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# Effect of $H_2S$ and Dimethyl Sulfide (DMS) on Growth and Enzymatic Activities of *Rhizoctonia solani* and its Implications for Sulfur-Induced Resistance (SIR) of Agricultural Crops

By

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With 4 figures

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Summary

YANG Z., HANEKLAUS S., DE KOK L.J., SCHNUG E. & SINGH B.R. 2006. Effect of $H_2S$ and dimethyl sulfide (DMS) on growth and enzymatic activities of *Rhizoctonia solani* and its implications for sulfur-induced resistance (SIR) of agricultural crops. – Phyton (Horn, Austria) 46 (1): 55 – 70, with 4 figures. – English with German summary.

Sulfur-Induced Resistance (SIR) was observed among others against infections of potatoes by *Rhizoctonia solani*. The release of reduced sulfur gases by plants may play an important role in plant defense against fungal pathogens. In a fumigation experiment the influence of increasing concentrations and the duration of fumigation

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with hydrogen sulfide ($H_2S$) and dimethyl sulfide (DMS) on colony growth and enzymatic activities of *R. solani* was determined in relation to the age of the fungal mycelium in order to evaluate the significance of the release of $H_2S$ and DMS for the protection of plants against fungal pathogens. With increasing $H_2S$ concentration the colony growth of two days old *R. solani* was significantly reduced, while with increasing duration of the fumigation a significant promotion of growth was found. Generally, the $H_2S$ concentration had no effect on the dehydrogenase and alkaline phosphatase activity of *R. solani*. In comparison, with increasing duration of fumigation both enzyme activities increased consistently up to six and two days after fumigation for two days and 5 days old *R. solani*. DMS seemed to have a detrimental effect on the alkaline phosphatase activity immediately after fumigation, but this was not consistently significant for the concentration and duration of fumigation. The effect of DMS on dehydrogenase activity of *R. solani* was not pronounced. The pathogen *R. solani* metabolized $H_2S$ and DMS in concentrations of up to 20 and 2 $\mu l \, l^{-1}$, respectively, within a period of up to 16 hours. Usually, up to six and two days after fumigation the alkaline phosphatase activity was the higher, the older the mycelium. In case of the dehydrogenase activity, in ten days old colonies significantly lower activities were found than in two and five days old mycelia. The results revealed that neither $H_2S$ nor DMS had a fungitoxic or fungicidal effect on *R. solani*. Thus it may be concluded that SIR for the soil-borne basidiomycete *R. solani*, was induced by other metabolites than $H_2S$ and DMS emissions of the infected plants.

## Zusammenfassung

YANG Z., HANEKLAUS S., DE KOK L.J., SCHNUG E. & SINGH B.R. 2006. Effekte von $H_2S$ und Dimethylsulfid (DMS) auf Wachstum und Enzymaktivitäten von *Rhizoctonia solani* und die Implikationen für schwefelinduzierte Resistenz (SIR) von landwirtschaftlichen Nutzpflanzen. – Phyton (Horn, Austria) 46 (1): 55 – 70, 4 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Schwefel-induzierte Resistenz (SIR) wurde unter anderem bei Infektionen von Kartoffeln mit *Rhizoctonia solani* beobachtet. Die Freisetzung reduzierter schwefelhaltiger Gase spielt möglicherweise eine wichtige Rolle für den Schutz von Pflanzen gegenüber pilzlichen Schaderregern. In einem Begasungsversuch mit *R. solani* wurde der Einfluss steigender Konzentrationen an $H_2S$ und DMS sowie Begasungszeiträume auf das Mycelwachstum und enzymatische Aktivitäten in Abhängigkeit vom Alter des Pilzmycels untersucht, um die Bedeutung von $H_2S$- und DMS-Freisetzungen für den Schutz der Pflanze gegenüber pilzlichen Schaderregern abzuschätzen. Mit steigenden $H_2S$-Konzentrationen nahm das Mycelwachstum signifikant ab, während mit fortschreitender Begasungsdauer das Wachstum signifikant gefördert wurde. Die $H_2S$-Konzentration hatte generell keinen Einfluß auf die Dehydrogenase- und alkalische Phosphatase-Aktivität von *R. solani*. Im Vergleich hierzu nahm die Aktivität beider Enzyme mit der Begasungsdauer konsistent bis zu zwei Tagen nach Beendigung der Begasung zu. Die Begasung mit DMS hatte tendenziell einen negativen Einfluß auf die Aktivität der alkalischen Phosphatase direkt nach Beendigung der Begasung, wobei dieser Effekt nicht einheitlich signifikant für verschiedenen Begasungsdauern und Konzentrationen war. Die Wirkung von DMS auf die Dehydrogenase-Aktivität war nicht sehr ausgeprägt. Der Erreger *R. solani* metabolisierte $H_2S$ und DMS in Konzentrationen von bis zu 20 bzw. 2 $\mu l \, l^{-1}$ innerhalb

