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Sulfur nutrition and its significance for crop resistance – a case study from Scotland

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Abstract

Severe sulfur deficiency causes a decrease in yield and has a negative impact on crop quality. Besides this, a higher susceptibility of crops to certain diseases was observed. Sulfur fertilization proved to lower disease incidence and severity of fungal infections in different crops. The sulfur metabolism provides several potential mechanisms by which plants are able to tackle biotic stress. The identification of these processes and adaptive control of sulfur induced resistance (SIR) against fungal diseases offers the opportunity to develop natural plant protection measures by means of targeted fertilization strategies. In the present paper, the results from a field experiment in Scotland are summarized, which reflect the influence of the sulfur nutritional status on sulfur-containing metabolites and infection with fungal diseases.

Key words: cysteine, glutathione, glucosinolate, Pyrenopeziza brassicae, sulfur induced resistance (SIR)

Introduction

Since the beginning of the 1980s severe sulfur (S) deficiency can be observed regularly under field conditions in the northern parts of Europe because of continuously decreasing S inputs to agroecosystems (Schnug and Pissarek, 1982; Schnug and Haneklaus, 1994). High S demanding cruciferous crops reacted first to a reduced S supply (Schnug and Pissarek, 1982) and about 10 - 15 years later, low demanding crops such as cereals and sugar beat also showed S deficiency (Schnug et al., 1993; Schnug et al., 2000). In Scotland, the infection of oilseed rape plants by fungal pathogens such as *Pyrenopeziza brassicae* (anamorph: *Cylindrosporium concentricum*) (*P. brassicae*) and *Leptosphaeria maculans* (anamorph: *Phoma lingam*) increased during the 1980s (Brokenshire et al., 1984). This phenomenon was attributed to the drastically reduced atmospheric S depositions in this region (Dore et al., 2003) as S was found to play a key role in the defense system of plants (Schnug and Ceynowa, 1990; Schnug et al., 1995a). Soil-applied S increased the resistance against various fungal diseases in different crops under greenhouse (Wang et al., 2003) and field conditions (Schnug et al., 1995a; Bourbos et al., 2000; Klikocka et al., 2004; Bloem et al., 2004; Salac et al., 2004). Based on these findings the concept of SIR (Sulfur Induced Resistance) was developed (Schnug, 1997).

The concept of Sulfur Induced Resistance (SIR)

The S metabolism of plants offers different possibilities to tackle with biotic stress. It includes an increased synthesis of natural compounds (e.g. H_2S, cysteine, methionine, glutathione), the degradation of glycosides (e.g. glucosinolates) and the synthesis of new compounds (e.g. phytoalexins; Figure 1) (Haneklaus et al., 2004). Supposedly, these S-containing defense compounds are released in a chain reaction triggered by the pathogen and controlled by the S status of the plant (Haneklaus et al., 2004, Figure 1).

Cysteine is the precursor of all relevant S-containing metabolites putatively involved in SIR (Figure 1) and therefore it might be assumed that cysteine is one of the cornerstones of plant resistance against pathogens. Previous studies have shown that the cysteine concentration in plant tissues is strongly related to the S nutritional status of plants (De Kok, 1990; Schnug, 1997) and that cysteine is enriched in resistant plant tissues (Vidhyasekaran, 2000). Cysteine can be rapidly degraded to H_2S or metabolized to other compounds that are putatively involved in pathogenesis (Figure 1).

H_2S is fungitoxic and plants have the ability to release H_2S and other gaseous S compounds into the atmosphere by different enzymatic reactions (Schroeder, 1993; Burandt et al., 2001; Bloem et al., 2004). Glutathione (γ-glutamyl-cysteinyl-glycine) (GSH) was found to accumulate rapidly in response to fungal attack (Vanacker et al., 2000; Bloem et al., 2004; Salac et al., 2004) and this was proven to be related to pathogenesis (Gullner and Kömives, 2001). Glucosinolates (GSLs) undergo hydrolysis, catalyzed by the enzyme myrosinase, to produce an array of products of which isothiocyanates are a major component (Luethy and Matile, 1984). These compounds, and other products of GSL hydrolysis, have been shown to be toxic or inhibitory to many

