots. Colours signify ranking of treatment effects within trials. Green: high effect. Yellow: medium effect. Orange: low effect. DAA=Days after application and GS=Growth stage at assessment.

% Control of *P. striiformis* Leaf 1 - 2015-16			**-**	**1,5 l/ha**	**1 l/ha**	**0,8 l/ha**	**1 l/ha**	**1 l/ha**	**3 l/ha**	**1,5 l/ha**	**1 l/ha**
Year-Trial-Contry	**GS**	**DAA**	**Untr.**	**EPX**		**PTH**	**MCA**	**TCA**	**EPX+MCA**		**PTH+TCA**
15-1-DNK	65	35	53,8	97	94	84	70	95	95	91	90
15-11-DEU	71	36	5,4	96	39	70	35	100	76	70	96
15-12-DEU	69	37	8,3	89	88	90	63	93	93	83	98
15-13-DEU	69	37	8,5	89	94	86	80	97	90	88	95
15-17-GBR	65	33	12,5	74	48	36	42	68	54	52	64
15-19-GBR	59	28	19,8	93	92	85	82	93	90	87	96
15-23-BEL	65	34	28,5	95	91	92	88	96	91	95	93
15-25-HUN	75	39	7,5	99	96	92	66	76	73	80	96
15-26-HUN	83-85	49	22,5	97	98	82	48	83	89	100	88
16-1-DNK	75	42	16,0	100	100	95	86	100	100	100	99
16-5-SCT	64-69	30	8,3	100	100	100	58	82	100	70	100
16-6-GBR	73	36	28,8	100	100	98	100	100	100	100	100
16-9-DEU	55	22	2,9	99	94	97	91	96	93	95	98
Average 2015			18,5	92,2	82,2	79,8	63,8	89,0	83,5	82,9	90,7
Average 2016			14,0	99,7	98,6	97,5	83,7	94,4	98,2	91,2	99,4
Average Total			17,1	94,5	87,3	85,2	69,9	90,7	88,0	85,4	93,3

Table S3: Summary of brown rust control from full rate of azoles assessed on 1st leaf in 6 trials carried out in 2015 and 2016. Control effects are summarized as percentage reduction of attack relative to untreated plots. The column "untr." represents attacks in untreated plots. Colours signify ranking of treatment effects for each trial. Green: high/best effect. Yellow: medium effect. Orange: low effect. DAA=Days after application and GS=Growth stage at assessment.

% Control of *P. triticina* Leaf 1 - 2015/16			**-**	**1,5 l/ha**	**1 l/ha**	**0,8 l/ha**	**1 l/ha**	**1 l/ha**	**3 l/ha**	**1,5 l/ha**	**1 l/ha**
Year-Trial-Contry	**GS**	**DAA**	**Untr.**	**EPX**		**PTH**	**MCA**	**TCA**	**EPX+MCA**		**PTH+TCA**
15-8-POL	75	46	3,0	87	80	58	82	62	87	77	68
15-9-POL	75	46	5,3	87	79	49	82	58	87	81	63
15-18-GBR	75	43	48,0	97	98	35	79	88	94	98	85
15-21-GBR	75	42	62,8	93	80	55	80	79	89	89	72
15-26-HUN	83-85	49	35,0	54	54	73	79	75	75	82	68
16-11-POL	75-77	39	6,2	99	97	84	77	53	96	97	89
Average 2015			30,8	83,5	78,2	54,0	80,4	72,4	86,3	85,4	71,2
Avrerage Total			26,7	86,1	81,3	59,0	79,8	69,1	87,9	87,3	74,1

Table S1. Detailed experimental background information on all trials in 2015. Abbreviations: “ Rep.”: replications; “Lat.”: lattitude in decimal degrees; “Long.”: Longitude in decimal degrees; “App. date”: application date; “App. GS”; “App. equip.”: application equipment.