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eines Zeitraumes von 16 Stunden. Prinzipiell konnte des Weiteren festgestellt werden, dass je älter das pilzliche Mycel zum Zeitpunkt der Begasung war, die alkalische Phosphatase-Aktivität bis zu zwei Tage nach Beendigung der Begasung deutlich erhöht war. Im Vergleich hierzu war die Dehydrogenase-Aktivität in Mycelien, die zum Zeitpunkt der Begasung 10 Tage alt waren, signifikant niedriger als in zwei und fünf Tage alten Kolonien. Die Ergebnisse zeigten, dass weder $H_2S$ noch DMS einen fungitoxischen bzw. fungistatischen Effekt auf *R. solani* ausübten. Daher kann davon ausgegangen werden, dass SIR, welche zuvor unter anderem für die bodenbürtigen Basidiomyceten *R. solani* und *R. cerealis* bestimmt wurde, sehr wahrscheinlich nicht durch entsprechende $H_2S$ und DMS Freisetzungen der infizierten Pflanzen ausgelöst wurde.

## Introduction

Since the beginning of 1980s, atmospheric S depositions have been declining drastically after clean air acts came into force, and severe sulfur (S) deficiency became a major nutritional disorder in Western Europe (SCHNUG & HANEKLAUS 1998). Increased infections of agricultural crops with fungal pathogens were observed and disease spread throughout regions that were never infected before (SCHNUG & CEYNOWA 1990). Sulfur fertilization applied as sulfate to the soil proved to have a significant effect on the infection rate and infection severity of different crops by fungal diseases (HANEKLAUS & al. 2005). For instance, in field-grown potatoes soil-applied S as sulfate significantly reduced infection rate and severity with *Rhizoctonia solani* (KLIKOCKA & al. 2005).

The term Sulfur-Induced Resistance (SIR) stands for the reinforcement of the natural resistance of plants against fungal pathogens through triggering the stimulation of metabolic processes involving sulfur by targeted fertilizer application strategies (HANEKLAUS & al. 2005). The mechanisms possibly involved in SIR may be related to processes of induced resistance (AGRAWAL & al. 2000), for example, via the formation of phytoalexins, glutathione, or the requirement of cysteine for the synthesis of salicylic acid by the ß-oxidation pathway (Fig. 1).

Another process is the release of reduced S gases such as $H_2S$ (Fig. 1), which is described in literature as being fungitoxic (REIFFENSTEIN & al. 1992), but no critical values for concentration and duration of fumigation in relation to different pathogens are available (BEAUCHAMP & al. 1984, BOERNER 1975, CARLILE & al. 2004). Endogenous production of $H_2S$ in plants or its release to the atmosphere might play a significant role in combating fungal infections. It may be speculated that the involvement of $H_2S$ in SIR will preferentially combat air-borne pathogens as cysteine biosynthesis is concentrated in chloroplasts and consequently $H_2S$ emitted from photosynthetically active tissue (SCHMIDT 2005). But infections by *R. solani* also pertain leaves and petioles (PRIYATMOJO & al. 2001, WINDELS & al. 1997).

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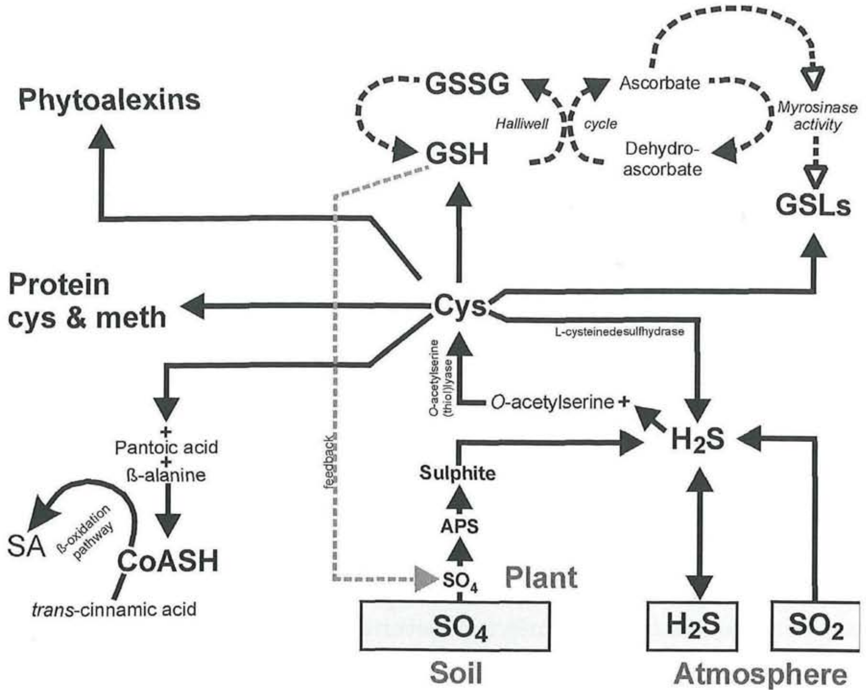