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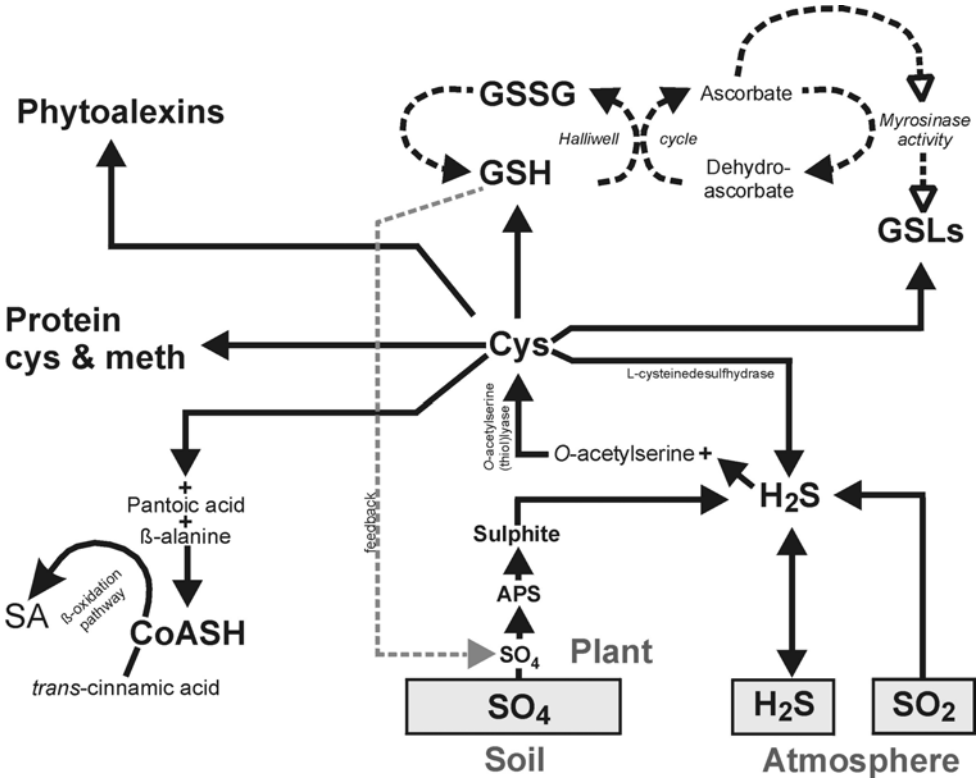


Figure 1:
Sulfur metabolites and pathways putatively involved in chain reactions of SIR in *Brassica* species (Haneklaus et al., 2004).

species of fungi and bacteria (Greenhalgh and Mitchell, 1976; Mithen et al., 1987; Doughty et al., 1991). Phytoalexin synthesis is induced after infection, involving *de novo* synthesis in the infected plant tissue (Hammerschmidt and Nicholson, 2000). The involvement of phytoalexins in SIR is obscure and can only be speculated from the dependency of their precursors on S. High levels of pathogenesis-related proteins were found to be related with enhanced disease resistance in plants (Bohlmann, 1999; van Loon, 1999). However, their possible role in SIR still requires empirical proof. The significance of the formation of elemental S in plants for defense has been discovered only recently (Williams et al., 2002), but the exact mode of action is still unclear.

Most investigations on the putative role of S-containing compounds in SIR were carried out *in vitro* and in pot experiments. Factors governing initialization and strength of SIR need to be tested, however, under field conditions in order to identify and regulate resistance mechanisms by means of targeted S applications. In a field experiment in Scotland, the influence of soil-applied S fertilization on disease incidence and severity of fungal diseases was tested and set in relation to extent and variation of S-containing metabolites in order to perceive triggers and magnitude of SIR.

Material and methods

Design of the field experiment

A quadri-factorial field experiment was carried out in 2001/2002 in Aberdeen, Scotland (W 2° 13`, N 57° 12`; 60 m a.s.l) on a loamy sand (Humic Podzol according to the FAO classification system). The plot size was 40 m^2. Plots were arranged in a split-plot design in four blocks. Two oilseed rape cultivars with different susceptibilities against *P. brassicae* were grown: *Bristol* (B; susceptible) and *Lipton* (L; resistant) (HGCA Recommended List WOSR 2003). For defining the growth stages (GS) of oilseed rape the BBCH scale was used (Strauss et al., 1994).

S was applied as K_2SO_4 to the soil at rates of 0 (S_0) and 100 kg S ha^{-1} (S_{100}). The K supply was balanced by fertilizing adequate amounts of KCl. The S

dose was split in two equal parts in autumn (GS 04) and in spring (GS 14 - 15). N was supplied as NH_4NO_3 at rates of 100 and 200 kg N ha^{-1}. 100 kg N ha^{-1} was applied to all plots at the start of the vegetation period (GS 14 - 15) and an additional 100 kg N ha^{-1} was fertilized at the beginning of stem elongation (GS 30) to those plots receiving a higher N dose.

Specific fungicides were used against *P. brassicae* infections. Either no fungicides were applied or the plots received 0.4 L ha^{-1} flusilazole (250 g L^{-1}) plus carbendazim (125 g L^{-1}) in autumn (GS 12) and in spring (GS 30), respectively.

Disease assessment

The development of *P. brassicae* was followed up during the whole growth period. Since visible symptoms of *P. brassicae* do not usually occur before February/March, during autumn-winter samples were taken every 1 to 2 weeks by randomly choosing 10 plants from non-treated fungicide plots. After incubating them in a damp chamber over night, the parameters disease incidence (%-age of plants infected) and disease severity (%-age of leaf area infected) were visually assessed. When macroscopic symptoms of infections by *P. brassicae* became visible in the field, the level of fungal infection was assessed visually and directly in all plots at monthly intervals. Besides assessing infections caused by *P. brassicae*, plants were also rated for other major fungal diseases (e.g. *Leptosphaeria maculans*, *Peronospora parasitica*, *Alternaria brassicae*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*).

