Effect of foliar-applied elemental sulphur on Fusarium infections in barley

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Abstract

Infection of cereals by Fusarium species is critical as Fusarium head blight yields regularly contamination of grains with mycotoxins, which is a serious threat to food safety. The efficacy of fungicides against Fusarium head blight is not complete and no alternative agro-technical measure proved to be adequate on its own to control infections by the pathogen, too. Elemental sulphur is a well-proven fungicide against various plant diseases. This is probably the first time that the effect of repeated elemental sulphur application on ear infections by *Fusarium culmorum* after artificial inoculation of the pathogen has been studied under field conditions. Macroscopic symptoms of Fusarium head blight were first visible 25 days after inoculation. Infection with *F. culmorum* had no influence on infection severity at any time. Weekly foliar applications of elemental sulphur from the start of anthesis yielded a reduction of the infection rate with Fusarium head blight by up to 32 %. Under high infection pressure, the application of elemental sulphur resulted in 13 % higher grain yields. The effectiveness of elemental sulphur proved to be comparable to other agro-technical measures.

Keywords: Barley, elemental sulphur, Fusarium culmorum, Fusarium head blight, infection rate

Zusammenfassung

Einfluss blattapplizierten Elementarschwefels auf den Befall von Gerste mit Fusarium

Die Infektion von Getreide mit Fusariosen ist kritisch, da diese regelmäßig mit einer Kontamination des Korns mit Mykotoxinen verbunden ist, was wiederum eine ernsthafte Gefährdung der Lebensmittelsicherheit darstellt. Der Einsatz von Fungiziden gewährleistet keinen vollständigen Schutz gegen Infektionen und auch kein anderes, alternatives agrotechnisches Verfahren erwies sich in seiner Wirksamkeit als ausreichend, um die Krankheit zu kontrollieren. Elementarschwefel ist ein erprobtes Fungizid gegen verschiedene Pflanzenkrankheiten. Die Untersuchungen zeigen vermutlich erstmals den Einfluss regelmäßiger Elementarschwefel-Applikationen auf Ähreninfektionen mit *Fusarium culmorum* nach künstlicher Inokulation des Schaderregers unter Feldbedingungen. Makroskopisch sichtbar wurden Ähreninfektionen frühestens 25 Tage nach Inokulation des Pathogens. Die Infektion mit *F. culmorum* hatte zu keinem Zeitpunkt einen Einfluss auf die Intensität des Befalls. Wöchentliche Applikationen von Elementarschwefel als Suspension ab Blühbeginn reduzierten die Infektionsrate der Ähren um bis zu 32 %. Parallel wurde der Ertrag bei hohem Befallsdruck durch den Einsatz von Elementarschwefel um 13 % gesteigert. Die Wirksamkeit von Elementarschwefel war vergleichbar mit anderen agrotechnischen Verfahren.

Schlüsselwörter: Ährenfusariose, Elementarschwefel, Fusarium culmorum, Infektionsrate

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1 Introduction

Fusarium head blight is a fungal disease in cereals, which is caused mainly by infection with Fusarium species. Hereby, the pathogenecity of *F. culmorum*, *F. graminearum* and *F. poa* is equal in barley and wheat (Hudec, 2006). Infection of agricultural crops by Fusarium species results in the contamination of foodstuff with mycotoxins such as deoxynivalenol (DON). Results with view to the efficiency of fungicide applications to protect the plant against Fusarium head blight infections are contradictory (D'Mello et al., 1998). Beyer et al. (2006) calculated a reduction of Fusarium infections of on average by 47 % after application of triazole fungicides at anthesis. Sub-lethal doses may, however, result in an increased production of mycotoxins (D'Mello et al., 1998). Various alternative approaches have been tested to reduce the DON content after infection of cereals with Fusarium species such as soil tillage operations, plant cultivation techniques including crop rotation and plant density and fertiliser practices, however, with diverging results (Beyer et al., 2006; Heier et al., 2005; Schaafsma and Tamburic-Ilinic, 2005). Cultivation of resistant varieties provides so far the best option for a low contamination of the harvest product compared to other approaches (Beyer et al., 2006; Koch et al., 2006).