Fig. 1. Sulfur metabolites and pathways putatively involved in sulfur-induced resistance of *Brassica* species (HANEKLAUS & al. 2005). CoASH Coenyme A, SA salicylic acid, GSH glutathione, GSSG oxidized glutathione, GSLs glucosinolates, APS adenosine-5'-phosphosulfate.

In addition to $H_2S$, the release of several other volatile reduced S compounds (carbonyl sulfide, dimethyl sulfide, carbon disulfide, and methylmercaptan) from various plant species have been identified (SCHROEDER 1993). Emissions from annual crops such as corn and soybean are mainly dimethyl sulfide (DMS), while deciduous trees emit equal amounts of $H_2S$ and DMS (ANDREAE & JAESCHKE 1992).

Hydrogen sulfide may be produced prior to or after cysteine formation in plant S metabolism (GIOVANELLI 1990), but there is still a question as to which enzymes catalyze the release of $H_2S$, and whether it will be consumed endogenously, or will be released to the atmosphere (SCHMIDT 2005). Two enzymes that could be responsible for the $H_2S$ release are L-cysteine desulfhydrase (LCD) and *O*-acetyl-L-serine(thiol)lyase (*O*AS-TL). L-cysteine desulfhydrase catalyzes the decomposition of cysteine to pyruvate, ammonia, and $H_2S$, while *O*AS-TL is responsible for the incorporation of inorganic S into the amino acid cysteine, which can be subsequently converted into other S containing compounds, such as methionine or glutathione. Hydrogen sulfide is evolved in a side-reaction because of the

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nature of the pyridoxal 5'-phosphate cofactor and the specific reaction mechanism of the *O*AS-TL protein (TAI & COOK 2000). In a molar ratio the enzyme formed about 25 times more cysteine than $H_2S$ per mg protein during the same incubation time (BURANDT & al. 2001). Therefore in vitro the reaction of *O*AS-TL is a net $H_2S$ consuming reaction. In samples from field experimentation with oilseed rape, BLOEM & al. 2004 found no significant differences in the *O*AS-TL activity in relation to infections by *Pyrenopeziza brassicae*. However, the glutathione and cysteine content as well as LCD activity were significantly higher and thus indicate a significantly higher release of $H_2S$ (BLOEM & al. 2004).

There is wide variation for specifications about the release of $H_2S$ that ranges from 0.04 ng $g^{-1}$ $s^{-1}$ in whole soybean plants on a dry matter basis (WINNER & al. 1981) to 100 pmol $min^{-1}$ $cm^{-1}$ in leaf discs of cucumber (SEKIYA & al. 1982a). Thus, $H_2S$ emissions of cut plant parts may be 500 times higher than in intact plants and experimental data needs to be critically evaluated against this background. No field data exists where the emission of gaseous reduced S was measured from living plants under different S nutritional conditions. Concerning $H_2S$ emissions in relation to the S supply and fungal infections data are not yet available, existing $H_2S$ measurements varied extremely, and critical values for the fungitoxicity of $H_2S$ and other reduced S gases are not known. The present investigations were aimed to assess the influence of concentration and duration of fumigation with $H_2S$ and DMS on growth and metabolic activity of the fungal pathogen *R. solani* in order to evaluate the potential significance of these metabolites for SIR.

## Material and Methods

### Experimental Design of the Fumigation Experiment

Actively growing culture of *Rhizoctonia solani* Kühn isolated from *Solanum tuberosum* (order No. 63010) from the DSMZ in Braunschweig (German Collection of Microorganisms and Cell Cultures) was used. A 40 g of potato dextrose agar medium was filled in each petri dish (9 cm in diameter) that was previously sterilized at 121° C for 2 hours. The plates were stored for 1 day in the clean bench to allow condensation to evaporate. *R.solani* mycelium was inoculated on the surface of the medium and grown in an incubation chamber at room temperature (21 – 22° C) for 2, 5 and 10 days in dark.