Sampling procedure

Younger, fully developed leaves of winter oilseed rape were randomly taken from each plot at the beginning of stem elongation (GS 50 - 53). Whole-leaf samples were split and either shock frozen in liquid nitrogen and finally freeze-dried, or dried in a ventilated oven at 60° C until constancy of weight. Additionally, leaf disc samples (16 mm) from leaf areas with visible symptoms of *P. brassicae* infections (+ infection) and without visible symptoms (- infection) were taken from the upper third of the crop. Leaf disc samples were shock frozen in liquid nitrogen before being freeze-dried.

Plant analysis

Oven-dried leaf samples were fine-ground to a particle size < 0.12 mm using a *Retsch* ultra-centrifugal mill and the total S content was determined by X-ray fluorescence spectroscopy according to Schnug and Haneklaus (1999). Freeze-dried leaf material and leaf disc samples were fine-ground in a coffee mill or a mortar, respectively prior to the analysis of organic S compounds. The free cysteine, GSH and GSL content were determined by HPLC analysis according to Hell and Bergmann (1990) and Rosa (1992), respectively.

Statistical calculations

For statistical analysis the SPSS software package version 10 was employed (SPSS, 1999). The GLM multivariate procedure was applied to assess the influence of the treatments on individual parameters. Cultivar, S, N and fungicide were tested as fixed factors. N and fungicide treatment delivered no statistical differences with respect to the investigated parameters and therefore their effect is not shown in the present paper. In order to test the influence of infections by *P. brassicae* on the cysteine and GSH content a one factorial ANOVA was carried out. The Student-Newman-Keuls test was used to determine which means were significantly different from each other at the 5 % significance level ($LSD_{5\%}$).

Results and discussion

Infections by *P. brassicae* were the most important fungal disease in winter oilseed rape in 2001/2002. Infections by *L. maculans* and *P. parasitica* were also found, but only at low levels. Disease progression throughout the vegetation period is illustrated for *P. brassicae* in Figure 2. Usually, *P. brassicae* infects winter oilseed rape plants soon after emergence of the seedlings (Gilles et al., 2000). During experimentation, first infections were found in mid-late October and maximum values for disease incidence and severity were determined in late March/April. At this time, the disease incidence was 91 % if no fungicides were applied (Figure 2). Values of > 25 % plants infected by *P. brassicae* at stem extension indicate a severe infection (Steed and Fitt, 2000). The corresponding value for disease severity was 13 % (Figure 2). In plots where fungicides were applied the disease incidence and severity of *P. brassicae* were lower compared to non-treated fungicide plots, but differences were not consistently significant. *P. brassicae* is a hemi-biotrophic fungus, which means that it becomes necrotrophic in the late developmental stage (Ashby, 1997), a characteristic that might be significant with view to processes involved in SIR (see below). At the time of leaf sampling (GS 50 - 53), additional infections by *L. maculans* (3 % plants infected; 0.01 % leaf area infected) and *P. parasitica* (13 % plants infected; 0.2 % leaf area infected) were found (data not shown).

Disease incidence and severity of *P. brassicae* were independent of the cultivar (Figure 2), though differences had been expected because of their divergent rating (HGCA Recommended List WOSR 2003). There is circumstantial evidence that resis-

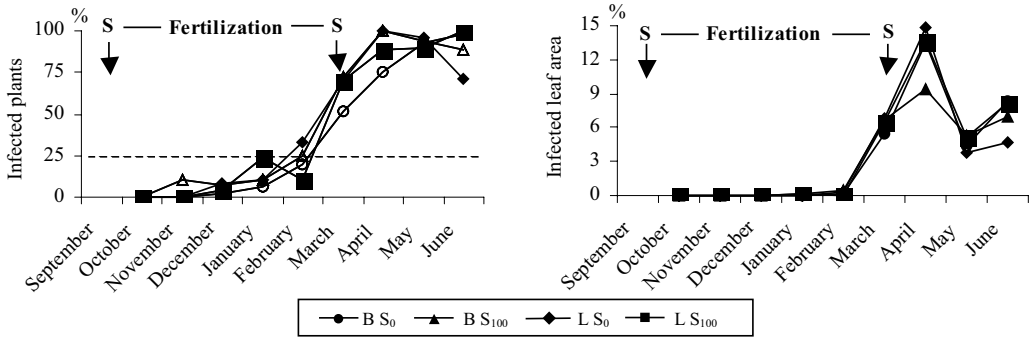


Figure 2:
Disease progression of *Pyrenopeziza brassicae* expressed by the percentage of infected plants (left) and the percentage of the infected leaf area (right) in winter oilseed rape in plots without fungicide applications in relation to cultivar and S rate.

tance against *P. brassicae* in new cultivars is overcome after a few years by changes in the metabolism of pathogen (Karolewski et al., 2004).

