Phyton (Austria)	**Vol. 29**	Fasc. 2	189–201	17. 11. 1989

Responses of Sulfur Metabolism in Plants to Atmospheric Hydrogen Sulfide *)

By

Luit J. De Kok **)

Received December 14, 1988

Summary

De Kok Luit J. 1989. Responses of sulfur metabolism in plants to atmospheric hydrogen sulfide. – Phyton (Austria) 29 (2): 189–201. – English with German summary.

H_2S is a phytotoxic gas, which can affect plant growth at relatively low levels (≥ 0.1 µl l^{-1}). Atmospheric H_2S was rapidly taken up by plant shoots. H_2S exposure resulted in a rapid accumulation of water-soluble non-protein sulfhydryl compounds in the shoots. In spinach shoots this accumulation was due to enhanced levels of cysteine and glutathione (in darkness also to τ-glutamyl-cysteine). The physiological consequenses of enhanced levels of sulfhydryl compounds, and its relation to the phytotoxicity of H_2S are discussed.

Zusammenfassung

De Kok Luit J. 1989. Wirkungen von atmosphärischem Schwefelwasserstoff auf den Schwefelstoffwechsel in Pflanzen. – Phyton (Austria) 29 (2): 189–201. – Englisch mit deutscher Zusammenfassung.

H_2S ist ein Gas, das in relativ geringen Konzentrationen das pflanzliche Wachstum beeinflussen kann. Atmosphärisches H_2S wird von Pflanzensprossen sehr rasch aufgenommen, es bewirkt eine rapide Anhäufung wasserlöslicher, nicht-proteingebundener Sulfhydrylverbindungen in den Sprossen. In Spinatpflanzen beruht die Akkumulation auf vermehrtem Cystein und Glutathion (im Dunkeln auch auf τ-Glutamyl-Cystein). Die physiologischen Folgen des erhöhten Gehalts an Sulfhydrylverbindungen und die Beziehungen zur Toxizität von H_2S werden diskutiert.

---

*) Lecture held on the FESPP meeting Split September 9, 1988.

**) Luit J. De Kok, Department of Plant Physiology, University of Groningen, P. O. Box 14, 9750 AA Haren, The Netherlands.

Introduction

H_2S is a major component in the global sulfur cycle. Natural sources of H_2S are anaerobic decomposition of sulfur-containing organic matter, volcanic activity and sulfur springs. However, there is also a variety of H_2S emissions due to industrial activities, which may be the cause of local high levels of H_2S in the ambient air. Examples are deposits from the utilization of coal, petroleum, natrual gas, and emission due to water pollution by organic sulfur compounds. E.g. in the southeastern part of the province of Groningen, The Netherlands, the atmosphere is locally polluted with high levels of H_2S (peak levels higher than 1 $\mu l\ l^{-1}$). Here the pollution of the canals with organic material drained off from farina factories, is the source of H_2S (De Kok & al. 1985).

H_2S is a phytotoxic gas which may reduce plant growth at relatively low levels ($\geq 0.1\ \mu l\ l^{-1}$, Thompson & Kats 1978, Krause 1979, De Kok & al. 1983b, Maas & al. 1985, Maas & al. 1987c). Besides growth reduction, atmospheric H_2S levels higher than $0.3\ \mu l\ l^{-1}$ may cause leaf lesions and defoliation (Oliva & Steubing 1976, Steubing & Jäger 1978, Thompson & Kats 1978). However, low levels of H_2S ($\leq 0.1\ \mu l\ l^{-1}$) may stimulate plant growth (Thompson & Kats 1978, De Kok & al. 1983b). The physiological and biochemical basis for the observed effects of H_2S on plants is largely unknown.

The present paper reviews and presents new data on the response of sulfur metabolism of plants with have to cope with H_2S in the atmosphere, and discusses the relation between a disturbed regulation of sulfur assimilation by H_2S and the phytotoxicity of this air pollutant.