### $H_2S$ Fumigation

Petri dishes with established colonies were put in 150 l cylindrical stainless steel cabinets with a polycarbonate top 2, 5 and 10 days after inoculation. 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 $h^{-1}$. Pressurized $H_2S$ diluted with $N_2$ was injected into the fumigation chamber. The $H_2S$ concentration in the chamber was controlled by an $SO_2$ analyzer (Model 9850) equipped with

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an $H_2S$ converter (Model 8770, Monitor Labs, Measurement Controls, Englewood, CO, USA). Four $H_2S$ concentrations: 0, 2, 4 and 20 µl $l^{-1}$ and three durations of fumigation: 1, 4 and 16 hours were tested for 2 and 5 days old colonies. Ten days old colonies were fumigated for 1 h with $H_2S$ concentrations of 0, 2, 4 and 20 µl $l^{-1}$. Each treatment had five replicates.

### DMS Fumigation

Pressurized liquid DMS was placed in the fumigation chamber and bottles containing liquid DMS exchanged to maintain a concentration of 2 µl $l^{-1}$. The DMS concentration was monitored by an $SO_2$ analyzer equipped with an $H_2S$ converter (as under $H_2S$ fumigation). Another chamber was aerated with ambient air. Five days old *R. solani* colonies were used for fumigating 1 and 4 hours in this experiment. Each treatment had three replicates.

### Vitality Assessment of *R. solani*

The influence of $H_2S$ fumigation on the vitality of the pathogen was determined by measuring the colony growth of 2 days old *R. solani* after fumigation. As *R. solani* grows concentrically the vitality test was carried out by metric measurement of the diameter of the colony until the whole area of the petri dishes was covered with fungal mycelium. The principal relationship between time and growth rate of *R. solani* is reflected in Figure 2.

The dehydrogenase and alkaline phosphatase activity were determined as indicators for the metabolic activity of the pathogen immediately after fumigation (immediately) and 2, 4, 6 days later. A corkscrew (0.5 cm diameter) was used to take equal mycelium/agar samples in a straight line from the center of the agar plates to

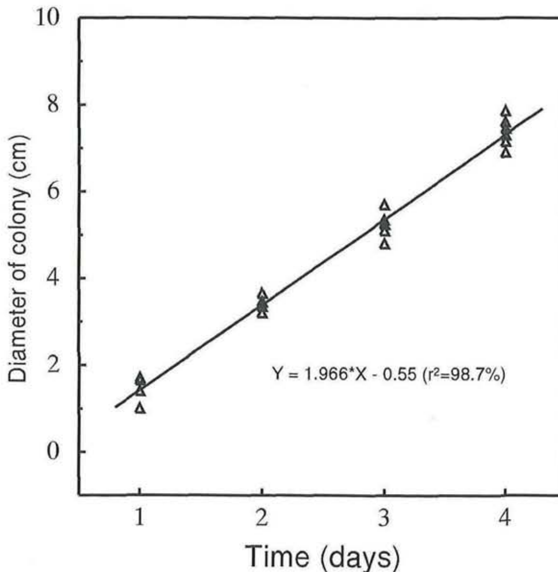


Fig. 2. Growth of *Rhizoctonia solani* colonies at 21° C.

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the outer parts of the mycelium. In an aliquot of 2 g, the dehydrogenase activity was determined by employing the substrate 2,3,5-triphenyltetrazolium chloride (TTC) (SCHINNER & al. 1996). In an aliquot of 1 g, the alkaline phosphatase activity was determined with substrate p-nitrophenyl phosphate (pNP) (SCHINNER & al. 1996). For each treatment blank values of pure agar plates were determined and in each case subtracted from the measurements of the inoculated dishes.

### Statistical Analysis

The statistical analysis was performed by the General Linear Model (GLM) procedure of the SPSS package program version 10 employing the LSD test ($\alpha = 0.05$) (SPSS 1999).

## Results

### Influence of $H_2S$ Fumigation on the Vitality of *R. solani*

The influence of $H_2S$ concentration and duration of fumigation on the colony growth of *R. solani* is shown in Table 1. The mean diameter of the fungal colonies before fumigation was 2.4 cm (cv – coefficient of variance 7.7%). With increasing $H_2S$ concentration the colony growth was significantly inhibited by the time the fumigation was terminated. This effect was to be seen also 1 and 2 days later and was consistent for all durations of fumigation. Compared to the control, the reduction of the colony growth decelerated on average from 29% by the time the fumigation ended to 17% and 12%, 1 and 2 days later, respectively. An inverse effect of the duration of fumigation was found in much as it significantly promoted the growth of the colony. This impact was the strongest immediately after fumigation with a mean diameter that was 80% higher when the mycelium was fumigated for the longest period of 16 hours and this value declined to 17% 2 days later (Table 1). The effects proved to be consistent for all $H_2S$ concentrations.