Since the S nutritional status of the plant was reported to have a strong impact on its natural resistance against pathogens (Schnug et al., 1995a), S was applied in autumn and spring in order to sufficiently supply the crop and to promote resistance mechanisms. However, in the present study the S applications did not influence disease progression of *P. brassicae* traceably (Figure 2), which indicates that S supply, S uptake, S resistance mechanisms and virulence of the pathogen did not fully coincide. Nevertheless, the data reflect changes in the plant S metabolism caused by S fertilization in combination with fungal infections, which contribute to uncover mechanisms underlying SIR. In this experiment special attention was paid to the metabolites cysteine, GSH and GSLs because of their direct dependence on the S supply (De Kok et al., 1981; Schnug, 1988; Schnug et al., 1995b; Schnug 1997) and their apparent link to SIR (Haneklaus et al., 2004).

The efficacy of S fertilization can be best verified by determining the total S content (Figure 3). S fertilization significantly increased the total S content from 4.2 mg S g^{-1} to 7.9 mg S g^{-1} in *Bristol* and from 4.2 mg S g^{-1} to 7.3 mg S g^{-1} in *Lipton* (Figure 3). In the control plots, the total S content in the leaf tissue was in the range of latent S deficiency (3.5 - 6.5 mg S g^{-1}), i.e. that though no macroscopic symptoms were visible, the S status was not sufficient for a high yielding crop (Schnug and Haneklaus, 1998). The S supply had no influence on disease incidence and severity of *P. brassicae* (Figure 2). This might indicate a temporal discrepancy between S fertilization and S uptake. Another explanation could be that the S doses were not sufficiently high to initiate SIR. Here, a regular S fertilization throughout the growing season might yield the desired effect.

Free cysteine and GSH are S-containing compounds of the primary plant metabolism. These metabolites were found to be involved in plant resistance against fungal pathogens (Vidhyasekaran, 2000; Gullner and Kömives, 2001). The effect of S fertilization and cultivar on the cysteine and GSH content in leaf discs infected and non-infected by *P. brassicae* is shown in Table 1. S fertilization increased the cysteine and GSH content in leaf discs, whereby differences were not consistently significant (Table 1). In greenhouse and field experiments, De Kok et al. (1981), Schnug et al. (1995b) and Bloem et al. (2004) found a significant relationship between S status and the cysteine and GSH content. Relevant in this context is that effects in Aberdeen might have been masked due to the smaller range of variation of the plant S status.

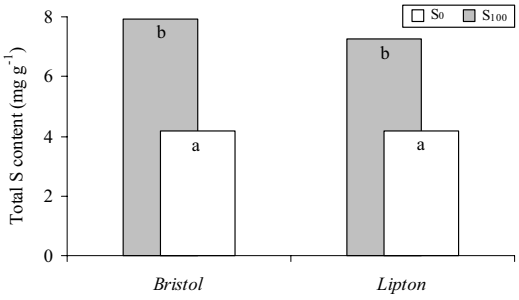


Figure 3:
Influence of S fertilization on the total S content in younger, fully developed leaves (d.w.) of two winter oilseed rape varieties at the start of stem elongation.

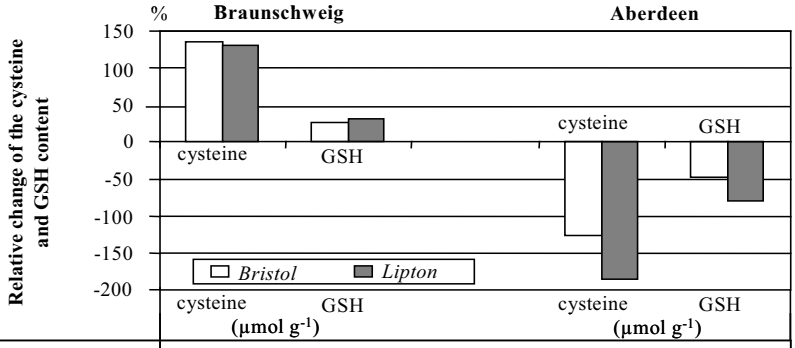
The increase in the content of cysteine and GSH was higher in infected leaf discs compared to non-infected leaf discs (Table 1). Differences in the cysteine and GSH content between the two cultivars were not significant (Table 1).

Table 1:
Influence of S fertilization on the cysteine and glutathione content in leaf discs (d.w.) of two winter oilseed rape varieties at the start of stem elongation.

