| Abh. Ber. Naturkundemus. Görlitz | Band 75 Heft 1 | S. 1 – 9 | 2003 |
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ISSN 0373-7586

# Influence of S-fertilisation on S-translocation in the host-pathogen-system winter wheat *Triticum aestivum*/powdery mildew *Blumeria graminis*

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**Abstract**

Powdery mildew (*Blumeria graminis* f. sp. *tritici*) is an obligate biotrophic pathogen, meaning that the host plant supplies nutrients to the fungus. It is known that nitrogen (N) can be taken up by the fungus in the form of di-amino acids that have been synthesised by the plants. However, whether the fungus uses sulphur amino acids or inorganic sulphate to meet its sulphur (S) requirements has remained unknown. Stable S-isotope analysis was used to evaluate the translocation of sulphur from the host plant into the pathogen. Sulphur has four naturally occurring stable isotopes. Compounds enriched with one of the S-isotopes can be used as tracers to follow the path of the substances.

Winter wheat (*Triticum aestivum* L., cv. Kanzler) grown in the field was subjected to natural infection with powdery mildew. The disease severity of S-fertilised plants was higher than in the non S-fertilised plants. Thus S-fertilisation had an influence on the disease caused by *B. graminis*. The sulphur concentration within the fungal mycelium and inside the corresponding underlying leaf areas varied, depending on the fungal development. Non-infected leaves contained less sulphur than the infected leaf areas. The isotope composition differed markedly – fungal mycelium was enriched in $^{34}S$ compared to the underlying leaf material. These results imply that a part of fertiliser sulphur had reached the pathogen itself.

**Zusammenfassung**

**Einfluss von Schwefeldüngung auf die Schwefeltranslokation im Wirt-Pathogen-System Winterweizen (*Triticum aestivum*)/Echter Mehltau (*Blumeria graminis*)** – Echter Mehltau (*Blumeria graminis* f. sp. *tritici*) ist ein obligat biotropher Parasit, d.h., dass der Pilz von der Wirtspflanze seine Nährstoffe bezieht. Obwohl bekannt ist, dass der Pilz Stickstoff (N) in Form von Diaminosäuren pflanzlichen Ursprungs aufnehmen kann, war bisher unklar, ob er seinen Schwefelbedarf aus S-haltigen Aminosäuren oder aus anorganischem Sulphat deckt. Eine Isotopenanalyse wurde eingesetzt, um die S-Translokation vom Wirt zum Pathogen zu untersuchen.

Im Feld angezogener Winterweizen (*Triticum aestivum* L., cv. Kanzler) war dem natürlichen Mehltaubefall ausgesetzt. Die Befallsdichte schwefelgedüngter Pflanzen war im Vergleich zu Pflanzen ohne Schwefeldüngung höher und zeigte somit einen Einfluss der S-Düngung auf das Befallsbild von *B. graminis*. Die Schwefelkonzentration im Myzel und im befallenen

Blatt waren je nach Entwicklungsstadium des Erregers unterschiedlich. Nichtbefallene Blätter enthielten weniger Schwefel als befallene Blattflächen. Die Isotopenzusammensetzung wies deutliche Unterschiede auf – der $^{34}S$-Gehalt des Myzels war gegenüber dem des befallenen Blattes erhöht. Die Ergebnisse deuten darauf hin, dass ein Teil des im Dünger enthaltenen Schwefels vom Pathogen aufgenommen wurde.

## 1. Introduction

Sulphur (S) is a major plant nutrient. For decades, it has been deposited on agricultural systems from the atmosphere as dry and wet deposition. SCHNUG (1991) reported that in 1955/1956 about 80 kg S/ha were deposited from the atmosphere. Between 1989 and 1994, these atmospheric depositions decreased substantially (GRÜNHAGE et al. 1992, ZIMMERLING 1994) to less than 20 kg S/ha a.

Agricultural systems need different amounts of sulphur, depending on the crop cultivated. Wheat plants, for example, need about 50 kg S/ha a during growth and development. With falling atmospheric supply, plants are no longer able to obtain their sulphur requirement by take up from the soil nor through uptake of atmospheric $SO_2$. Furthermore, the soil reservoir is no longer refilled and sulphur deficiency becomes evident, making sulphur fertilisation necessary.