### $H_2S$ Fumigation and Dehydrogenase Activity

The mean dehydrogenase activity before fumigation was 11 μg TPF $g^{-1}$ 24 $h^{-1}$ (cv 53.3%) and 114 μg TPF $g^{-1}$ 24 $h^{-1}$ (cv 20.2%) in 2 and 5 days old colonies, respectively. The $H_2S$ concentration in relation to the duration of the fumigation and colony age had no significant influence on the dehydrogenase activity of *R. solani*. On average the mean dehydrogenase activity for all treatments increased from 12 μg TPF $g^{-1}$ 24 $h^{-1}$ immediately after fumigation to 309 μg TPF $g^{-1}$ 24 $h^{-1}$ 6 days after fumigation in 2 days old colonies. In comparison, in 5 days old colonies an increase from 128 to 263 μg TPF $g^{-1}$ 24 $h^{-1}$ in the dehydrogenase activity was found only up to 2 days after fumigation. The lowest value with 109 μg TPF $g^{-1}$ 24 $h^{-1}$ was determined 6 days after fumigation.

A significant inhibitory effect of the $H_2S$ concentration was found only 2 days after fumigation in 10 days old mycelium. Here, a decrease on average from 62 to 36 μg TPF $g^{-1}$ 24 $h^{-1}$ was found.

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Table 1. Influence of $H_2S$ concentration and duration of fumigation on the colony growth of *Rhizoctonia solani* fumigated two days after inoculation. LSD least squared difference.

| Duration (h) | Conc. (µl $l^{-1}$) | Colony growth of *R. solani* (cm) Immediately after fumigation | 1 day after fumigation | 2 days after fumigation |
|---|---|---|---|---|
| 1 | 0 | 0.3 | 1.8 | 3.5 |
| 1 | 2 | 0.2 | 1.9 | 3.5 |
| 1 | 4 | 0.2 | 1.7 | 3.4 |
| 1 | 20 | 0.1 | 1.4 | 3.0 |
| | $LSD_{5\%}$ | 0.1 | 0.1 | 0.1 |
| 4 | 0 | 0.8 | 2.4 | 4.4 |
| 4 | 2 | 0.6 | 2.1 | 4.0 |
| 4 | 4 | 0.6 | 2.1 | 4.0 |
| 4 | 20 | 0.5 | 2.0 | 3.8 |
| | $LSD_{5\%}$ | 0.2 | 0.3 | 0.2 |
| 16 | 0 | 1.0 | 2.7 | 4.3 |
| 16 | 2 | 0.9 | 2.5 | 4.1 |
| 16 | 4 | 1.0 | 2.5 | 4.0 |
| 16 | 20 | 0.9 | 2.3 | 3.9 |
| | $LSD_{5\%}$ | 0.2 | 0.2 | 0.4 |

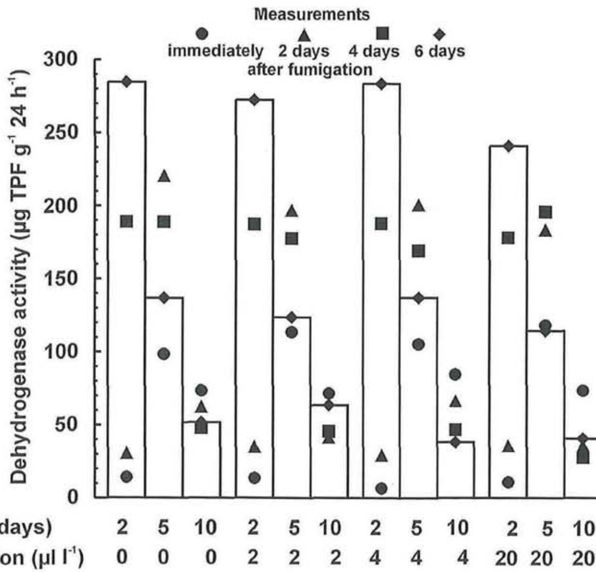


Fig. 3. Influence of increasing $H_2S$ concentrations on the dehydrogenase activity of *R. solani* after 1 hour fumigation in relation to the age of the fungal mycelium.

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The influence of increasing $H_2S$ concentrations on the dehydrogenase activity in relation to the age of the fungal mycelium for different sampling dates after fumigation is shown in Figure 3. The dehydrogenase activity, 6 days after fumigation, decreased distinctly with the age of the mycelium. The 10 days old fungal mycelium showed the lowest values already after 4 days of fumigation. Immediately and 2 days after fumigation, a consistently highest activity was found in 2 days old colonies.