Treatment		Cysteine (µmol g^{-1})			Glutathione (µmol g^{-1})		
		min	max	mean	min	max	mean
				+ Infection			
Bristol	S_0	0.10	0.44	0.27	4.2	10.3	7.2
	S_{100}	0.30	0.64	0.47	7.6	13.7	10.7
Lipton	S_0	0.01	0.35	0.18	2.6	8.7	5.6
	S_{100}	0.22	0.56	0.39	7.1	13.1	10.1
$LSD_{5\%}$				0.24			4.26
				- Infection			
Bristol	S_0	0.73	0.83	0.78	11.3	13.3	12.3
	S_{100}	0.84	0.94	0.89	13.1	15.1	14.1
Lipton	S_0	0.70	0.80	0.75	12.5	14.5	13.5
	S_{100}	0.83	0.93	0.88	13.8	15.8	14.8
$LSD_{5\%}$				0.74			1.41

In Figure 4, the influence of infections by *P. brassicae* on the cysteine and GSH content at two experimental sites, in Aberdeen (Scotland) and Braunschweig (Germany) is shown. When plant material was visually infected by *P. brassicae*, a significant 2.5-fold and 1.6-fold decrease of the cysteine and GSH content, respectively was found in Aberdeen (Figure 4; Table 1). In contrast, in experiments with the same cultivars in Braunschweig in 2002, infections by *P. brassicae* resulted in an increase of the cysteine and GSH content at the site of infection (Figure 4; Bloem et al., 2004). Additionally, the activity of the enzyme L-cysteine desulfhydrase increased (Bloem et al., 2004). Other researchers also showed that fungal infections generally yield an increase in the GSH content (Vanacker et al., 2000; Gullner and Kömives, 2001; Williams et al., 2002). Two scenarios are possible which could explain these different findings. Firstly, on sites with a higher S supply, reflected in higher total S concentrations (4.8 mg S g^{-1} in Braunschweig *vs*. 4.2 mg S g^{-1} in Aberdeen), a correspondingly higher cysteine (0.7 µmol g^{-1} in Braunschweig vs. 0.5 µmol g^{-1} in Aberdeen) and GSH content (12.1 µmol g^{-1} in Braunschweig vs. 9.7 µmol g^{-1} in Aberdeen) can be found in the leaf tissue. Besides this, an increased synthesis of GSH on the Braunschweig site was obviously related to a certain disease severity (Salac et al., 2004). In comparison in Aberdeen, where a continuous and consistently high infection severity for *P. brassicae* existed, particularly from the start of the vegetation period onwards, and the S status being sub-optimum, more S is bound in cysteine and GSH in non-infected tissues. In the infected plant tissues these metabolites were eventually consumed during metabolic protection processes thus yielding significantly lower values.

Secondly, the possibility exists that the plant tissue was severely and lastingly damaged by the pathogen resulting in a shift of anabolic in the favour of catabolic processes. Previous investigations revealed no differences between dry weights of leaves in inoculated and non-inoculated pea leaves by *Mycosphaerella pinodes* (Garry et al., 1996). Necrotic leaf areas are composed of dead cells and assuming a complete degradation and/or translocation of cysteine and GSH in/from necrotic plant tissue, this would imply that if ≤ 50/60 % (*Bristol/Lipton*) and ≤ 17/33 % (Bristol/*Lipton*) of the leaf disc area is impaired by *P. brassicae* at the time of sampling (see Figure 2), a significant decrease in the cysteine and GSH content might be expected in visually infected leaf discs whereby causal reasons remain speculative (see above). In other words, only if > 60 % of the leaf disc area in case of cysteine and > 32 % in case of GSH is severely impaired by *P. brassicae*, reflected in corresponding necroses, the decreases may be attributed to metabolic changes in



		Braunschweig (2002)		Aberdeen (2002)	
		cysteine (μmol g^{-1})	GSH (μmol g^{-1})	cysteine (μmol g^{-1})	GSH (μmol g^{-1})
Bristol	- Infection	0.59 a	10.8 a	0.84 a	13.2 a
	+ Infection	1.39 b	13.7 b	0.36 b	8.95 b
Lipton	- Infection	0.57 a	11.6 a	0.82 a	14.2 a
	+ Infection	1.31 b	15.3 b	0.28 b	7.86 b

Figure 4:
Influence of infections by *P. brassicae* on the cysteine and GSH content in leaf discs (d.w.) of two winter oilseed rape varieties at the start of stem elongation in Braunschweig (2002) and Aberdeen (2002) (source for Braunschweig: Bloem et al., 2004).

the decaying leaf tissue. These simple calculations reveal that the latter scenario may apply for leaf tissues severely impaired by the pathogen.

