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# The potential of sulfur induced resistance against plant diseases of oilseed rape

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**Abstract**

The contribution of essential plant nutrients to resistance against diseases saw some interest in the past, but research in this field has been limited by its complexity and its practical significance unrecognized due to the availability of effective pesticides. The term Sulfur Induced Resistance (SIR) denotes the reinforcement of the natural resistance of plants against fungal pathogens through triggering the stimulation of metabolic processes involving sulfur by targeted sulfate-based and soil-applied fertilizer strategies. The disease index, calculated from infection rate and infection severity, was reduced by sulfur fertilization up to 50% in greenhouse and 35% in field experiments. The sulfur metabolism in plants offers several options to combat fungal attacks. Plants emit sulfur-containing volatiles such as $H_2S$ which is commonly rated as being fungitoxic. So far, however, dose/effect measurements were non-existent and first measurements revealed that basidiomycetes such as *Rhizoctonia* ssp. metabolize $H_2S$ up to concentrations of 20 µl $l^{-1}$ $H_2S$ within 16 h, while growth of *Sclerotinia sclerotiorum* was inhibited by the same concentration after 24 h. Other mechanisms involved in defense from biotic stress that are provided by the S metabolism of plants involve glutathione and here the glutathione/ascorbate cycle, cysteine, phytoalexins and glucosinolates. The results from comparative field experiments in northern Germany and Scotland revealed that sulfur fertilization significantly increased the total sulfur, cysteine and glutathione content on a sulfur deficient site. Infections with light leaf spot (*Pyrenopeziza brassicae*), stem canker (*Leptosphaeria maculans*) and downy mildew (*Peronospora parasitica*) were the most severe diseases in Scotland. A significant increase in the cysteine and glutathione content was found in visually infected (*P. brassicae*) leaf tissue in northern Germany, while the same oilseed rape cultivars showed a 2.5-fold and 1.6-fold decrease in these metabolites in Scotland. An increased concentration of these components reveals enhanced defense mechanisms, while a reduction may indicate that these were either consumed for defense, or being the result of a successful establishment of the pathogen. Research in SIR revealed that there are sulfur-based crop and pathogen specific resistance mechanisms. A model is presented, which reflects chronologically synthesis of sulfur metabolites and related biochemical pathways which are putatively triggered by SIR in oilseed rape.

**Key words:** Elemental sulfur, $H_2S$, *Sclerotinia sclerotiorum*, sulfur fertilization

## Introduction

In 2005, 8,176 t of fungicides were consumed on conventional farms in Germany (Anon, 2006). Sustainable agricultural production should take advantage of any attempt to exploit procedures of disease control, which are ecologically sound. The targeted use of mineral nutrients offers such a possibility to enhance resistance against pathogens. Here, the direct toxicity of nutrients (elemental S, Cu) and indirect impairment by minerals (Si) needs to be distinguished from nutrient-mediated, resistance mechanisms, which were observed for all essential macro and micronutrients, Si and Al (Datnoff et al., 2006). Schnug (1997) predicted that the decline in atmospheric S depositions and S nutritional status of agricultural crops since the mid 1980s might have serious consequences for the stability of current ecosystems and in this regard referred to the advancing susceptibility of oilseed rape against fungal pathogens such as light leaf spot (*Pyrenopeziza brassicae*). S fertilization yielded a reduction of the disease index for various host/pathogen relationships from 5 - 50% and 17 - 35% in greenhouse and field experiments, respectively (Haneklaus et al., 2006a). The term Sulfur Induced Resistance (SIR) denotes the reinforcement of the natural resistance of plants against fungal pathogens through triggering the stimulation of metabolic processes involving S by targeted sulfate-based and soil-applied fertilizer strategies (Haneklaus et al., 2006b). Research in SIR revealed that there are S-based, pathogen-specific crop resistance mechanisms. The S metabolism in plants offers several options to combat fungal attacks, for instance by the emission of $H_2S$ which is commonly rated as being fungitoxic. It is the aim of this contribution to address the significance of different S metabolites in disease resistance during pathogenesis with special view to the possible role of $H_2S$.