The duration of the fumigation yielded significant effects on the dehydrogenase activity (Table 2). Generally in 2 days old colonies, the dehydrogenase increased with the duration of fumigation. In 5 days old colonies a significant decrease was determined after 4 and 6 days of fumigation (Table 2). These effects proved to be consistent over all $H_2S$ concentrations.

Table 2. Influence of the duration of fumigation with $H_2S$ on the dehydrogenase activity of *Rhizoctonia solani* in relation to the age of the fungal colony (data represent cell means over all tested $H_2S$ concentrations).

| Duration (h) | Time (days after fumigation) | 2 days old colonies | | 5 days old colonies | |
|---|---|---|---|---|---|
| | | Dehydrogenase activity (μg TPF $g^{-1}$ $24h^{-1}$) | | | |
| | | Fumigated | Non-fumigated | Fumigated | Non-fumigated |
| 1 | Immediately | 11 | 13 | 112 | 74 |
| 4 | Immediately | 14 | 12 | 103 | 110 |
| 16 | Immediately | 9 | 19 | 173 | 212 |
| $LSD_{5\%}$ | | 4 | 7 | 15 | 39 |
| 1 | 2 | 34 | 30 | 187 | 205 |
| 4 | 2 | 35 | 35 | 243 | 245 |
| 16 | 2 | 81 | 84 | 341 | 336 |
| $LSD_{5\%}$ | | 8 | 9 | 28 | 28 |
| 1 | 4 | 194 | 185 | 176 | 196 |
| 4 | 4 | 188 | 216 | 192 | 216 |
| 16 | 4 | 316 | 310 | 132 | 107 |
| $LSD_{5\%}$ | | 26 | 41 | 17 | 36 |
| 1 | 6 | 265 | 284 | 125 | 137 |
| 4 | 6 | 389 | 344 | 100 | 119 |
| 16 | 6 | 352 | 358 | 98 | 76 |
| $LSD_{5\%}$ | | 41 | 72 | 21 | 55 |

## $H_2S$ Fumigation and Alkaline Phosphatase Activity

The mean alkaline phosphatase activity was 50 (cv 24.3%) μg pNP $g^{-1}$ $h^{-1}$ and 337 (cv 36.5%) μg pNP $g^{-1}$ $h^{-1}$ in 2 and 5 days old colonies, respectively before fumigation. In 2 days old colonies the mean alkaline phosphatase activity for all treatments increased from 43 μg pNP $g^{-1}$ $h^{-1}$ immediately after fumigation to 450 μg pNP $g^{-1}$ $h^{-1}$ 6 days after fumigation. The corresponding

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values for 5 days old colonies were from 331 to 515 µg pNP $g^{-1}$ $h^{-1}$. The lowest values were found for both ages of the mycelium immediately after fumigation. A significant promoting effect of the $H_2S$ concentration was found immediately after fumigation in 10 days old mycelium. Here, an increase on average from 436 to 514 µg pNP $g^{-1}$ $h^{-1}$ was found.

With increasing $H_2S$ concentration the alkaline phosphatase activity decreased from 412 µg pNP $g^{-1}$ $h^{-1}$ in the control to 311 µg pNP $g^{-1}$ $h^{-1}$ in the 20 µl $H_2S$ $l^{-1}$ treatment immediately after fumigation of 5 days old *R. solani* colonies. For other dates of sampling and 2 days old colonies, the $H_2S$ concentration had no significant influence on the enzyme activity.

In Figure 4 the influence of increasing $H_2S$ concentrations after fumigation on the alkaline phosphatase activity at different ages of the fungal mycelium at various sampling dates is shown. Immediately after fumigation the alkaline phosphatase activity proved to be distinctly lower in 2 and 5 days old colonies than in 10 days old ones. The 5 days old colonies after 2 days of fumigation and the 2 days old colonies after 6 days of fumigation always showed the highest alkaline phosphatase activity (Fig. 4).

In the 2 and 5 days old colonies, the alkaline phosphatase activity increased with prolonged duration of fumigation for all sampling dates (Table 3).