H_2S uptake by plants

Atmospheric H_2S was rapidly taken up by plants and could be used a sulfur source (Faller 1972, Brunold & Erismann 1974, 1975). Cope & Spedding 1982 and Taylor & al. 1983 observed high H_2S fluxes into the leaves upon short-term exposure (30 and 240 min) to low atmospheric H_2S levels (0.04 and $0.12\ \mu l\ l^{-1}$). The leaf conductance for H_2S influx depended on the metabolic activity and only at a high metabolic activity it was close to stomatal conductance (Cope & Spedding 1982). De Kok & al. 1989 observed that the H_2S flux to shoots was almost linear with concentration up to around $0.3\ \mu l\ l^{-1}$ H_2S; at higher concentrations it reached a maximum. The H_2S flux to shoots proceeded predominantly via the stomata and was rather constant up to 4 days of exposure to both 0.2 and $0.8\ \mu l\ l^{-1}$ H_2S. Up to an atmospheric H_2S concentration about $0.3\ \mu l\ l^{-1}$, the shoot conductance for H_2S influx of various species varied between 60 to 90% of the H_2S influx predicted from shoot conductance for aqueous vapor efflux (stomatal conductance). It was proposed that a rapid incorporation of H_2S into com-

pounds of the assimilatory sulfur pathway was responsible for the high H_2S absorption by the plants (COPE & SPEDDING 1982, DE KOK & al. 1989).

Accumulation of sulfur compounds upon H_2S exposure

In general, prolonged exposure of plants to H_2S resulted in an increased sulfur content of the leaves (BRUNOLD & ERISMANN 1974, STEUBING & JÄGER 1978, THOMPSON & KATS 1978). Long-term exposure of *Spinacia oleracea* L. and *Beta vulgaris* L. to 0.03, 0.1 and 0.3 $\mu l\ l^{-1}$ H_2S resulted in an increase in water-soluble sulfhydryl compounds of the shoots (DE KOK & al. 1986). The level of accumulation depended on the H_2S concentration. Although ageing of the plants caused a decrease of the actual sulfhydryl content of the shoots, the H_2S induced increase in sulfhydryl content was observed during the entire 6 weeks exposure period. A similar H_2S induced accumulation was observed in shoots of *Zea mays* L. after two weeks of exposure (DE KOK & al. 1983b).

Short-term exposure of spinach to H_2S resulted in a rapid accumulation of water-soluble non-protein sulfhydryl compounds in spinach shoots (DE KOK & al. 1985, MAAS & al. 1987; Table 1). Only prolonged H_2S fumigation (longer than two days) resulted in substantially enhanced sulfate levels in the shoots (MAAS & al. 1985, 1987a, c, Table 1). Already after one hour of exposure of spinach to 0.25 $\mu l\ l^{-1}$ H_2S, a significant increase in sulfhydryl content was noticed. In general, at this concentration maximum accumulation, up to 3- to 4-fold of that in control plants, was observed after

Table 1.

Effect of H_2S on the levels of water-soluble non-protein sulfhydryl compounds and sulfate in spinach shoots. Plants were exposed to H_2S according to DE KOK & al. 1986b and sulfhydryl and sulfate contents were determined as described by MAAS & al. 1987a.

	Initial value	Exposure 2 days	4 days
SH content ($\mu mol\ g^{-1}$ f. wt.)[1]			
0 $\mu l\ l^{-1}$ H_2S	0.30 ± 0.02	0.27 ± 0.03	0.25 ± 0.03
0.25 $\mu l\ l^{-1}$ H_2S	—	0.75 ± 0.12	0.92 ± 0.12
Sulfate content ($\mu mol\ g^{-1}$ f. wt.)[1]			
0 $\mu l\ l^{-1}$ H_2S	3.77 ± 0.30	3.35 ± 0.37	3.19 ± 0.17
0.25 $\mu l\ l^{-1}$ H_2S	—	4.18 ± 0.42	5.64 ± 0.25

[1]) Means of 3 measurements with 3 shoots each.

24 h of exposure (De Kok & al. 1985, Maas & al. 1987a). Even exposure of spinach in the dark, under conditions where the stomata were not closed, a H_2S-induced increase in sulfhydryl content of the shoots could be noticed (De Kok & al. 1985).

The level of sulfhydryl accumulation in shoots depended on the H_2S concentration and for spinach it was maximum at 1 $\mu l\ l^{-1}$ (5-fold of that of untreated plants) after 24 h of exposure (Maas & al. 1987b). H_2S-induced sulfhydryl accumulation was also affected by temperature during the exposure (Maas & al. 1987a, b), e.g. at 15°C the sulfhydryl level was 1.6-fold higher than that at 25°C after 48 h of exposure to 0.25 $\mu l\ l^{-1}$ H_2S (Maas & al. 1987). However, the light intensity during the H_2S exposure did not affect the level of sulfhydryl accumulation (De Kok & al. 1985, Maas & al. 1987b).

