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Azoles Have Different Strengths and Perform Diversely Across Europe

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INTRODUCTION

Leaf diseases cause major yield losses in winter wheat every year across Europe. Septoria leaf blotch – STB (caused by *Zymoseptoria tritici)* is the most serious leaf disease in Northern Europe, but also yellow rust – YR (due to *Puccinia striiformis*) and brown rust – BR (due to *Puccinia triticina)* are known to cause major problems in some regions and seasons (Jørgensen et *al*., 2014). In recent years increasing problems with fungicide resistance in the populations of *Z. tritici* have caused concerns for future control options (Cools & Fraije, 2013). Azoles have been used for more than 35 years but are still seen as the backbone of disease management and provide moderate to good control of STB depending on locality and the specific azole used. Due to differences in disease pressures and fungicide availability across Europe the patterns of fungicide use varies greatly and because of this, fungicide efficacy is also expected to vary considerably. With the aim of investigating the differences in azoles performances between different regions of Europe, a EUROwheat project was initiated in 2015.

MATERIALS AND METHODS

Twenty-six trials were carried out in 2015 across different locations in Europe, covering different climate zones and agricultural practices. The trials were carried out by local scientific organisations in Poland, Germany, France, Belgium, Hungary, Ireland, UK, Lithuania and Denmark. Standard procedures and assessment methods were applied using a randomized plot design, a minimum plot size of 10 m^2 and 3-4 replicates. Moderate to susceptible cultivars were chosen, which could provide good levels of attack aiming at having Septoria tritici blotch (*Zymoseptoria. tritici*), yellow rust *(Puccinia striiformis)* or brown rust *(Puccinia triticina)* 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 and 250 l/ha. Spraying was carried out at flag leaf emergence (GS 37-39) and in 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 2 weeks before the main treatments. Fungicides were provided by BASF and all products were tested at full and half rates (Table 1).

Per cent leaf area attacked by specific diseases was assessed at regular intervals after applications following EPPO guideline (1/26 (4)). Focus was put on assessments carried out at 30-50 days after application (DAA) at GS 73-75. 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 TGW assessments.

Table 1 Fungicide doses (l/ha) and amount of active ingredient (g/ha) used per treatment.

Trt. No.	Product	l/ha	Active ingredient	g/ha
1	Untreated	-	-	-
2	Opus Max	1.5	epoxiconazole	125
3		1		83
4		0.75		62.5
5	Proline 250 EC	0.8	prothioconazole	200
6		0.4		100
7	Caramba 90	1	metconazole	90
8		0.5		45
9	Folicur 250 EW	1	tebuconazole	250
10		0.5		125
11	Osiris	3	epoxiconazole +	112.5 + 82.5
12		1.5	metconazole	56 + 41.3
13	Prosaro 250 EC	1	tebuconazole +	125 + 125
14		0.5	prothioconazole	62.5 + 62.5

In order to understand the control profiles from the specific trial sites leaf samples of STB were collected at GS 65-75 from all sites and forwarded for characterization. CYP51 mutation profiling of local *Z. tritici* populations was carried out by pyrosequencing and QPCR by BASF and EC_{50} values to the four azoles were measured on single isolates by Epilogic. All data were collected locally by the subcontractors and forwarded to AU-Flakkebjerg. All data 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 F-test.

RESULTS

Disease severities and treatment effects were highly variable across the 9 countries and 26 trials involved in the project. However, general trends regarding treatment effects were observed. In 15 trials STB developed sufficiently for ranking of product performances and a total summary is given in Table 2. However, at several sites the disease pressure was too low which obscured the patterns of product potencies. Due to the high variability, particularly for the control of STB only few summaries of more than one trial were possible. Thus, data were predominately evaluated separately by country and/or region.

Table 2 Average preventive (leaf 1) and curative (leaf 2) % control by triazoles in septoria, yellow rust and brown rust dominated trials at GS 69-75, DAA 32-59.

			% Control, GS 69-75, DAA 32-59					
			Epoxi.	Prothio.	Met.	Tebu.	Epoxi. + met.	Tebu. + prothio.
Disease	Trials	Leaf	125 g/ha	200 g/ha	90 g/ha	250 g/ha	112.5 + 82.5 g/ha	125 + 125 g/ha
Septoria	15	1	75	77	72	64	86	78
	17	2	68	62	63	54	77	69
Yellow rust	13	1	93	82	71	91	89	92
	13	2	77	83	77	86	84	83
Brown rust	7	1	84	54	79	77	87	75

For control of STB the products all provided better preventive than curative control. Overall the best control of STB was provided by epoxiconazole or prothioconazole used alone or the co-formulations epoxi.+met. and tebu.+prothio. Looking at national data, products performed very differently (Table 3). For example, in France and Ireland metconazole gave better control of STB than in other countries providing high effects (70-90%) relative to other countries (40-70%). The opposite was true of the curative control of STB by prothioconazole and epoxiconazole, which in these countries stood out as being relatively weak (40-60% compared to 60-90% in the other countries). Furthermore, tebuconazole performed very well in Ireland and Belgium (ca. 70%), whereas this product performed poorly in other countries (ca. 50%).