The results of the $H_2S$ fumigation experiment reveal that despite the fact that increasing $H_2S$ concentrations reduced the colony significantly,

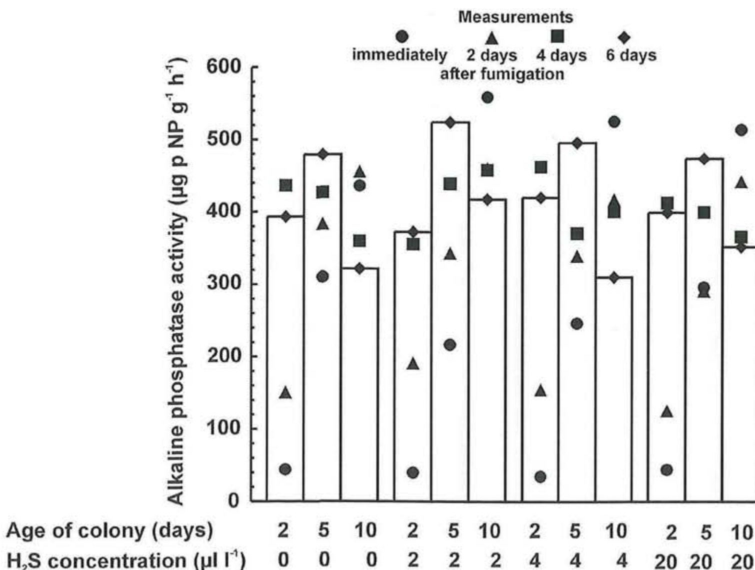


Fig. 4. Influence of increasing $H_2S$ concentrations on the alkaline phosphatase activity of *R. solani* after 1 hour fumigation in relation to the age of the fungal mycelium.

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an inhibitory effect on the dehydrogenase and alkaline phosphatase activity was not seen. The fact that the duration of fumigation had a stimulating effect on the colony growth and that the enzyme activities increased generally over time suggested that $H_2S$ was metabolized by the pathogen. Additionally, differences in the dehydrogenase and alkaline phosphatase activity of the fungal mycelium were related to the age of the colony.

Table 3. Influence of the duration of fumigation with $H_2S$ on the alkaline phosphatase activity of *Rhizoctonia solani* in relation to the age of the fungal colony (data represent cell means over all tested $H_2S$ concentrations).

| Duration (h) | Time (days after fumigation) | 2 days old colonies | | 5 days old colonies | |
|---|---|---|---|---|---|
| | | Phosphatase activity (µg p NP $g^{-1}$ $h^{-1}$) | | | |
| | | Fumigated | Non-fumigated | Fumigated | Non-fumigated |
| 1 | Immediately | 42 | 44 | 279 | 322 |
| 4 | Immediately | 33 | 35 | 225 | 260 |
| 16 | Immediately | 50 | 69 | 500 | 653 |
| $LSD_{5\%}$ | | 6 | 13 | 49 | 69 |
| 1 | 2 | 152 | 149 | 321 | 365 |
| 4 | 2 | 131 | 148 | 450 | 454 |
| 16 | 2 | 317 | 278 | 516 | 471 |
| $LSD_{5\%}$ | | 28 | 41 | 60 | 116 |
| 1 | 4 | 417 | 436 | 413 | 394 |
| 4 | 4 | 438 | 415 | 379 | 349 |
| 16 | 4 | 671 | 579 | 419 | 533 |
| $LSD_{5\%}$ | | 56 | 129 | 38 | 132 |
| 1 | 6 | 397 | 393 | 498 | 479 |
| 4 | 6 | 433 | 432 | 532 | 501 |
| 16 | 6 | 515 | 576 | 530 | 522 |
| $LSD_{5\%}$ | | 57 | 59 | 50 | 74 |

## DMS Fumigation and Enzyme Activities

The DMS concentration of 2 µl $l^{-1}$ reduced the alkaline phosphatase activity only immediately after fumigating for 1 and 4 hours (Table 4). However, at all dates after fumigation, the effect was statistically not significant. The effects of DMS duration on alkaline phosphatase activity were not consistent at all sampling dates after fumigation (Table 4). Fumigation with DMS seemed to increase the dehydrogenase activity, but the effect was not significant (Table 4). In contrast to fumigation with $H_2S$, DMS yielded an inhibitory effect on the dehydrogenase activity when fumigated for 4 instead of 1 hour (Table 4).

The results of both fumigation experiments reveal that neither $H_2S$ nor DMS proved to be fungitoxic for *R. solani* at concentration of up to

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20 μl $l^{-1}$ and 2 μl $l^{-1}$, respectively. Higher enzymatic activities of older fungal mycelium may indicate differences in the susceptibility against fumigation with $H_2S$ and DMS, which is related to the stage of development of the pathogen.

