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Anti-resistance strategies for fungicides against wheat pathogen *Zymoseptoria tritici* with focus on DMI fungicides

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ABSTRACT

Septoria tritici blotch (STB) caused by the fungal pathogen *Zymoseptoria tritici* is a global threat to sustainable wheat production. Applications of fungicide against STB are regarded as an essential means to minimise yield losses, however, fungicide resistance is developing and affecting fungicide efficacy greatly. Only a few fungicide classes are available for STB control. DMI fungicides are seen as the main group, but increasing problems with resistance is challenging their use. In 2015 and 2016, field trials were conducted testing fungicide spray strategies using one, two or three applications, alternations, mixtures of different DMIs and DMI mixed with other modes of action including a SDHI and a multi-site inhibitor. The strategies were tested for disease control, their impact on yield and their effect on resistance build-up, measured as CYP51 alterations responsible for decreased sensitivity and efficacy of DMIs. Strategies consisting of three treatments provided adequate control of STB and significant yield increases. The best results with regard to yield and control were attained by a diversified DMI strategy also including the SDHI boscalid and the multi-site inhibitor folpet. Spraying once or twice lowered selection yet compromised STB control and yield. CYP51 alteration I381V was the most predominated in all samples. Frequencies of alterations D134G, V136A and S524T increased significantly following applications with DMI fungicides. The more diversified a strategy, the less it selected for CYP51 alterations. EC_{50} values for epoxiconazole showed a tendency to be higher post-treatment in 2015. The results presented in this study encourage the adoption of mixing and alternating fungicides into spray strategies to minimise the risk of resistance build-up and to prolong the effective life of fungicides.

1. Introduction

Winter wheat (*Triticum aestivum*) is one of the major cereal crops in Europe. In 2014, the crop was cultivated on approximately 27 million hectares, achieving yields of seven to nine tonnes per hectare in highly intensified cropping systems, as known in north-western Europe (Anonymous, 2016b). An essential factor ensuring high yields is well-timed disease management throughout the growing season. For many years *Parastagonospora nodorum* was the dominant disease in winter wheat in Europe (Bearchell et al., 2005). Since the early 1980s, the ascomycete *Zymoseptoria tritici* (*Z. tritici*) causing septoria tritici blotch (STB) has taken over and is now regarded as the most important disease in wheat (O'Driscoll et al., 2014) with yield losses in the range of 10–50% depending on the region and yearly disease pressure. Several agronomical practices e.g. late sowing and planting of resistant cultivars (Thomas et al., 1989; Gladders et al., 2001; Brown et al., 2015) have shown some potential keeping disease levels low and minimising epidemics. In most years, however, STB management is heavily reliant on frequent fungicide applications (O'Driscoll et al., 2014).

In north-western Europe, two to four fungicide sprays are commonly applied to winter wheat for the control of fungal diseases such as STB (Jørgensen et al., 2008; Fones and Gurr, 2015). For disease management of STB in winter wheat, compounds belonging to four fungicide classes are available in Europe: (1) quinone outside inhibitors (QoIs), (2) sterol 14α-demethylation inhibitors (DMIs), (3) succinate dehydrogenase inhibitors (SDHIs) and (4) multi-site inhibitors. For many years now, DMI fungicides epoxiconazole and prothioconazole have been the most widely used fungicides for STB control throughout Europe. In recent years, declining field efficacies have been observed for both fungicides (Anonymous, 2014; Wieczorek et al., 2016) which raises major concern of a total loss of efficacy of this important fungicide class.

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Fungicide resistance to DMIs has been associated with three molecular mechanisms: (1) mutations in the DMI target gene *CYP51* causing amino acid alterations in the CYP51 enzyme (Cools and Fraaije, 2013), (2) overexpression of the target gene *CYP51* (Chassot et al., 2008; Cools et al., 2012) and (3) an enhanced efflux of the cell (Omrane et al., 2015). Some of these CYP51 alterations have shown to affect the performance of specific DMIs e.g. I381V for tebuconazole (Fraaije et al., 2007); others show a more broad effect on the efficacy of several DMIs (Mullins et al., 2011). All three mechanisms can occur in combination in a single strain at the same time, rendering it difficult to control (Kirikyali et al., 2017). In the northern European *Z. tritici* population, alterations of the CYP51 contribute the most to reduced sensitivity to DMIs, whereas overexpression and enhanced efflux are rare (Heick et al., 2017). The rapid development and spread of resistant strains are a real threat to wheat production, however, yearly efficacy testing of fungicide compounds carried out in most European countries shows that DMI fungicides still provide significant control of STB in the field (Jørgensen et al., 2017).