Trial 2015	**Target disease**	**Country**	**Location**	**Variety**	**Harvest plot area (m2)**	**Rep.**	**Lat.**	**Long.**	**Harvest date**	**Sowing date**	**App. date**	**App. GS**	**Precrop**	**Nozzle type**	**Pressure (bar)**	**Volume water (l/ha)**
1	YR	Denmark	Flakkebjerg	Substance	22,50	4	55,33	11,39	17-08-15	29-09-14	20-05-15	33-37	OSR	teejet9504	2,4	150
2	STB	Denmark	Flakkebjerg	Hereford	22,50	4	55,32	11,39	14-08-15	05-09-14	20-05-15	37-39	OSR	teejet9504	2,4	150
3	BR	Denmark	Flakkebjerg	Mariboss	22,50	4	55,32	11,37	19-08-15	09-09-14	22-05-15	37-39	OSR	teejet9504	2,4	150
4	BR	Denmark	Holeby	Mariboss	18,50	4	54,71	11,54	22-08-15	20-09-14	22-05-15	37-39	OSR			
5	BR	Lithuania	Dotnuva	Magnifik	20,00	4	55,41	23,87	11-08-15	18-09-14	08-06-15		Pea	HAR ISOLD-02-110	2,5	300
6	STB	Poland	Nagradowice	Astoria	12,00	4	52,32	17,15	15-07-15	15.09.14	29-04-15	37	OSR	TEEJet XR 11003	2,5	200
7	YR	Poland	Nagradowice	Astoria	12,00	4	52,32	17,16	15-07-15	15.09.14	29-04-15	37	OSR	TEEJet XR 11003	2,5	200
8	STB	Poland	Łany Wielkie	Zyta	15,00	4	50,28	18,56	04-08-15	05.10.14	11-05-15	37	OSR	TEEJet XR 11003	0,2	300
9	BR	Poland	Łany Wielkie	Turnia	15,00	4	50,28	18,55	03-08-15	05.10.14	11-05-15	37	OSR	TEEJet XR 11003	0,2	300
10	STB	France	Boigneville	Pakito	12,25	3	48,34	2,37	17-07-15	15-10-14	07-05-15	39	Horsebean	LECHLER ; verte Cal. 015	2.8	218
11	STB	Germany	Sickte	JB Asano	30,00	4	52,08	10,65	03-08-15	14-10-14	13-05-15	37	OSR	ID 120 02	4,0	300
12	YR	Germany	Lafferde	JB Asano	30,00	4	52,23	10,24	04-08-15	08-10-14	11-05-15	37	W wheat	ID 120 02	4,0	300
13	BR	Germany	Evensen	JB Asano	30,00	4	52,58	9,53	06-08-15		11-05-15	37	W wheat	ID 120 02	4,0	300
14	STB	Germany	Fraunberg Bavaria	JB Asano	18,00	4	48,35	11,97	22-07-15	13-10-14	18-05-15	41	Clover	AW 11002	2,5	200
15	YR	Germany	Weihensteph an Bavaria	JB Asano	13,13	4*	48,40	11,72	06-08-15	10-10-14	18-05-15		Horsebean	AIR-MIX 11003	2,5	300
16	STB	UK	Terrington	Santiago	40,00	4	52,67	0,29	10-09-15	30-11-14	21-05-15	39	OSR	F02/110	2	200
17	YR	UK	Terrington	Kielder	40,00	4	52,79	0,28	29-08-15	26-11-14	14-05-15	37	OSR	F02/110	2	200
18	BR	UK	Terrington	Crusoe	40,00	4	52,79	0,28	29-08-15	26-11-14	28-05-15	43	OSR	F02/110	2	200
19	STB	UK	Berwick upon Tweed	Solstice	20,40	4	55,67	-2,03	06-09-15	22-09-14	19-05-15	Mix	OSR	FLAFAN	3	200