Glucosinolates are S-containing secondary compounds, which are protective against fungal pathogens (Mithen et al., 1987; Schnug and Ceynowa, 1990; Doughty et al., 1991; Zukalová and Vašák, 2002). Alkenyl GSLs are supposed to take part in the general resistance of plants against fungal pathogens, whereas the synthesis of indole and aromatic GSLs may be involved in the induced resistance (Zukalová and Vašák, 2002). So far, however, no relationship between GSL content or GSL profile in vegetative tissues and crop resistance has been verified (Chen and Andreasson, 2001). Three predominant alkenyl GSLs were detected in leaves of winter oilseed rape in the present study: glucobrassicanapin (4-pentenyl glucosinolate), gluconapin (3-butenyl glucosinolate) and progoitrin (2-hydroxy-3-butenyl glucosinolate) (Table 2). Glucobrassicin (3-indole methyl glucosinolate) and gluconasturtiin (2-phenyl ethyl glucosinolate) were the main indole and aromatic GSLs, respectively found in the vegetative tissue (Table 2). S applications increased the individual and total GSL content in younger leaves of winter oilseed rape at the start of stem elongation in both varieties, but differences proved to be statistically not significant (Table 2). Schnug (1997) found a significant close correlation between S status (from severe to excess S supply) and GSL content (from 3 μmol g^{-1} to 52 μmol g^{-1}) in younger, fully developed leaves of *B. oleracea*. The total GSL content ranged from 2.8 μmol g^{-1} to 5.4 μmol g^{-1} in *Lipton* and *Bristol* (Table 2), which is fairly low compared to values of up to 7.8 μmol g^{-1} found for the variety *Cobra* by Booth et al. (1991).

Glucobrassicanapin, which was found to have biocidal properties (Peterka and Schlosser, 1989), was the predominant alkenyl GSL in the leaf tissue of winter oilseed rape (Table 2). As its concentration was not influenced by the S supply, its significance in preformed resistance appears negligible. As a response to infection an increased indole and aromatic GSL content was determined in the plant tissue (Doughty et al., 1991; Giamoustaris and Mithen, 1995). Besides the degradation of GSLs, a selective accumulation of indole and aromatic GSLs could be mediated physiologically and might contribute to the resistance of plants (Haneklaus et al., 2004).

The cultivars *Bristol* and *Lipton* differed significantly in the progoitrin content (Table 2), but this GSL has no antifungal properties (Mithen et al., 1987; Peterka and Schlosser, 1989). The mean progoitrin content in the leaf tissue was 0.7 μmol g^{-1} for *Lipton* and 0.4 μmol g^{-1} for *Bristol* (Table 2).

Conclusions

Alternative plant protection measures are gaining increasing interest for conventional and organic farming systems. Up till now nutrient induced resistance mechanisms are well known (Datnoff et al., 2003), but still of minor importance in agricultural production. Sulfur induced resistance (SIR) was first observed for oilseed rape by Schnug et al. (1995a) and will be of high relevance in S-deficient production areas. This, however, requires targeted S fertilization strategies, which prompt SIR on production

Table 2:
Influence of S fertilization on the individual and total glucosinolate (GSL) content in younger, fully developed leaves (d.w.) of two winter oilseed rape varieties at the start of stem elongation.

Treatment		Glucobrassicanapin (µmol g^{-1})			Gluconapin (µmol g^{-1})			Progoitrin (µmol g^{-1})		
		min	max	mean	min	max	mean	min	max	mean
Bristol	**S_0**	1.9	3.1	2.5	0.38	0.63	0.51	0.12	0.46	0.29
	S_{100}	2.1	3.3	2.7	0.40	0.65	0.53	0.25	0.59	0.42
Lipton	**S_0**	1.5	2.7	2.1	0.32	0.56	0.44	0.50	0.83	0.67
	S_{100}	1.9	3.2	2.6	0.31	0.57	0.44	0.58	0.98	0.76
$LSD_{5\%}$				0.83			0.17			0.23

Treatment		Glucobrassicin (µmol g^{-1})			Gluconasturtiin (µmol g^{-1})			Total GSL (µmol g^{-1})		
		min	max	mean	min	max	mean	min	max	mean
Bristol	**S_0**	0.11	0.19	0.15	0.19	0.33	0.26	2.8	4.8	3.8
	S_{100}	0.15	0.24	0.19	0.26	0.40	0.33	3.3	5.3	4.3
Lipton	**S_0**	0.10	0.18	0.14	0.22	0.36	0.29	2.9	4.8	3.9
	S_{100}	0.11	0.20	0.15	0.25	0.39	0.32	3.3	5.4	4.4
$LSD_{5\%}$				0.06			0.09			1.34

fields. In this context, the presented research work revealed that:
- S fertilization increased the cysteine, GSH and GSL content;
- disease incidence and severity during the vegetative period obviously play a major role in SIR as changes in the GSH and cysteine content showed corresponding variations;
- for initializing SIR, the S supply needs to follow the actual metabolic demand, which means that: (a) doses higher than the physiological demand might be required; (b) split doses need to be applied in order to match the S demand for S induced processes against fungal infections.

