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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 treatments 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 ranking of the azoles tested 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, either as epoxiconazole + metconazole or prothioconazole + tebuconazole. This was especially clear in the western parts of Europe with high frequencies of CYP51 mutations D134G, V136C and S524T in local *Z. tritici* populations. Effectiveness of all single azoles decreased from 2015 to 2016 except for tebuconazole and azole mixtures, the mixtures providing more robust control across all sites and sensitivities. The average EC_{50} values for *Z. tritici* from the trial sites measured for the four azoles showed different levels of cross-resistance and similarly did the efficacy ranking from the azoles. 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%. The greatest yield responses were measured at the sites where yellow rust was the primary disease controlled.

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1. 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 prevent yield loss. Septoria leaf blotch (STB) caused by *Zymoseptoria tritici* is seen as the most serious leaf disease in Northern Europe (Fones and Gurr, 2015), but also yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia triticina*) are known to cause major problems depending on region and season (Jørgensen et al., 2014).

Four major fungicidal modes of action (MoA) are available for management of leaf diseases in wheat: (1) quinone outside inhibitors (QoI), (2) sterol 14α-demethylation inhibitors (DMI), often referred to as azoles, (3) succinate dehydrogenase inhibitors (SDHI) and (4) multi-site inhibitors. Amongst these, following the widespread proliferation of QoI resistance in north-European *Z. tritici* populations, target site-specific systemic fungicides such as the DMIs and SDHIs 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). The DMIs consist of azoles, which represent triazoles, the triazolinthione derivate 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 azoles are often applied 1–4 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 but in recent years major changes in the sensitivity of the populations have been observed across Europe (Dooley et al., 2016a; Stammler & Semar, 2011, Heick et al., 2017).

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: changes in the target enzyme caused by mutations in the CYP51 gene, overexpression of the CYP51 gene 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 and Fraaije, 2013). The many 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 *Z. tritici*, which have been identified, can have different sensitivities to the various DMIs (Leroux et al., 2007; Cools and Fraaije, 2013).

The changes seen in field 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 (Heick et al., 2017; Jørgensen et al., 2017). In spite of major shifts occurring in the field populations, their impact on 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 and Fraaije, 2013; Kildea, 2016; Ahdb, 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). 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 and Fraaije, 2013; Buitrago et al., 2014; Leroux and Walker, 2011).

In the current study the overall aim was to generate an updated dataset of the efficacy profiles of four azoles commonly used for control of the major foliar diseases affecting wheat across Europe. More specifically these 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 across Europe. (3) 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

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

Trt. No.	Product	l/ha	Active ingredient	g/ha (% N)
1	Untreated	–	–	–
2	Opus Max	1.5	Epoxiconazole (EPX)	125 (100%)
3		1		83.3 (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	112.5 + 82.5 (182%)
12		1.5	(EPX + MCA)	56.3 + 41.3 (91%)
13	Prosaro 250 EC	1	Tebuconazole + Prothioconazole (TCA + PTH)	125 + 125 (112%)
14		0.5		62.5 + 62.5 (56%)