Such changes in nutrient supply of plants can directly effect development and growth of plant pathogens. Obligate biotrophic pathogens e.g. powdery mildew (*Blumeria graminis* f. sp. *tritici*) feed exclusively on their hosts via haustoria in the host's epidermal cells (BRAUN 1995), and hence depend on the host's nutritional status (GÖTZ 1996) to get nutrients and metabolites. Changes in the nutrient supply of the plants may therefore directly effect growth, development and spread of the pathogen. Consequently, sulphur fertilisation could possibly either lead to a reduction of the infection or enhance the pathogen's growth, leading to more severe infections in the field.

Mechanistically, it is not clear how sulphur is transferred from the host to the pathogen. Its supply can be assured by the uptake of amino acids as e.g. in the case of nitrogen (BOYLE et al. 1994 ined., IMC 5 Vancouver, Congress Volume, SCHMIDT et al. 1994, GÖTZ 1996), or by using the inorganic S-pool of the host.

To evaluate the effect of sulphur on the development and spread of a pathogen, analysis of the stable S-isotope composition in different compartments of an agroecosystem seems to be a reasonable method of identifying S-sources.

Stable S-isotope analysis proved to be a valuable tool for identifying sources of sulphur (WINNER et al. 1978, KROUSE & GRINENKO 1991). Sulphur consists naturally of four stable isotopes present at different relative abundances. During chemical, physical and biological processes, these isotopes are turned over at different rates (THODE 1991) leading to sulphur pools of different isotope composition. In order to trace sulphur within a given system, the primary condition is a distinct difference between the isotope composition of the sulphur in the source and that in the sink. Sulphurous fertiliser enriched in the $^{34}S$-isotope applied to the field induced change in S-isotope composition of the soil sulphur pool and has been used to trace the fertiliser on its way through the host-pathogen system. The S-isotope composition of sulphurous compounds extracted from host or pathogen is expected to provide information on the origin of sulphur translocated within the system.

A field study was also carried out to evaluate the influence of sulphur fertilisation on disease severity and development of the obligate biotrophic fungus *Blumeria graminis* f. sp. *tritici* (powdery mildew) on winter wheat (*Triticum aestivum* L. cv. Kanzler) following natural infection. Plant growth, disease severity and pathogen development was evaluated regularly during the season. Stable S-isotope analysis was used to evaluate whether sulphur from the $^{34}S$-enriched fertiliser participated in the changes in sulphur supply to the pathogen.

## 2. Materials and methods

Winter wheat (*Triticum aestivum* L. cv. Kanzler), highly susceptible to *Blumeria graminis* (DC.) Speer f. sp. *tritici* Marchal (mildew susceptibility: 9; Beschreibende Sortenliste, Bundessortenamt 1993), was cultivated in a field where N-fertilisation was carried out according to local farming practise. While two plots remained as control areas, another two were additionally fertilised using ASS (Ammonium nitrate sulphate) equivalent to 50 kg S/ha. This fertiliser was enriched in $^{34}S$ ($\delta^{34}S$-value = +9,4 ‰). One of each out of these plots was treated with fungicide.

The abbreviations used are as follows:

ASS: Ammonium nitrate-sulphate-boron

KAS: Nitro chalk, no S present

U: no fungicide used

G: treated with fungicide

Tab. 1 Abbreviations used for the different treatments

| treatment | N-fertilisation | S-fertilisation | fungicide application |
|---|---|---|---|
| KASU | Yes | no | untreated |
| KASG | Yes | no | applied |
| ASSU | Yes | yes | untreated |
| ASSG | Yes | yes | applied |

Plants in the field were subjected to naturally occurring infection with *Blumeria graminis*. The evaluation of disease severity (in % of leaf area) was carried out according to GÖTZ (1996).

### Sample collection:

Soil, plant and fungal samples were collected from all plots at different developmental stages of both the plants and the fungus. At different developmental stages of the fungus, mycelium was separated from the underlying leaf without destroying the epidermal layer.

These now mycelium-free leaf areas were punched out (Ø 1,0 cm). The samples were lyophylised and ground to fine powder prior to analysis (VENSCHOTT & BOYLE 1994). The fungal mycelium stages analysed represent the increasing ripening of the cleistothecia from white mycelium with primordia (gM1) that still has close contact to the host, to white mycelium with black fruit bodies (gM3), at which stage the contact to the host is interrupted (GÖTZ & BOYLE 1998).