References

Ashby AM (1997) A molecular view through the looking glass: the *Pyrenopeziza brassicae – Brassica* interaction. Adv Bot Res 24:32-70

Bloem E, Riemenschneider A, Volker J, Papenbrock J, Schmidt A, Salac I, Haneklaus S, Schnug E (2004) Sulphur supply and infection with *Pyrenopeziza brassicae* influence L-cysteine desulfhydrase activity in *Brassica napus* L. J Exp Bot 55(406):2305-2312

Bohlmann H (1999) The role of thionins in the resistance of plants. In: Datta SK and Muthukrishnan S (eds) Pathogenesis-related proteins in plants. CER Press, Inc., pp 207-234

Booth EJ, Walker KC, Schnug E (1991) Effect of site, foliar sulphur and nitrogen application on glucosinolate content and yield of oilseed rape (*Brassica napus* L.). Proc. 8th Int. Rapeseed Congress, Saskatoon, Canada, pp 567-572

Bourbos VA, Skoudridakis MT, Barbopoulou E, Venetis K (2000) Ecological control of grape powdery mildew (*Uncinula necator*) http://www.landwirtschaftmrl.badenwuerttemberg.de/la/lvwo/kongress/SULFUR.htlm

Brokenshire T, Channon AG, Wale S (1984) Recognizing oilseed rape disease. Publication 135, The Scottish Agricultural Colleges

Burandt P, Papenbrock J, Schmidt A, Bloem E, Haneklaus S, Schnug E (2001) Genotypical differences in total sulphur contents and cysteine desulfhydrase activities in *Brassica napus* L. Phyton 41:75-86

Chen S, Andreasson E (2001) Update on glucosinolate metabolism and transport. Plant Physiol Biochem 39:743-758

Datnoff L, Elmer W, Huber D (2003) Mineral nutrition and plant diseases. APS Press, St. Paul, MN

De Kok LJ, De Kam JL, Tánczos OG, Kuiper PJC (1981) Sulfate induced accumulation of glutathione and frost tolerance of spinach leaf tissue. Physiol Plant 53:435-438

De Kok LJ (1990) Sulfur metabolism in plants exposed to atmospheric sulfur. In: Rennenberg H et al. (eds) Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Fundamental, Environmental and Agricultural Aspects. SPB Academic Publishers, The Netherlands, pp 111-130

Dore A, Heywood E, Vieno M, Fournier N, Smith R, Weston K, Hall J, Sutton M (2003) Modeling the transport and deposition of sulphur and reduced and oxidized nitrogen in the UK. Status Report to DEFRA

Doughty KJ, Porrter AJR, Morton AM, Kiddle G, Bock CH, Wallsgrove RM (1991) Variation in the glucosinolate content of oilseed rape (*Brassica napus*) leaves. II. Response to infection by *Alternaria brassicae* (Berk.) Sacc Ann Appl Biol 118:469-477

Garry G, Tivoli B, Jeuffroy MH, Citharel J (1996) Effects of Ascochyta blight caused by *Mycosphaerella pinodes* on the translocation of carbohydrates and nitrogenous compounds from the leaf and hull to the seed of dries-pea. Plant Pathol 45:769-777

Giamoustaris A, Mithen R (1995) The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests. Ann Appl Biol 126:347-363

Gilles T, Evans N, Fitt BDL, Jeger MJ (2000) Epidemiology in relation to methods for forecasting light leaf spot (*Pyrenopeziza brassicae*) severity on winter oilseed rape (*Brassica napus*) in the UK. European J Plant Pathol 106:593-605

Greenhalgh JR, Mitchell ND (1976) The involvement of flavour volatiles in the resistance to downy mildew of wild and cultivates forms of *Brassica oleracea*. New Phytologist 77:391-398

Gullner G, Kömives T (2001) The role of glutathione and glutathione-related enzymes in plant-pathogen interactions. In: Grill D et al. (eds) Significance of Glutathione in Plant Adaptation to the Environment. Kluwer Acad. Publ., The Netherlands, pp 202-239

Hammerschmidt R, Nicholson RL (2000) A survey of plant defense responses to pathogens. In: Agrawal AA et al. (eds) Induced Plant Defenses against Pathogens and Herbivores. APS Press, Minnesota, USA, pp 55-71, ISN: 0-89054-242-2

Hell R, Bergmann L (1990) Gamma-glutamylcysteine synthetase in higher plants: catalytic properties and subcellular localization. Planta 180:603-612

Haneklaus S, Bloem E, Schnug E (2004) Interaction of sulfur and plant disease. In: Datnoff L et al. (eds) Mineral Elements and Plant Disease. APS Press, Minneapolis, MN, USA (in press)

HGCA Recommended List WOSR (2003) http://www.oilseedrape.com/pages/trial/dis_res_tables.htm

Luethy B, Matile P (1984) The mustard oil bomb: Rectified analysis of the subcellular organisation of the myrosinase system. Biochem Physiol Pflanzen 179:5-12

Karolewski Z, Arkell DH, Fitt BDL (2004) Field and controlled environment assessment of winter oilseed rape resistance to light leaf spot (*Pyrenopeziza brassicae*). Biannual Meeting 2004 IOBC Working group "Integrated protection in oilseed crops", 30th and 31st of March, UK

Klikocka H, Haneklaus S, Bloem E, Schnug E (2004) Influence of sulfur fertilization on infection of potato tubers (*Solanum tuberosum*) with *Rhizoctonia solani* and *Streptomyces scabies*. J Plant Nutr (in press)

Mithen RF, Lewis BG, Heaney RK, Fenwick GR (1987) Resistance of leaves of *Brassica* species to *Leptosphaeria maculans*. Trans British Mycol Soc 88:525-531