Trial 2015	Target disease	Country	Location	Variety	Harvest plot area (m2)	Rep.	Lat.	Long.	Harvest date	Sowing date	App. date	App. GS	Precrop	Nozzle type	Pressure (bar)	Volume water (l/ha)
20	YR	UK	Caythorpe	Cordiale	20,40	4	53,07	-0,55	24-08-15	02-10-14	14-05-15	39	S barley	FLAFAN	3	200
21	BR	UK	Wye	KWS Santiago	18,00	4	51,25	-1,25	08-08-15	20-10-14	26-05-15	39	S beans	FLAFAN	1,4	204
22	STB	Ireland	Carlow	Cordiale	23,00	4	52,86	-6,91	17-08-15	01-10-14	22-05-15	39	W Oats	Teejet 110 03	2	220
23	YR	Belgium	Donmartin	JB Asano	19,00	4	50,62	5,36	03.08.15	02-10-14	13-05-15	39	Potato	Teejet HR 110	1.6	200
24	BR	Belgium	Braffe	KWS Ozon	19,00	4	50,54	3,57	06-08-15	23-10-14	12-05-15	39	Potato	Teejet HR 110	1.6	200
25	STB	Hungary	Szeged	GK Körös	10,00	4	46,29	20,10	07-07-15	08-10-14	23-04-15	37-39	Pea	Lechler "IS 80-04"	3,5	200
26	BR	Hungary	Szeged	GK Petur	10,00	4	46,29	20,10	07-07-15	08-10-14	23-04-15	37-39	Pea	Lechler "IS 80-04"	3,5	200

*Yield data has 4 replications, but disease severity data consists of one number per treatment representing an average of subsamples from all replication per treatment.

Table S2. Detailed experimental background information on all trials in 2016. Abbreviations: " Rep.": replications; "Lat.": lattitude in decimal degrees; "Long.": Longitude in decimal degrees; "App. date": application date; "App. GS"; "App. equip.": application equipment.

Trial 2016	Target path.	Country	Location	Variety	Harvest plot area (m²)	Rep.	Lat.	Long.	Harvest date	Sowing date	App. date	App. GS	Precrop	Nozzle type	Pressure (BAR)	Volume water (l/ha)
1	YR	Denmark	Flakkebjerg	Ambition	22,5	4	55,32	11,39	11-08-16	15-09-15	23-05-16	37	OSR	Minidrift	2,4	200
2	STB	Denmark	Horsens	Hereford	18,1	3	55,86	9,76	17-08-16	22-09-15	26-05-16	37	W OSR	Flat fan	1,7	200
3	STB	France	Aubigny	Selekt	11,6	3	47,40	2,46	26-07-16	30-09-15	09-05-16	38	OSR	LECHLER	2,5	206
4	STB	France	Bergerac	Sy Moisson	11,6	3	44,85	0,52	30-06-16	28-10-15	11-04-16	37	Grain corn	LECHLER	2,8	196
5	STB	Scotland	East Lothian	Myriad	19.8	4	55,90	-2,84	07-09-16	29-09-15	01-06-16	39	W OSR	Lurmark FF 02F80	2.0	220
6	YR	UK	Cambridge	Solistice	18,0	4	52,24	0,10	16-08-16	03-10-15	19-05-16	39	W Bean	ARINDH 03	2.0	200
7	STB	UK	Rosemaund	Santiago	18,1	4	52,08	-2,73	13-08-16	27-09-15	20-05-16	39	S beans	03F110	2,5	200
8	STB	Germany	Büddenstedt	Biscay	10,0	4	52,15	11,03	09-08-16	30-10-15	25-05-16	39	Sugar beet	ID 120 02	4,0	300
9	STB	Germany	Fraunberg	JB Asano	18,0	4	48,34	11,98	28-07-16	13-10-15	11-05-16	37	Woat	AM11002	2,5	200
10	STB	Poland	Sosnicowice	Fidelius	15,0	4	50,27	18,55	16-08-16	05-10-15	18-05-16	39	S OSR	FLAFAN	2,0	200
11	BR	Poland	Lany Wilkie	Turnia	15,0	4	50,28	18,56	04-08-16	03-10-15	19-05-16	37-39	W OSR	FLAFAN	2,0	200
12	STB	Ireland	Teagasc	Cordiale	20,0	4	52,86	-6,94	09-08-16	07-10-15	23-05-16	39	W Oats	Teejet 110 03 Flat Fan	2,0	220
13	STB	Latvia	Peterlauki	Zentos	21,0	4	56,54	23,73	08-08-16	09-30-15	17-05-16	37	W OSR	COHOSW	3,0	250
14	STB	Hungary	Martonvásár	MV Nádor	-	4	47,18	18,49	-	20-10-15	10-05-16	49	Maize	albuz cvi-twin 11002	4,0	250