**S-analyses:**

S-concentration was determined using an automated S-analyser (Leco SC 132). Stable S-isotope analysis was carried out as described in GIESEMANN et al. (1994) on an elemental analyser coupled to an isotope ratio mass spectrometer (Carlo Erba NA 1500 and Finnigan MAT Delta S).

The organic and inorganic sulphur fraction in all samples was achieved using the method of JÄGER & STEUBING (1971), where an aliquot of each sample is combusted in a stream of oxygen to evolve $SO_2$ out of the organic fraction while inorganic S remains in the ashes. Sulphur from both fractions was precipitated as $BaSO_4$ and S-isotope analysis carried out on this sulphate.

## 3. Results and Discussion

The plants from plots with additional fungicide application showed lower disease severity (ASSG, KASG, Fig. 1) and a higher total shoot growth than plants from plots without fungicide. *Blumeria graminis* f. sp. *tritici* showed infection by vegetative and generative mycelium according as described by to GÖTZ et al. (1996; Fig. 1). Disease severity caused by both mycelium types is higher in S-fertilised plots (ASS), as compared to non S-fertilisation (KAS), irrespective of the presence or lack of fungicide treatment. In both only N-fertilised plots (+/– fungicide) the parasite expressed a nearly identical disease severity. Thus S-fertilisation increased the disease severity of powdery mildew on wheat. As known from GÖTZ & BOYLE (1998), the development of generative mycelium depends on the physiological state and fitness of the host plant and seems to be forced by S-fertilisation (Fig.1) independent of fungicide application.

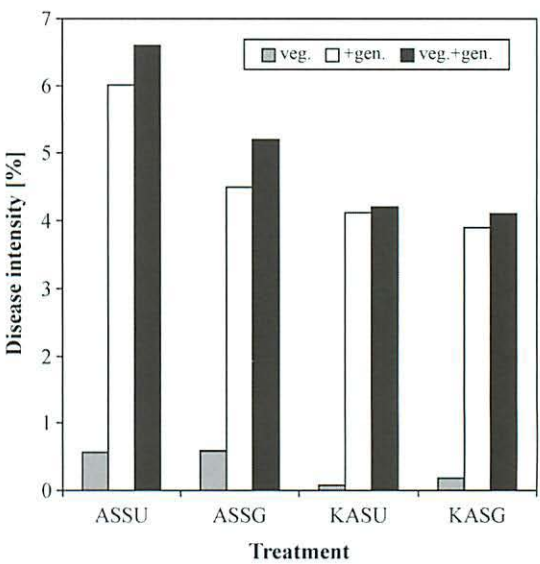



Fig. 1 Disease intensity [%] of developmental stages of *Blumeria graminis*. Mean of the last three evaluations, n=10 ASS: N+additionally S-fertilised, KAS: only N-fertilised, U: no fungicide application, G: fungicide application

Because of this independence, the following data represent results from plots without fungicide application (ASSU / KASU). The total sulphur concentration of non-infected control leaves was lower than that of the leaf area directly influenced by the earliest (youngest) generative fungal stage (gM1) (Fig. 2). The parasite seems to induce a strong sink, not only in the directly infected leaf area but also in the mycelium itself. However, the additional S-fertilisation on the ASS plots seems to lead to a S-concentration in the leaves which is high enough to fulfil the parasite's needs. While the S-concentration in leaves from plants grown on the non S-fertilised plot dropped substantially in the course of fruit body development (gM1/gM2), it remained nearly constant in the samples from the S-fertilised plot. This might be indicative of too little sulphur available for an ongoing rapid fungal development in the non S-fertilised plot. Regarding the mycelia, a similar effect became visible: we found higher S-concentrations in the maturing mycelia (gM2/gM3) from S-fertilised plants than in those from leaves of non-S-fertilised plants.