Peterka S, Schlosser E (1989) *In vitro* activity of glucosinolates against *Leptosphaeria maculans* in comparison to the glucosinolate content and susceptibility of seedlings of different *Brassica* spp. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 54 (2a):439-446

Rosa EAS (1992) Glucosinolates in cabbage – a study of their variation throughout the growing season. PhD Thesis Universidade de Tras-Os-Montes E Alto Douro, Vila Real

Salac I, Bloem E, Haneklaus S, Schnug E (2004) Relation between spatial variability of plant physiological parameters in oilseed rape and infection with *Leptosphaeria maculans*. Phyton 44(2):219-233

Schnug E, Pissarek HP (1982) Kalium und Schwefel, Minimumfaktoren des schleswig-holsteinischen Rapsanbaus. Kali-Briefe (Büntehof) 16:77-84

Schnug E (1988) Quantitative und qualitative Aspekte der Diagnose und Therapie der Schwefelversorgung von Raps (*Brassica napus* L.) unter besonderer Berücksichtigung Glukosinolatarmer Sorten. Habilitationsschrift

Schnug E, Ceynowa J (1990) Crop protection problems for double low rape associated with decreased disease resistance and increased pest damage. Proc. Conf. on Crop Protection in Northern Britain, Dundee, pp 275-282

Schnug E, Haneklaus S, Murphy D (1993) Impact of sulfur supply on the backing quality of wheat. Asp Appl Biol 36:337-345

Schnug E, Haneklaus S (1994) Sulphur deficiency in *Brassica napus*: biochemistry, symptomatology, morphogenesis. Landbauforschung Völkenrode 144:1-31

Schnug E, Booth E, Haneklaus S, Walker KC (1995a) Sulphur supply and stress resistance in oilseed rape. Proc. 9th Int. Rapeseed Congress, 4-7 July, Cambridge, UK, pp 229-231

Schnug E, Haneklaus S, Borchers A, Polle A (1995b) Relations between sulphur supply and glutathione, ascorbate and glucosinolate concentrations in *Brassica napus* varieties. J Plant Nutr Soil Sci 158:67-70

Schnug E (1997) Significance of sulphur for the nutritional and technological quality of domesticated plants. In: Cram WJ et al. (eds) Sulphur Metabolism in Higher Plants: Molecular, Ecological and Nutritional Aspects. Backhuys Publ., The Netherlands, pp 109-130

Schnug E, Haneklaus S (1998) Diagnosis of sulphur nutrition. In: Schnug E and Beringer H (eds) Sulphur in Agroecosystems. Kluwer Acad. Publ., The Netherlands, pp 1-38

Schnug E, Haneklaus S (1999) Diagnosis of the nutritional status and quality assessment of oilseed rape by X-ray spectroscopy. Proc. 10th Int. Rapeseed Congress, 26-29 Sept, Canberra, Australia (CD-ROM)

Schnug E, Bloem E, Haneklaus S (2000) Schwefelmangel – jetzt auch in Rüben? Top Agrar 3:122-125

Schroeder P (1993) Plants as source of atmospheric sulphur. In: De Kok LJ et al. (eds) Sulphur Nutrition and Sulphur Assimilation in Higher Plants: Regulatory, Agricultural and Environmental Aspects. SPB Acad. Publ., The Netherlands, pp 253-270

SPSS (1999) SPSS Base 10.0. Application guide. SPSS Inc., USA

Steed J, Fitt BDL (2000) Forecasting light leaf spot on winter oilseed rape. HGCA Project Report No. OS41

Strauss R, Bleiholder H, Van der Boom T, Buhr L, Hack H, Hess M, Klose R, Meier U, Weber E (1994) Einheitliche Codierung der Phänologischen Entwicklungsstadien mono- und dikotyler Pflanzen. Ciba. Geigy AG, Basel

Vanacker H, Carver TLW, Foyer CH (2000) Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hypersensitive response in the barley-powdery mildew interaction. Plant Physiol 123:1289-1300

van Loon, L. C. (1999) Occurrence and properties of plant pathogenesis-related proteins. In: Datta SK and Muthukrishnan S (eds) Pathogenesis-related proteins in plants. CRC Press, Inc., pp 1 - 20

Vidhyasekaran P (2000) Physiology of disease resistance in plants. Volume II. CRC Press, Inc.

Wang J, Zhang J, Ma Y, Wang Li Yang L, Shi S, Liu L, Schnug E (2003) Crop resistance to diseases as influenced by sulphur application rates. Proc. 12th World Fertiliser Congress, 3-9 August 2001, Beijing, China, pp 1285-1296

Williams JS, Hall SA, Hawkesford MJ, Beale MH, Cooper RM (2002) Elemental sulphur and thiol accumulation in tomato and defence against a fungal vascular pathogen. Plant Physiol 128:150-159

Zukalová H, Vašák J (2002) The role and effect of glucosinolates of *Brassica* species - a review. Rostl Vyr 48:175-180