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Biological control of yellow rust of wheat (*Puccinia striiformis*) with Serenade®ASO (*Bacillus subtilis* strain QST713)

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ABSTRACT

Yellow rust (*Puccinia striiformis* f. sp. *tritici*) is an important disease in wheat causing significant yield reductions, if not effectively controlled. The biofungicide *Bacillus subtilis* strain QST 713 suspension concentrate (Serenade®ASO) was investigated for its potential for yellow rust control in winter wheat field trials. Serenade®ASO reduced severity of yellow rust significantly, providing up to 60% control at BBCH growth stage 65–69, under moderate disease pressure. Under high disease pressure reductions were more variable and provided less than 30% control. An increase in the number of applications of biofungicide from two to four per season tended to improve disease control, although differences were not always significant. With a few exceptions no clear dose-response was seen between using 1, 2, 4, 6 or 8 l/ha applied 4 times at 8–10-day intervals. Yield responses were positive, but responses to biofungicide were only significant in a few cases, and in all cases the level of control and yield responses were significantly lower compared with using prothioconazole as chemical control. An outdoor pot trial using artificial inoculation tested preventive and curative application of Serenade®ASO at three dose rates. This trial confirmed the lack of a clear dose response but showed that timing had a major impact on control, with the best control obtained at the day of inoculation. This study revealed that Serenade®ASO cannot stand alone in the control of yellow rust. More research is needed to develop integrated disease management strategies which also include biofungicides.

1. Introduction

Wheat is a dominant cereal crop worldwide and very important as a staple food resource. Multiple diseases can attack the crop and the disease yellow rust caused by *Puccinia striiformis f. sp. tritici* is seen as one of the major threats to wheat production. *P. striiformis* is a biotroph pathogen on wheat commonly found in cooler and wetter regions including Asia, North America, Australia and Europe. It can cause yield reductions between 5 and 50% depending on the year, region and developmental stage of wheat at which the attack occurs (Singh et al., 2015). Following significant epidemics major economic losses have been measured in Europe, Australia and the US (Beddow et al., 2015; Murray and Brennan, 2009).

Yellow rusts have been a major focus for research and breeding due to the ability of the fungi to overcome race-specific resistance genes within a few years, causing major changes in pattern of epidemics and subsequent yield losses. Traditionally, yellow rust has been less prevalent than other wheat diseases in many countries due to efficient resistance in commonly grown cultivars. However, the dynamics of the *P. striiformis* pathogen to generate new races has caused sudden epidemics in cultivars previously regarded as resistant, and particularly since 2010 detection and fast spread of new aggressive races have caused severe losses (Hovmøller et al., 2015; Wellings et al., 2012). This development has increased the pressure on producing new resistant cultivars in order to manage yellow rust, but it has also put pressure on other control measures such as improved cropping practice and use of fungicides. In case of outbreaks of yellow rust, fungicide treatments are usually recommended as soon as the disease is detected in the field in order to prevent a severe epidemic (Jørgensen et al., 2014). Several fungicides belonging to the chemical groups of triazoles, strobilurins and SDHI's are known to be effective against yellow rust. If treatment is applied at the very early stages of attack, reduced dose rates can be applied and provide good control of yellow rust, whereas delayed treatments have proved to be less cost effective (Jørgensen and Nielsen, 1994).

Fortunately, no evidence of fungicide resistance in *P. striiformis*

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has been found so far, but the risk of development of resistance can not be ruled out due to intense use of fungicides over decades (Oliver, 2014). Chemical control relies on few modes of action, which may increase selection pressure and eventually lead to resistance development. This calls for investigating alternative control measures including biological control products, which are also generally seen as more environmentally sound solutions.

Bacillus subtilis is a rhizobacterium that can form endospores and can produce several different antibiotics (Stein, 2005). These are primarily formed during endospore formation in low concentrations, and there has been some uncertainty as to whether disease control is directly linked to the action of antibiotics (Kilian et al., 2000; Leifert et al., 1995). The mode of action of *Bacillus* species is described by Frac and Jezierska-Tys (2010) as microbial disrupters of pathogen cell membranes. Some of the effect may be indirect as some findings suggest that *B. subtilis* also has the ability to induce plant mediated resistance in the host plant (Ongena et al., 2007).