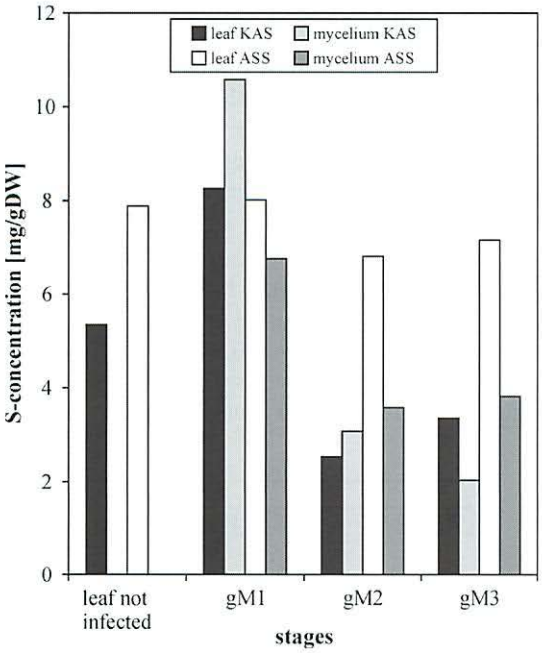



Fig. 2 S-concentration [mg/gDW] in leaf and mycelium of different developmental stages of *Blumeria graminis* sampled from S-fertilised (ASS) and non-S-fertilised (KAS) plots

At gM3 both treatments showed higher S-concentration in the leaves than in the mycelia. We assume that at this developmental stage, a translocation of sulphur in direction of the pathogen is reduced (GÖTZ & BOYLE 1998) and hence a slight accumulation of S-compounds in the influenced leaf areas becomes visible. The S-levels observed in non-infected controls, however, is not reached (Fig. 2).

The application of S-fertiliser resulted in a higher sulphur support of the leaves and higher pool sizes in nearly all stages determined. The concentration was also somewhat higher in the further-developed generative mycelia. The plants of these plots were better able to support the infected sink leaves with sulphur. The pronounced development of the cleistothecia beginning at gM2 is independent of further support with nutrients by the host (GÖTZ et al. 1996). This might enable the plants to increase defence reactions on the basis of nutrient accumulation, as e.g. encasement of the haustoria (GÖTZ & BOYLE 1998). Thus the nutritional barrier between host and parasite becomes larger. This results in an increase in S-pool sizes in the directly influenced leaf areas with a parallel reduction of sulphur concentration in the mycelia.

Comparable investigations based on stable S-isotope analysis led to the same conclusions. The plant organs of wheat from the S-fertilised plots showed more positive $\delta^{34}$S-values in dependence of physiological and developmental stage than those of plants from non-fertilised plots. As the sulphur added to the system through fertilisation is enriched in $^{34}$S, samples containing this sulphur should show more positive $\delta^{34}$S-values compared to samples from non-fertilised plots.

Investigations of the $\delta^{34}$S-values in different developmental stages of the mycelium led to more positive $\delta^{34}$S-values when fungal samples originated from the fertilised plot (Fig. 3, ASS). Mycelium as well as its directly influenced leaf areas and samples from non-fertilised control plants however, showed lower $\delta^{34}$S-values (Fig. 3, KAS). The observed continuous increase of $\delta^{34}$S-values in leaf and fungus samples with developmental stage, from the fertilised plot, is due to a continuous uptake and translocation of fertiliser sulphur during plant growth.

The generative mycelium stage 1 (gM1) and the leaf area it directly influenced was depleted in $^{34}$S, leading to negative $\delta^{34}$S-values (Fig. 3). This means that more $^{32}$S-isotopes are present in these samples from non-fertilised plots. Preliminary studies showed that during plant metabolism, sulphur fractionation takes place, leading to an organic S-fraction with more negative $\delta^{34}$S-values than in the inorganic S-fraction, where $^{34}$S is passively enriched (Krouse et al. 1992). The depletion of $^{34}$S in both mycelium and leaves of gM1 from the non S-fertilised plot could presumably be caused by a specific withdrawal of the organic S-fraction by the fungus due to a lack of sulphur in the plants. The fungus will accumulate the $^{32}$S-isotope especially in the physiologically active young generative mycelium (gM1), resulting in negative $\delta^{34}$S-values. As the plant is not able to translocate inorganic S rapidly into the directly infected leaf areas, these regions are – for a certain period of time – depleted in sulphur. During the following developmental stages the plant supplies more inorganic sulphur to the fungal mycelium to ensure the nutrient supply for the growing cleistothecia, leading to more positive $\delta^{34}$S-values in samples of gM2 (Fig. 3, KAS).