The products were generally much more effective in their control of YR (ca. 80-90%) compared to STB (ca. 60-70%). This was especially the case for epoxiconazole and tebuconazole, but also for the mixtures epoxi.+met. and tebu.+prothio. Metconazole was seen as the weakest product for control of YR. The most effective treatments against BR were epoxiconazole and the mixture epoxi.+met. (>80%), whereas the control from prothioconazole was clearly inferior (ca. 50%).

The trials gave positive and significant yield increases (Table 4). Higher yield increases were achieved by treatments in trials dominated by YR (rel. 122-142), than those dominated by STB (rel. 106-112) or BR (rel. 105-118). Overall tebu.+prothio., epoxi.+met. and epoxiconazole gave the highest relative yields of ca. 117-118 each, whereas metconazole and tebuconazole treatments resulted in the lowest relative yields of 113 and 114 respectively.

Table 3 Percent control of STB on leaf 2 at growth stage (GS) 71-85, 37-58 days after application (DAA) in 12 trials located in 7 different countries. Colours signify ranking of treatment effects within each trial (i.e. treatment rankings are not compared between trials). Green: high treatment effect. Yellow: medium treatment effect. Orange: Low treatment effect. Red: Disease severity of untreated plots (%).

					Untr.	Epoxi.	Prothio.	Met.	Tebu.	Epoxi. + met.	Tebu. + prothio.
Country	Trial id.	Leaf	GS	DAA	-	125 g/ha	200 g/ha	90 g/ha	250 g/ha	112.5 + 82.5 g/ha	125 + 125 g/ha
Denmark	2	2	75	47	72.5	76	79	62	55	83	75
Denmark	3	2	75	46	58.8	60	52	45	43	60	53
Denmark	4	2	75	43	40.0	75	63	47	47	71	56
Poland	6	2	75	58	5.3	45	59	62	62	69	50
Poland	8	2	75	46	17.5	90	63	56	62	91	65
France	10	2	75	41	79.7	58	48	69	57	81	72
Germany	15	2	75	37	30.0	80	93	77	50	87	73
Ireland	22	2	85	42	74.9	60	38	84	69	86	77
Belgium	23	2	87	50	35.5	28	63	46	72	85	74
Belgium	24	2	70	42	28.3	56	70	57	58	64	66
Hungary	25	2	75	39	45.0	83	56	47	11	89	58
Hungary	26	2	75	39	50.0	72	60	67	70	90	75
Average control - Leaf 2 [%]					44.8	65	62	60	55	80	66

Table 4 Average yield and yield increase (dt/ha) of Septoria tritici blotch (STB), yellow rust (YR), brown rust (BR) dominated trials and all 26 trials (rel. yields also presented).

		Untr.	Epoxi.	Prothio.	Met.	Tebu.	Epoxi. + met.	Tebu. + prothio.	
Disease	Trials	-	125 g/ha	200 g/ha	90 g/ha	250 g/ha	112.5 + 82.5 g/ha	125 + 125 g/ha	LSD
Septoria	15	93.9	+9.5	+10.1	+7.7	+7.1	+9.8	+10.4	1.6
Yellow rust	7	69.0	+26.9	+22.7	+18.7	+24.6	+21.5	+29.0	3.5
Brown rust	4	86.6	+14.2	+5.1	+10.7	+11.2	+13.5	+10.6	3.7
All trials	26	81.3	+ 13.8	+ 12.0	+10.4	+11.4	+13.5	+14.3	1.7
		100	117	115	113	114	117	118	2.5

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

The analyses of the different populations of *Z. tritici* revealed variable distributions of CYP51 mutations. Out of the 6 investigated CYP51 mutations, the most prolific mutation found was I381V, which was detected in more than 90% of all investigated populations (Table 5). The least frequently detected mutations were V136C and S524T. V136C was detected with a frequency of 0-34%, with the highest frequency in Central UK. 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% in all locations except Belgium (0%), Central UK (0%) and Hungary where frequencies were around twice as high as in other locations. The two mutations D134G and V136A were detected at comparable frequencies in the medium range at most localities. The exceptions were south Poland and Hungary with 0%.