## Materials and Methods

*$H_2S$ fumigation experiment.* The influence of $H_2S$ fumigation on mycelial growth of *Sclerotinia sclerotiorum* (Libert) De Bary, isolate 1.1 collected from oilseed rape in Biestow in 1997 was tested in relation to the concentration of the $H_2S$ concentration and duration of fumigation. The isolate was grown on potato dextrose agar (PDA) and kept at 4° C for 24 h before fumigation. *S. sclerotiorum* mycelium was then transferred to 150 l stainless steel cabinets with a polycarbonate top

(Buchner et al., 2004). The temperature inside the chamber was maintained at 20 ± 2° C day and night with a relative humidity of 90 ± 5%. The air exchange in the chamber was 300 l $h^{-1}$. *S. sclerotiorum* mycelium was exposed to 0, 4, 10 and 20 µl $l^{-1}$ $H_2S$ as described by Buchner et al. (2004) for 24 and 48 h. The vitality of the pathogen was determined by measuring the colony growth (cm) before fumigation (0 h) and growth increments (cm) after 24h and 48 h. Each treatment had 5 replicates.

*In situ soil experiment.* The soil material used for the experiment was a glacial fluvial sand, predominantly silty-loamy sand. The soil was autoclaved at 121° C for 2 hours and 2 g of it was put into 20 ml glass tubes. *Rhizoctonia solani* (Kühn) R. Schneider IMB 11662 purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig) was grown on PDA. From 5 days old *R. solani* colonies an area of 0.8 $cm^2$ was transferred to inoculate each soil sample. S was applied as water disperse elemental S (Kumulus®) and gypsum ($CaSO_4 \cdot 2H_2O$) at a rate of 30 mg S $g^{-1}$ soil. Thiobacilli were applied either on their own or in combination with the S fertilizers. Thiobacilli were extracted from soil (1:1, soil: distilled water, v/v), which contained a thiobacilli population number of 45,750 $g^{-1}$ soil. From this suspension 0.15 ml were applied to the soil in combination with the S application. The soil moisture content was adjusted to 60% of the water holding capacity during the whole incubation period. Glass tubes were sealed with parafilm in order to warrant aerobic conditions. The moisture content was maintained by adding daily adequate amounts of distilled water. Each treatment had 5 replicates. The dehydrogenase and alkaline phosphatase activity were determined directly after uniform homogenization of the soil material with the fungal mycelium, S fertilizer and thiobacilli and after 2, 4 and 6 days of inoculation. Analytical procedures for the determination of the enzymatic activities were according to Yang et al. (2006).

*Statistical analysis.* The statistical analysis was performed by the General Linear Model (GLM) procedure employing the LSD test ($\alpha$=0.05).

## Results and Discussion

Research in SIR indicates that underlying mechanisms and pathways are related to the host/pathogen relationship. For the following S metabolites an involvement in resistance against fungal pathogens was found: total S, cysteine, glutathione, PR-proteins, phytoalexins and glucosinolates. The influence of S fertilization on selected S-containing metabolites that are linked to resistance appearances in relation to fungal infections with *Pyrenopeziza brassicae* was tested in long-term field experiments with oilseed rape in northern Germany and Scotland from 2001 to 2006 (Bloem et al., 2007). S fertilization significantly increased the total S, cysteine, glutathione (GSH) and glucosinolate (GSL) content in the leaf tissue of oilseed rape at stem extension. Visible infections with *P. brassicae* resulted in a significant increase of the cysteine content, whereby S fertilization yielded an extra increase. With cysteine being the precursor of all other S metabolites this might be an important key to SIR. At the same time the glutathione content was significantly reduced in the infected leaf tissue, whereby S fertilization weakened this effect. Infection-related changes in the GSH content are obviously related to different phases in the host/pathogen relationship. Infections with *P. brassicae* significantly increased the L-cysteine desulfhydrase activity (Bloem et al., 2004). Bloem et al. (2007) could show that S fertilization yielded an increased emission of $H_2S$ emission of oilseed rape in the field. In experiments with grape plants during the initial phase of infections by *Uncinula necator* when symptoms were yet not visible plants released elevated levels of $H_2S$; this effect seems to be independent of the S supply (Bloem et al., 2007).