Interactions between mineral elements and plant diseases are well known for essential macro and micro plant nutrients, and aluminium and silicon. The term Sulphur Induced Resistance (SIR) denotes the reinforcement of the natural resistance of plants against fungal pathogens through triggering the stimulation of metabolic processes involving sulphur by targeted sulphate-based and soil-applied fertiliser strategies (Haneklaus et al., 2007). Mechanisms involved in SIR involve those discussed for elemental sulphur (S^0) such as direct toxicity and toxicity of its reduction product hydrogen sulphide (Haneklaus et al., 2007). The fungicidal effect of foliar-applied S^0 has been exploited since the end of the nineteenth century (Hoy, 1987). S^0 proved to be effective against rust and powdery mildew (Coleno, 1987; Cook, 1987; Hoy, 1987; Bourbos et al., 2000; Reuveni, 2001), but was also successfully used against other diseases such as downy mildew in cereals (Hoy, 1987), common scab of potato (Vlitos and Hooker, 1951; Mortvedt et al., 1963) and Alternaria black spot of oilseed rape (Anon, 1988). So far, no information is available about the influence of foliar-applied S^0 on infections by Fusarium head blight. It was the aim of the presented investigations to determine the influence of repeated applications of S^0 on infection rate and severity of barley inoculated with *F. culmorum* under field conditions.

2 Materials and methods

In 2006, a field trial was conducted at the experimental station of the Federal Agricultural Research Centre (FAL) in Braunschweig (E 10°27′, N 52°18′). The climate is temperate and characterised by frequent changes in temperature, humidity, and winds. During experimentation the mean temperature and mean sunshine hours per month were 8.8° C and 151 hours. Precipitation varied between 15.8 and 54.8 mm per month with a mean value of 37 mm. The long-term mean annual precipitation is 620 mm. Values for relative humidity ranged from 60.4 to 89.6 % (mean 79 %). The soil type is a Cambisol with a loamy sand soil texture (6.5 % clay; 47 % sand), characterised by low water retention capacity and high rates of leaching. The soil pH value was 5.5.

Winter barley (cultivar *Theresa*) was sown on September 20, 2005. Seed density was 350 grains m^{-2}. S was soil-applied as sulphate at a rate of 15 kg ha^{-1} as Korn-Kali before sowing and 50 kg ha^{-1} together with the first nitrogen rate at start of the vegetation period (BBCH 25 according to Strauss et al. (1994)) and start of stem elongation (BBCH 30) in spring as ammonium sulphate saltpetre to warrant a sufficient S supply of the crop. In total, 190 kg ha^{-1} N was applied in three rates at BBCH 25, 30 and 69.

Plots were arranged in a split-block design. Each block had a size of 120 m^2. Two factors (foliar-applied S^0 and inoculation with *F. culmorum*) with two treatments (with and without S^0 and inoculation, respectively) were tested. In this experiment, S^0 was foliar-applied weekly at rates of 4.8 kg ha^{-1} S^0 in form of a suspension (1 kg S^0 in 62.5 l water) using Kumulus^R with 80 % S^0 and starting one day before inoculation with *F. culmorum* at BBCH 63. S^0 was applied in total five times, the last application being administered four weeks before harvest.

Plants were inoculated with *F. culmorum* (isolates 36 - 38) at BBCH 63 and 65 by spraying 1 l ha^{-1} of a solution containing ≥ 400,000 conidiospores ml^{-1}.

Visual rating of infections by Fusarium head blight was carried out weekly with the start of anthesis (BBCH 63). From each block, in total 20 ears from 5 locations were taken for scoring from an area of about 1 - 2 m^2 at different samplings. At harvest (BBCH 90) no Fusarium damaged kernels were found for the different treatments.

The infection rate was calculated by the following formula:

$$IR\ (\%) = \left(\frac{A}{B}\right) * 100$$

with IR (%) = Infection Rate (%); A = number of infected spikelets/sections of ears; B = total number of ears being rated.

For the determination of the infection severity a visual scoring was carried out on a scale from 1 to 5 according to Anon (1974):

1 = No symptoms
2 = Bleaching of single spikelets (1 – 25 % of ear infected)
3 = Bleaching of complete sections of ear (26 – 50 % of ear infected)
4 = Bleaching of complete sections of ear (51 – 75 % of ear infected)
5 = Bleaching of complete sections of ear (76 – 100 % of ear infected)

Grain and straw yield was determined by middle plot threshing (BBCH 90). Grain was dried at 30 °C and straw at 60 °C until constancy of weight. Grain yield refers to a dry matter content of 86 %.

The experimental data were analysed with the GLM procedure of Cohort, version 3.034. Tukey's HSD test was applied to ascertain any significant differences between group means. Limits of significance for all critical ranges were set at $P < 0.05$.