Table 4. Influence of the dimethyl sulfide (DMS) concentration and duration of fumigation on the dehydrogenase and alkaline phosphatase activity of 5 days old *Rhizoctonia solani* colonies.

| | | Dehydrogenase activity (μg TPF $g^{-1}$ $24h^{-1}$) | | | |
|---|---|---|---|---|---|
| | | | Days after fumigation | | |
| Duration | DMS (μl $l^{-1}$) | Immediately | 2 | 4 | 6 |
| 1 | 0 | 51 | 197 | 261 | 123 |
| 1 | 2 | 54 | 182 | 280 | 184 |
| | $LSD_{5\%}$ | 34 | 61 | 38 | 142 |
| 4 | 0 | 35 | 211 | 345 | 238 |
| 4 | 2 | 40 | 215 | 334 | 222 |
| | $LSD_{5\%}$ | 40 | 87 | 101 | 144 |
| | | Alkaline phosphatase activity (μg p NP $g^{-1}$ $h^{-1}$) | | | |
| Duration | DMS (μl $l^{-1}$) | Immediately | 2 | 4 | 6 |
| 1 | 0 | 192 | 331 | 354 | 423 |
| 1 | 2 | 169 | 344 | 491 | 435 |
| | $LSD_{5\%}$ | 40 | 115 | 224 | 149 |
| 4 | 0 | 176 | 388 | 576 | 524 |
| 4 | 2 | 140 | 466 | 466 | 524 |
| | $LSD_{5\%}$ | 58 | 231 | 242 | 60 |

## Discussion

Fumigation of mycelia from *R. solani* with S gases was carried out so far only with $SO_2$ (LORENZINI & al. 1992). These studies focused on the detrimental effects of acid rain in polluted areas. In plant metabolism, $H_2S$ may interfere with the respiration as it is effectively bound to cytochromes and a similar mechanism is assumed for fungal pathogens (BEFFA 1993, DE KOK & al. 2002, SCHMIDT 2005). In literature, it is mentioned that $H_2S$ is fungitoxic, but information on the required concentration and duration of exposure for individual fungal pathogens is not available. Thus fumigation of fungal mycelium with $H_2S$ and DMS might provide key information not only about critical thresholds for $H_2S$ and DMS toxicity for fungal pathogens, but also for metabolic plant specifications.

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 & al. 1996) and the alkaline phosphatase activity was determined in previous investiga-

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tions to quantify the inhibitory effect of fungicides, or used for isoenzyme analysis of *R. solani* (BEAM & al. 1977, MOHAMMADI & al. 2003). Generally, an inhibitory effect on the growth of the pathogen is reflected in reduced enzyme activities.

The results of the fumigation experiment revealed that neither $H_2S$, nor DMS had a fungitoxic or fungicidal effect. *Rhizoctonia solani* showed even increased metabolic activities over time when fumigated with $H_2S$. The results may be explained in several ways. Firstly, a minor influence of $H_2S$ and DMS could not inhibit both growth and enzymatic activities of *R. solani*. Secondly, both dehydrogenase and alkaline phosphatase activity increase with the growth of the mycelium (the data were not shown here), resulting in higher enzymatic activities for longer durations of fumigation. The duration of fumigation with DMS had an inhibitory effect on both enzymes, but this effect was not consistent for all sampling dates (Table 4).

Recent studies from BURANDT & al. 2001 showed that there was a significant relationship between the activity of $H_2S$ releasing enzymes and the infestation with fungal disease for field crops. A variable amount of $H_2S$ release was also found in several field crops (SEKIYA & al. 1982b, BLOEM & al. 2005). However, in the present investigations $H_2S$ concentrations were about 270 times higher than those, which may reduce plant growth (DE KOK & al. 2002). For basidiomyceta it is known that the pathogen can degrade $H_2S$ and DMS by oxidization to sulfate and dimethylsulfoxide, respectively (PHAE & SHODA 1991). The degradation of elevated levels of $H_2S$ and DMS was completed within 2 hours (PHAE & SHODA 1991). The results of the present study support these findings. Therefore, a fungicidal or fungitoxic effect of $H_2S$ seems unlikely at least for infections with *R. solani*, though factors such as the age of the fungal colony and the nutritional status of the pathogen may influence the results obtained. Differences in the dehydrogenase and alkaline phosphatase activity of the fungal mycelium in relation to the age of the colony point to this fact (Table 2 and 3, Fig. 3 and 4). In this context KASSEMEYER 2003 found the highest efficiency of foliar-applied elemental S against *Uncinula necator* when it was applied in the early stage of infection, after adhesion of conidia and mycelia, but before penetration of appressoria.

These results bear relevant implications for SIR. Hydrogen sulfide and DMS emissions of plants are supposedly not involved in SIR against fungal pathogens belonging to the class of basidiomycetes. The identification of cross-talks between host and pathogen specific metabolic pathways which are triggered by the S nutritional status of the host will be the key to SIR and thus a successful substitution of crude chemicals by biological know-how in agriculture. In conclusion, SIR, which was shown previously for the soil-borne basidiomycetes *R. solani* (KLIKOCKA & al. 2005) must be induced by other metabolites than $H_2S$ and DMS emissions of the infected plants.

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