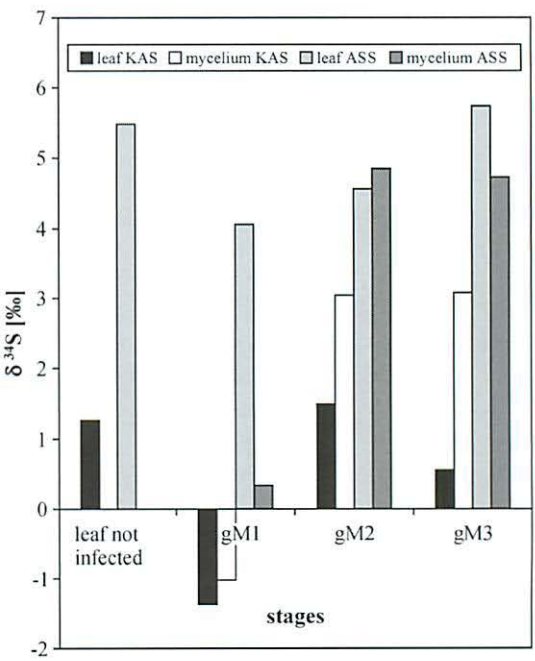
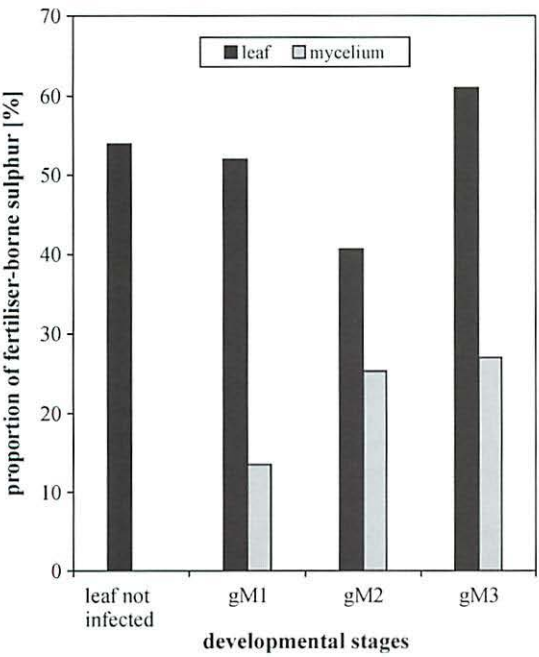



Fig. 3 $\delta^{34}$S-value of leaf and mycelium of different developmental stages of *Blumeria graminis* as influenced through fertilisation with $^{34}$S-enriched fertiliser

Calculating the proportion of sulphur derived from the $^{34}S$-enriched fertiliser in the mycelium of the different developmental stages as well as in the associated leaf areas (Fig. 4) will give a clue as to where fertiliser sulphur was going. In the non-infected control leaves harvested from the fertilised plot, more than 50 % of the sulphur could be calculated to be of fertiliser origin. Infection by powdery mildew caused a reduction in the proportion of sulphur deriving from the fertiliser in the leaf areas influenced directly, while this proportion increased in the fungus. This effect was more pronounced with greater development of the parasite.

The stage gM2, expressing brownish fruit bodies, takes more sulphur from its host to support the need of nutrients during this developmental stage. During gM3, the leaf replenishes its S-reservoirs again using fertiliser-derived inorganic sulphur. This becomes visible in an increase of the fertiliser-derived sulphur proportion in the mycelium (Fig. 4). BOYLE (1996) suggested that the sulphur supply of this obligate biotrophic fungus mainly originates from the inorganic sulphur fraction of its host. Because of the pronounced thickness of the encasement of the haustoria at gM3, the translocation from the host to the pathogen was more or less brought to a standstill. The nutrients accumulated in the mycelium and will then be transported into the cleistothecia, but the leaves still behave as a sink. This led to an accumulation of total sulphur as well as the $\delta^{34}S$-values in the directly influenced infected leaf areas, as expressed by the proportion of fertiliser-derived sulphur of 61 % (Fig. 4).




Our data verify that the method of determining the stable S-isotope distribution on the basis of natural S-isotope-variability is suitable for acquiring information on the sulphur source of obligate biotrophic fungi such as powdery mildew. The results suggest the hypothesis that the pathogenic fungus not only acts as a sink for S itself, but also affects the sulphur metabolism of the host.

Fig. 4 Proportion of fertiliser-borne sulphur [%] in leaf and mycelium of different developmental stages of *Blumeria graminis*

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Manuscript accepted: 28 November 2003

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