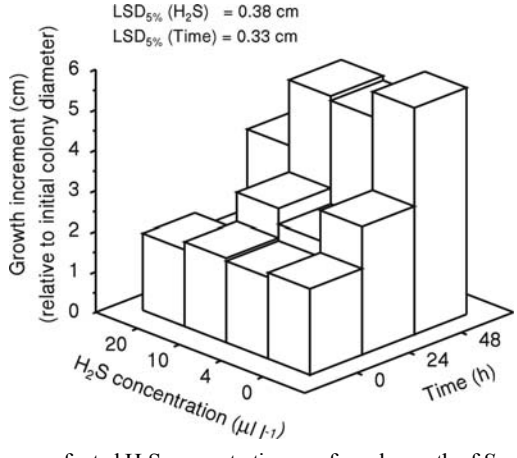


Fig. 1. Influence of rated $H_2S$ concentrations on fungal growth of *S. sclerotiorum*.

$H_2S$ is presumably highly fungitoxic (Beauchamp et al., 1984, Carlile et al. 2004), but no critical values are available for $H_2S$ concentrations and duration of fumigation in relation to different pathogens. Fumigation experiments showed that even with a very high atmospheric level of 20 µl $l^{-1}$ the colony growth of *Rhizoctonia solani* was only reduced by 17 % (Yang et al., 2006). Moreover an inverse effect of the duration of fumigation was found in such a way that longer duration of fumigation significantly promoted colony growth. The pathogen *R. solani* was able to metabolize $H_2S$ in concentrations of up to 20 µl $l^{-1}$ within a period of up to 16 hours (Yang et al., 2006). While the basidiomycete *R. solani* metabolized $H_2S$, growth of *S. sclerotiorum* was significantly reduced after 24 h and 48 h at all $H_2S$ concentration; only at 10 µl $l^{-1}$ $H_2S$ differences were statistically not significant after 24 h (Fig. 1). Before, in a greenhouse experiment a significant decrease of the disease index in relation to the supply was found for *S. sclerotiorum* infections of oilseed rape (Wang et al., 2003).

Comparing these data with that from $H_2S$ emissions from plants it is evident that even an excessive S supply might not increase emissions so high that they are fungitoxic (Bloem et al., 2007). However, it is possible that increased $H_2S$ emissions are concentrated only around the site of infection and thus not yet measurable. Riemenschneider (2006) for instance has shown that plants contained $H_2S$ concentrations of 4-10 µM in the mesophyll of leaves of *Arabidopsis thaliana*.

A further step to appraise the role of $H_2S$ in SIR was a simple *in situ* soil experiment with elemental S applications. The pathogen *R. solani* was selected deliberately for this experiment as SIR was verified before for infections of potatoes with stem cancer under field conditions (Klikocka et al., 2005). Thus relatively stronger effects were expected for *R. solani* when for instance compared with *S. sclerotiorum* infections of oilseed rape (see above). Elemental S, which is an approved fungicide, may unfold its toxicity by direct contact, the disturbance of redox reactions after penetration of the fungal cell wall and release of cytotoxic $H_2S$ and the toxicity of its oxidation/reduction products $SO_2/H_2S$ outside the fungal hyphae (Heitefuss, 1975). Thus, a fungicidal effect would indicate that $H_2S$ at the periphery of the fungal mycelium was not involved as this compound only exists in relevant concentrations under anaerobic conditions.

Different S sources were added to soil inoculated with *R. solani* and the metabolic activities of the pathogen determined. Generally, an inhibitory effect on the growth of the pathogen is reflected in reduced enzyme activities. The dehydrogenase activity based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to the red-colored formazan (TPF) is often used to determine the microbial activity in soils (Schinner, 1996) and was used here to quantify the influence of the treatments on the vitality of the pathogen. The alkaline phosphatase activity was determined previously to quantify the inhibitory effect of fungicides (Beam et al., 1977).

Elemental S, with and without thiobacilli, amendments to the soil inoculated with *R. solani* resulted in a dehydrogenase activity that was distinctly higher than in the soil only inoculated with the pathogen directly after start of the experiment and 2 days later (Fig. 2). The peak value 2 days after inoculation when elemental S was applied might indicate metabolism of this S compound. After 6 days, however, elemental S reduced the dehydrogenase activity significantly.