The evolution of fungicide resistance in *Z. tritici* poses a severe threat to profitability and sustainability of wheat production worldwide. Numerous studies have identified the major drivers for fungicide resistance and how to mitigate them in anti-fungicide-resistance management strategies in theory (van den Bosch et al., 2014; Hobbelen et al. 2010, 2011a, 2013, 2014, 2011b, van den Bosch et al., 2011; Grimmer et al., 2014). Key principles of anti-resistance strategies without compromising disease control include the optimal application timing, the adjustment of the number of applications, the appropriate dose and the application of fungicide mixtures and/or alterations of active ingredients with different modes of action (MoAs) (van den Bosch et al., 2014; Dooley et al., 2016b; van den Berg et al., 2013; Mavroeidi and Shaw, 2006). All principles should be taken into account when designing a practicable anti-resistance strategy. In addition, the sensitivity status of the pathogen population is another important factor that can influence the success of any strategy. Van den Bosch et al. (2011) defined three phases of fungicide resistance evolution: an initial 'emergence phase', in which a resistant strain arises for the first time by spontaneous mutation or invasion from another population. Followed by a 'selection phase', in which the resistant strain is present in the population and increases in frequency over time due to selection pressure imposed by fungicide applications. In the final 'adjustment phase', the resistant strain has established itself and accounts for a large proportion of the population. According to the authors, anti-resistance strategy can only be successful when employed in the 'selection phase'; a prevention of the rise of a resistant strain is impossible and once established in a population, resistant strains can only be managed to some degree by agronomical practices (van den Bosch et al., 2011).

There is an urge to extend the effective life of those active ingredients that are available at the moment. Further decline in efficacy, new restricted criteria for the registration of new active ingredients imposed by authorities (endocrine disruptors; hazard vs. risk) and the development of highly resistant pathogens will constrain the current situation further (Hillocks, 2012; Jess et al., 2014).

There is a general lack of applied studies showing the effect of anti-fungicide-resistance strategies in practice, and only a few studies have been published measuring the effect of spray strategies in the field (Dooley et al., 2016b, 2016c). In this work we use the Danish disease management situation, i.e. a limited armoury of fungicides and a moderately DMI-resistant *Z. tritici* population (Heick et al., 2017), as starting point to assess the following hypotheses: (1) a limited spectrum of fungicides provides good control against *Z. tritici*; (2) Diversified spray strategies, i.e. using fungicide mixtures and alteration of fungicides, counteract the selection of CYP51 alterations, and thus selection for DMI insensitivity. To test these hypotheses, field trials were carried out testing commercially available fungicides including DMIs, a SDHI, a multisite inhibitor and the biofungicide *Bacillus subtilis* (*B. subtilis*), in different combinations and at different sites. The effect of the different fungicide strategies on CYP51 alterations was assessed by pyrosequencing and qPCR.

2. Material and methods

2.1. Trial design and fungicide application

A total of four field trials was conducted during 2014/15 and 2015/16 at two locations in Denmark (Table 1). All trials were laid out as complete randomised block design with four replicates containing nine treatments and an untreated control. Plot size was 12.5–22 m^2. Table 2 gives an overview of the six products and their active ingredients used in this study. Table 3 shows the different treatments and their timings; growth stage (GS) 31–32, GS 37–39 and GS 59–65 (Zadoks et al., 1974). Application rates were half the label rate, as commonly recommended in Denmark. All fungicides were applied using a plot sprayer in 150 L ha^{-1} water at low pressure with flat fan nozzles. With respect to other management issues the crop was treated using standard cultural practices.

2.2. Disease and yield assessments

Zymoseptoria tritici developed naturally at all sites and was the dominating foliar disease in 2015 and 2016. In both years, disease severity was medium to severe. Field trials at the Flakkebjerg site were irrigated to avoid drought and to promote disease development during a dry period from May to June in both years. Foliar diseases were assessed several times during the season as per cent diseased leaf area on flag leaf and 2nd leaf. Disease assessments at GS 75 and GS 77, the latter as per cent green leaf area (GLA), are regarded as the key assessments for statistical analyses. No other diseases were present and influenced the results in the four trials. All trials were harvested and yield (adjusted to 15% moisture content) and yield increase (both dt ha^{-1}) were measured.

Table 1
Trial site details, cultivar information, dates and growth stage (GS), at which fungicide applications and disease assessments took place.

Site		Year	Cultivar	Susceptibility	Attack of STB	First application		Second application		Third application		Disease assessment	
Name	Coordinates (lat, lon)			to STB[a]		Date	GS	Date	GS	Date	GS	Date	GS
Flakkebjerg	55.326724, 11.390078	2014/15	Hereford	very susceptible	moderate	24-04-2015	31	06-05-2015	33	04-06-2015	51	11-07-2015	75
Hadsten	56.323629, 10.101383	2014/15	Nakskov	moderate	high	22-04-2015	31	22-05-2015	37	16-06-2015	59	16-07-2015	75
Flakkebjerg	55.331065, 11.384772	2015/16	Hereford	very susceptible	high	04-05-2016	32	19-05-2016	37	07-06-2016	65	09-07-2016	75
Horsens	55.858976, 9.756632	2015/16	Hereford	very susceptible	high	01-05-2016	32	18-05-2016	37	06-06-2016	55	07-07-2016	75

[a] www.sortsinfo.dk.