3 Results and discussion

S^0 is both, an approved fungicide and an approved fertiliser. A particular advantage of a foliar application of S^0 from nutritional point of view is its continuous, slow oxidation to sulphate, followed by a continuous uptake of sulphate and utilisation in plants (Schnug et al., 1998). In comparison, foliar sulphate applications yielded a rapid and strong uptake, and immobilisation of sulphate in the vacuoles (Schnug et al., 1998). In numerous field experiments the use of S^0 yielded better results than a sulphate fertiliser (Klikocka et al., 2005). The reason was the fungicidal next to its nutritional effect.

In the present field experiment, no visible symptoms of Fusarium head blight were visible before, 6 and 14 days after inoculation. Inoculation of winter barley with *F. culmorum* resulted in visible symptoms of infection earliest 25 days after inoculation (Figure 1).

Figure 1:
Spikelets of winter barley infected by *F. culmorum*

Infection with Fusarium blight head never affected more than 25 % of the spikelets so that there was no variation in the disease severity according to the categories set up by Anon (1974). The fact that inoculation with the pathogen did not result in a more severe infection might be related to the choice of test crop as barley is less susceptible than winter wheat because of its compact flowering and earlier ripening; additionally, relative humidity of only 60 - 70 % from May to July was supposedly sub-optimum for germination and further development of *F. culmorum*.

Grain yield was reduced on average by 13 % in the inoculated plots if no S^0 was applied (Table 1). Grain yield was still 5 % higher in plots that were not inoculated, but received S^0 (Table 1). It can be assumed that the effect of S^0 in this experiment was explicitly fungicidal as the S supply of the crop was provided for by soil applied sulphate applications.

The main effects of S^0 application and inoculation with *F. culmorum* on visible infections of spikelets were quantified and results of the ANOVA are summarised in Table 2.

Table 1:
Influence of foliar S^0 application and inoculation with *F. culmorum* on infection rate (%) of winter barley with Fusarium head blight three and four weeks after inoculation and yield parameters

Weekly S^0 application[1]	Inoculation with *F. culmorum*	Infection Rate (%) BBCH 73	Infection Rate (%) BBCH 77	Grain yield (dt ha^{-1})	Straw yield (dt ha^{-1})
without	without	35	48	72.9	43.4
without	with	34	58	65.2	36.4
with	without	28	37	76.6	43.4
with	with	19	38	74.6	47.2

[1]first S^0 application one week before inoculation

Table 2:

Relative quantification of main effects (%) and statistical significances (F-test) for the influence of S^0 application and inoculation with *F. culmorum* on infection rate of winter barley with Fusarium head blight three and four weeks after inoculation

Source of variance	Infection rate at BBCH 73	Infection rate at BBCH 77
Weekly S^0 application ($+S^0/-S^0$)	-32 *	-29 **
Inoculation with *F. culmorum* (+I/-I)	+16 ns	-13 ns
S^0 application * Inoculation	ns	ns

Repeated, weekly S^0 application, in total five, reduced the infection rate with *F. culmorum* by about 30 % (Table 2). Inoculation with *F. culmorum* did not significantly influence the infection rate of winter barley (Table 2). A tendency towards higher infection rates in plots that had been inoculated before was found irrespective of the S^0 application only at BBCH 77 (Table 1). Though spraying of the pathogen was handled carefully, propagation of the conidiospores thereafter by wind cannot be excluded.

The efficacy of S^0 to reduce the infection rate under high infection pressure was comparable to that of soil tillage operations and crop rotation on the DON content (Beyer et al., 2006). Relevant is in this context that visual rating of infection can not substitute the measurement of DON as there seems to be no general relationship between both parameters (Paul et al., 2005). This means that further research is required to quantify the influence of repeated S^0 applications on the DON content of grain.

The efficacy of S^0 against Fusarium head blight was either related to the direct toxicity of S^0 or that of its reduction product hydrogen sulphide outside the fungal hyphae, or reduction of S^0 to hydrogen sulphide after entering the fungal cell (Boerner, 1997). In previous experiments it was shown that sulphur dioxide had no effect on the pathogen (Mansfield et al., 1991).

4 Conclusions

The results of the presented study, which investigated the influence of repeated foliar S^0 applications on infection rate of barley with Fusarium head blight, previously inoculated with *F. culmorum*, revealed that this traditional fungicide seems to be effective against this pathogen, too. Further research is required to verify the results obtained for other cereal crops, under natural field conditions and last but not least for varieties that differ in their resistance against *F. culmorum*. Additionally, rates and frequency of S^0 applications which are required for yielding a significant fungitoxic effect need to be elaborated.

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