EC_{50} values for the 4 azoles showed similarly major variation across the different localities. Ireland and UK had relative high values for all 4 azoles and Hungary had lower values. Other countries had a more variable picture.

Table 5 Frequence of CYP51 mutations (%) based on leaf samples from untreated plots collected at GS 65-75 and EC_{50} values for 4 main azoles. Green: no mutation/low EC_{50}. Yellow: low frequency/medium EC_{50}. Orange: Medium frequency/medium to high EC_{50}. Red: High frequency/high EC_{50}.

		Frequence of mutations (%)						EC50 (mg/l)			
Country	**Trial id.**	**D134G**	**V136A**	**V136C**	**A379G**	**I381V**	**S524T**	**Epoxi.**	**Met.**	**Tebu.**	**Prothio. -desthio**
DK2 Flak	2	17	28	0	30	91	2	0.15	0.17	4.67	0.03
DK3 Flak	3	37	43	21	14	89	1	0.28	0.13	2.11	0.04
DK4 Lolland	4	47	52	0	19	95	1	0.29	0.13	1.26	0.07
Germany JKI	12	22	24	18	16	98	8	0.45	0.26	2.84	0.09
Germany Bavaria	14	22	29	0	34	98	8	0.21	0.17	3.86	0.03
France	10	40	47	0	10	89	3	0.16	0.07	1.76	0.04
Belgium	24	62	64	28	0	94	6	0.31	0.10	0.37	0.09
Ireland	22	33	73	22	27	88	51	0.82	0.46	2.37	0.18
North UK	19	33	48	14	16	100	34	0.99	0.41	2.74	0.23
Middle UK	20	33	38	34	0	100	29	0.57	0.39	5.43	0.11
Middle UK	16	NA	NA	NA	NA	NA	NA	0.66	0.53	4.75	0.14
South UK	21	15	35	20	14	97	30	0.55	0.53	5.97	0.10
Poland, north	6	39	44	22	28	96	4	NA	NA	NA	NA
Poland, south	8	0	10	11	13	94	2	0.13	0.08	3.84	0.02
Hungary 1	25	0	0	0	50	76	0	0.05	0.05	1.61	0.01
Hungary 2	26	0	0	0	73	95	0	0.05	0.06	2.82	0.01

DISCUSSION

Data collected from 26 trials carried out in 2015 confirm that azoles still provide significant effects on major wheat diseases. For control of STB the performance is however variable accross Europe reflecting different intensitiy and historical use pattern. Variability was also identified in *Z. tritici* populations patterns of CYP51 mutations and in their sensitivity to azoles measured as EC_{50} values in *in vitro* tests.

Over the past 15 years a significant number of mutations in the CYP51 gene has emerged and been documented (Cools & Fraaije 2013; Leroux & Walker 2011). The mutations in the *Z. tritici* populations occur in combinations and the populations described in this paper reflect the overall dominance of mutations, but do not indicate how specific haplotypes are composed. Several of the specific genotypes are known to have variable impacts on particular DMIs.

Stammler et *al*. (2008) found, similarly to this study, I381V to be the most widely distributed CYP51 mutation throughout Europe (Table 4).

Danish, German and French trials had quite similar mutation frequency profiles and intermediate EC_{50} values. Hungary differed distinctly from all other locations as this country only had few mutations and low EC_{50} values for all 4 azoles probably reflecting less intensive use of azoles in this country. Ireland and UK also had unique profiles with high frequencies of S524T and the highest EC_{50} values for all 4 azoles. This confirms other findings where the mutation S524T in combination with several other mutations (V137F or V136A) has increased and reduced the sensitivity to commonly used DMI's like prothioconazole and epoxiconazole (Cools et *al*. 2013; Kildea et *al*. 2014, Leroux & Walker 2011). Belgium had high proportions of D134G and good performance of tebuconazole, which confims that haplotypes carrying D134G are more sensitive to tebuconazole, similarly confirmed with the low EC_{50} for tebuconazole. Belgium and France had low EC_{50} values for metconazole, also reflected by a better control of this active ingrediant in comparison with other azoles. The presented data confirm the importance of azoles both as single product but also as mixing partners. Although cross resistance is described for this group, the data presented clearly verifies the need for diversity in order to obtain good control and reduce the selection pressure. The trials are to be continued in 2016 covering additional geographic areas which were not included in 2015.

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