Sulfhydryl accumulation in spinach shoots upon H_2S exposure was due to enhanced levels of cysteine and glutathione (GSH; De Kok & al. 1988, Buwalda & al. 1988, Table 2). However, upon dark exposure also substantial quantities of γ-glutamyl-cysteine accumulated (Buwalda & al. 1988). A comparison of the response of the different leaves of spinach demonstrated, that young leaves were characterized by a high sulfhydryl content (Table 2). After a 24 h exposure to 0.25 $\mu l\ l^{-1}$ H_2S, young leaves also accumulated higher levels of sulfhydryl compounds than older leaves. However, relative accumulation expressed as ratio of sulfhydryl content of H_2S exposed leaves to untreated leaves was constant for leaves of different age (around 3.4). Differences in sulfhydryl content between leaves could be only partially explained by differences in dry matter content and were more likely an illustration of the metabolic activity of the tissue. Young leaves were also characterized by a high amino acid content. The amino acid content was not affected after a 24 h exposure to 0.25 $\mu l\ l^{-1}$ H_2S. In light GSH was the major sulfhydryl compound present in untreated spinach leaf tissue (Buwalda & al. 1988, De Kok & al. 1988). In untreated spinach plants, the cysteine content accounted for only 12% or less of the water-soluble non-protein sulfhydryl compounds (Buwalda & al. 1988, De Kok & al. 1988; Table 2). However, after a 24 h exposure to 0.25 $\mu l\ l^{-1}$ H_2S, the cysteine content could account for up to 50% of the sulfhydryl compounds (De Kok & al. 1988, Buwalda & al. 1988; Table 2.). In both young and older spinach leaves there was a more than 10-fold increase in the cysteine content of upon H_2S exposure (Table 2). In older leaves cysteine was the major sulfhydryl compound which accumulated after a 24 h H_2S exposure (65% of the accumulated sulfhydryl compounds), however, in young leaves this was GSH (67% of the accumulated sulfhydryl compounds; GSH content was calculated by subtracting the cysteine content from the total sulfhydryl content; Table 2).

Oxidized glutathione level was rather low in spinach shoots (about 0.03 $\mu mol\ g\ fresh\ weight^{-1}$) and its content was only increased 2-fold after a 48 h of exposure to 0.25 $\mu l\ l^{-1}$ (De Kok & al. 1986a). The glutathione

reductase activity was not substantially affected by H_2S exposure (De Kok & al. 1986a).

Short-term H_2S exposure did not affect the water-soluble protein sulfhydryl content (De Kok & al. 1985).

When the H_2S exposure was ceased, the accumulated sulfhydryl compounds in spinach shoots disappeared rapidly (De Kok & al. 1985, 1986a, Maas & al. 1987b). The major fraction of the accumulated sulfhydryl compounds had disappeared within 48 h after the termination of the H_2S pre-exposure. The rate of decrease was not significantly affected by temperature (15, 20 and 25°C), photo fluence rate (60 and 170 μmol m^{-2} s^{-1})

Table 2.

Effect of H_2S on the levels of water-soluble non-protein sulfhydryl compounds and amino acids in leaves of 26 days old spinach plants. Plants were exposed in continuous light for 24 hours under conditions as described by De Kok & al. 1988. Sulfhydryl groups were determined according to De Kok & al. 1988 and amino acids as described by Stuiver & al. 1988.