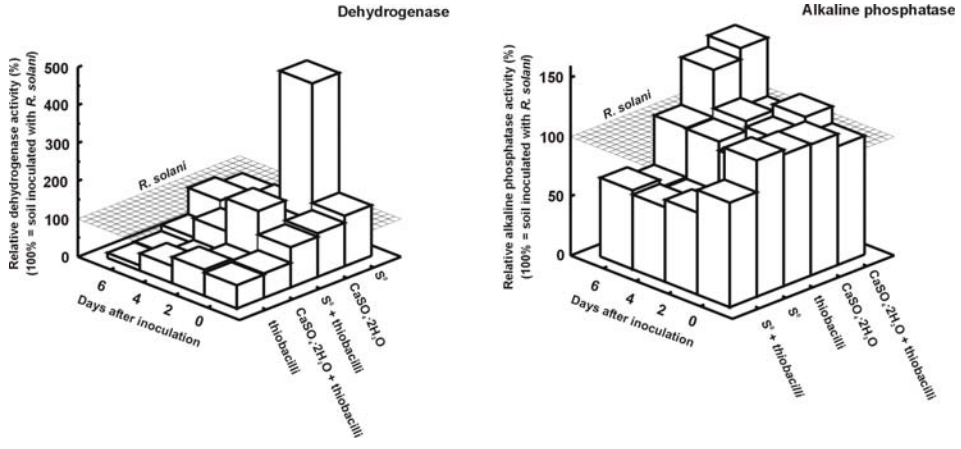


Fig. 2. Influence of S form and thiobacillus amendment on the relative dehydrogenase and alkaline phosphatase activity in a sterilized soil inoculated with *R. solani*. (100% dehydrogenase activity = 44, 38, 73, 64 µg TPF $g^{-1}$ 24 $h^{-1}$ 0, 2, 4 and 6 days after inoculation; 100% alkaline phosphatase activity = 340, 556, 471, 345 µg NP $g^{-1}$ $h^{-1}$ 0, 2, 4 and 6 days after inoculation).

All treatments with thiobacilli showed a drastic decrease of the dehydrogenase activity with the lowest value of only 13% compared to a soil inoculated with *R. solani* (Fig. 2). The inhibitory effect of exclusive thiobacilli applications compared to the other treatments was significant at all dates.

The amendment of thiobacilli had only a minor inhibitory effect on the alkaline phosphatase activity (Fig. 2). The alkaline phosphatase activity increased, however, over time when gypsum was applied, either with, or without thiobacilli. The strongest and significant repressive effect was found for elemental S applications, with and without thiobacilli, 2, 4 and 6 days after inoculation. Here, the alkaline phosphatase activity was reduced at maximum by 40% compared to the control treatments.

The results of the *in situ* experiment suggest a direct inhibitory effect of elemental S on *R. solani*, which is corroborated by the experiments of Klikocka et al. (2005), where elemental S reduced the infection rate and severity of potatoes infestated with the pathogen. Even more intriguing is the finding of Cooper et al. (1996) that elemental S deposits in vascular plant tissue was only found in tomato varieties, which were resistant against *Verticillium dahliae*. Such elemental S depositions were rapidly induced in *Solanaceae*; in *Brassicaceae* elemental S depositions can be found constitutively (Cooper, 2006).

## Conclusions

In field experiments, the maximum potential of SIR against infections with fungal pathogens equaled a reduction of the disease index by 35% (Haneklaus et al., 2006a). In case of oilseed rape, by SIR the yield potential could be more or less fully maintained (Schnug et al., 1995). Though S fertilization significantly increased $H_2S$ emissions of oilseed rape, it is obviously the potential of the plant to release $H_2S$ during the initial stage of infection that is relevant for defence (Bloem et al., 2007).

Hereby, pathogens show differences in their susceptibility against $H_2S$ (Fig. 1; Yang et al., 2006). Additionally, the *in situ* experiment suggests that elemental S yields a direct toxic effect, but as elemental S depositions in oilseed rape are rated as being constitutive (Cooper and Williams, 2004), their involvement in SIR seems to be of minor relevance. The results emphasize that the effect of soil-applied S on infections with fungal diseases is not universal, rather involves a crop type dependent and pathogen-triggered sequence in the synthesis of various S-metabolites.

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