Serenade®ASO is a broad-spectrum biofungicide that contains *Bacillus subtilis* strain QST 713 and has been approved for use in the European Union (Reg. (EC) No 839/2008). Its worldwide utilisation covers all kinds of fungal diseases in diverse crops (Fischer et al., 2013). The fungicide was registered in Europe targeting mainly *Botrytis cinerea* on outdoor grown lettuce and strawberries, aubergine/eggplant, tomato and paprika in greenhouses and *Erysiphe heracle* and *Alternaria dauci* on carrot. The effectiveness of the related endophytic *Bacillus subtilis* strain E1R-j for the control of yellow rust has previously been reported (Li et al., 2013); however, no studies have been conducted on yellow rust control by the *Bacillus subtilis* strain QST 713. The objective of this study was to i) evaluate the efficacy and consistency of *B. subtilis* QST 713 against yellow rust under field conditions, ii) investigate the dose-response relationship both under field and semi-field conditions and iii) identify the importance of timing and intensity when applying *B. subtilis* QST 713 for control of yellow rust.

2. Materials and methods

2.1. Field trials

A total of four field trials were conducted in winter wheat at Flakkebjerg research station (55.3253 N 11.3913 W) on a fine clay loam soil in the growing season 2013/14 and 2014/15. Disease developed naturally during both seasons and severity was regarded as moderate in 2014 and severe in 2015. The weather in both seasons started with mild winters giving good possibilities for inoculum of yellow rust to survive the winter. In 2014 the disease developed from late April and gave rise to moderate levels of disease. In 2015 yellow rust was established already in the autumn and further development started already in February. These early attacks led to very high and significant infections in susceptible crops in 2015. An overview of the weather conditions can be found in the supplementary material. The four independent trials were conducted using the susceptible cultivars Ambition and Baltimor in 2014 and Ambition and Substance in 2015. The experimental set-up was a completely randomised block design with four replicates, a plot size of 22.5 m^2 (9 m length and 2.5 m width) and with 25 cm space between the plots. Plots were sown with a plot sowing machine at 2–4 cm depth aiming at 400 seeds per m^2. Apart from fungal treatment crop management was conducted according to common crop practice. Chemical control was performed with prothioconazole 250 g/l, (Proline EC250, Bayer CropScience) and the biofungicide used was *B. subtilis* QST 713 10^{12} colony forming units per l (CFU/l), (Serenade®ASO, Bayer CropScience). The products were applied with a self propelled sprayer (Speedy 2500) operating at a speed of 4.5 km/h, and a boom height of 40 cm. The boom was fitted with Teejet 9504 nozzles, operating at a pressure of 2.4 bar and delivering a volume rate of 150 l/ha. Fungicide applications included six treatments plus an untreated control and four treatments plus untreated control in 2014 and 2015, respectively (Table 1). The number of included doses was reduced in 2015 due to the limited dose-response seen in 2014. Treatments and assessments followed a similar schedule in both years (Table 2). Disease assessment was carried out visually as percentage of yellow rust coverage of green leaves evaluated at specific leaf layers at intervals of ten days, starting at the first application and finishing at senescence, following European plant protection standards (EPPO/OEPP (2012) PP 1/26(4)). For the statistical analyses of disease severity, three representative time points (BBCH (Lancashire et al. (1991)) 39, 51 and 69 in 2014 and BBCH 33, 49 and 65 in 2015) were chosen to illustrate the performances of the treatments (Table 2).

The trials were harvested using a plot combine harvester. Yield responses in t/ha were adjusted to 15% moisture.

2.2. Semi-field trial

The pot trial was conducted in a covered outdoor area in 8 l pots (semi-field) in 2015. Each pot was watered individually with an automatic drip irrigation system and temperature conditions were similar to the ones described for the field trials. Twenty seeds per pot of the spring wheat variety Trappe, known for its susceptibility to *P. striiformis*, were sown in each pot. A spring wheat cultivar was chosen as it does not need a vernalisation period. *P. striiformis*

Table 1
Description of treatments used in the four field trials. Replicate trials in 2014 and 2015 followed the same protocol.

Year	Treatment	Product[a]	Application rate (l/ha)	Application time points[b]
2014	1	Untreated		
2014	2	Chemical control	0.8	AD
2014	3	Biofungicide	1	AD and ABCD
2014	4	Biofungicide	2	AD and ABCD
2014	5	Biofungicide	4	AD and ABCD
2014	6	Biofungicide	6	AD and ABCD
2014	7	Biofungicide	8	AD and ABCD
2015	1	Untreated		
2015	2	Chemical control	0.8	AD
2015	3	Biofungicide	4	ABCD
2015	4	Biofungicide	6	ABCD
2015	5	Biofungicide	8	ABCD

[a] Chemical control: 250 g/l prothioconazole (Proline EC250, Bayer CropScience); Biofungicide: 10^{12} CFU/l, *B. subtilis* QST 713 (Serenade®ASO, Bayer CropScience).
[b] Dates of application time points (A to D) are given in Table 2.