		Leaves			
		1st pair	2nd pair	3th pair	Combined
Fresh weight[1])	(g)	1.54 ± 0.40	2.18 ± 0.51	0.93 ± 0.35	4.69 ± 0.49
Dry matter content[2])	(%)	7.1 ± 0.1	9.8 ± 0.6	13.1 ± 0.5	9.6 ± 0.4
Amino acid content[2]) (μmol g^{-1} f. wt.)[2])					
0 μl l^{-1} H_2S		2.8 ± 0.2	7.1 ± 1.0	9.8 ± 0.5	6.1 ± 0.3
0.25 μl l^{-1} H_2S		2.7 ± 0.3	6.9 ± 0.1	10.8 ± 0.2	5.8 ± 0.0
SH content (μmol g^{-1} f. wt.)[2])					
0 μl l^{-1} H_2S		0.17 ± 0.01	0.33 ± 0.04	0.46 ± 0.01	0.31 ± 0.01
0.25 μl l^{-1} H_2S		0.60 ± 0.04	1.13 ± 0.14	1.58 ± 0.14	1.02 ± 0.07
Cysteine content (μmol g^{-1} f. wt.)[2])					
0 μl l^{-1} H_2S		0.02 ± 0.00	0.04 ± 0.01	0.03 ± 0.02	0.03 ± 0.00
0.25 μl l^{-1} H_2S		0.30 ± 0.02	0.45 ± 0.07	0.40 ± 0.01	0.40 ± 0.04
Calculated GSH content (μmol g^{-1} f. wt.)[2])					
0 μl l^{-1} H_2S		0.15 ± 0.01	0.29 ± 0.04	0.43 ± 0.03	0.28 ± 0.01
0.25 μl l^{-1} H_2S		0.30 ± 0.02	0.66 ± 0.06	1.18 ± 0.14	0.63 ± 0.03

[1]) Means of 18 measurements;

[2]) means of 3 measurements with 3 shoots each.

or H_2S concentration (0.25 and 0.5 $\mu l\ l^{-1}$, MAAS & al. 1987b). There was no increase in the oxidized glutathione content in spinach shoots (it even decreased), and no detectable emission of H_2S by shoots was noticed after the H_2S exposure was terminated (DE KOK & al. 1986a). The latter indicated that desulfhydration of cysteine was not significantly involved in the decrease of sulfhydryl compounds. It was suggested that the excessive sulfhydryl compounds (cysteine and GSH) were rapidly metabolized and used e.g. for protein synthesis (DE KOK & al. 1986).

In order to obtain insight into the regulatory aspects of H_2S-induced sulfhydryl accumulation spinach leaf discs were simultaneously exposed to atmospheric H_2S and incubated with sulfate (liquid phase) or to H_2S and inhibitors of sulfate reduction and GSH synthesis (Table 3). Exposure of spinach leaf discs to H_2S in light resulted in comparable strongly increased levels of sulfhydryl compounds than that observed in intact leaves. After a 24 h exposure to 0.5 $\mu l\ l^{-1}$ H_2S there was a twelve-fold and two-fold increase in cysteine and GSH content, respectively.

Incubation of spinach leaf discs with high concentrations of Na_2SO_4 resulted in an increased de novo reduction of sulfate and its subsequent incorporation into sulfhydryl compounds (DE KOK & al. 1981, 1988, DE KOK & KUIPER 1986; Table 3). A 24 hour incubation of spinach leaf discs with 25 mM Na_2SO_4 (at this concentration sulfhydryl accumulation was maximum) in the light resulted in a about six-fold increase in the cysteine and two-fold increase in the GSH content (Table 3). A combined exposure of leaf discs to Na_2SO_4 and H_2S demonstrated, that the H_2S- and the sulfate-induced sulfhydryl accumulation were not additional; it was intermediate. This indicated, that the sulfide produced by sulfate reduction and the atmospheric sulfide absorbed by the leaf discs competed for the same substrates utilized for the synthesis of cysteine and GSH. The observation that also the cysteine accumulation was intermediate (nine-fold) indicated that H_2S- and sulfate-induced sulfhydryl accumulation occurred simultaneously.

Incubation of spinach leaf discs with 0.25 mM selenate, an inhibitor of sulfate reduction (DE KOK & KUIPER 1986), resulted in a substantial decrease in the GSH content, however, the cysteine content was not affected (Table 3). Higher concentrations of selenate even strongly decreased sulfhydryl content, but also resulted in visible injury of the leaf discs. The observed selenate-induced decrease in GSH content indicated a rapid turnover of GSH in the light. Apparently under conditions where sulfate reduction is reduced, GSH may be utilized as sulfur source. Incubation of spinach leaf discs with selenate reduced strongly both H_2S-induced cysteine and GSH accumulation (Table 3). This may indicate that due to inhibition of sulfate reduction by selenate, part of the absorbed atmospheric H_2S was directly utilized for synthesis of other sulfur containing compounds e.g. proteins.

Incubation of spinach leaf discs with 1 mM buthionine sulfoximine (BSO), a selective inhibitor of τ-glutamyl-cysteine synthetase (MEISTER 1983), slightly reduced sulfhydryl compound content, however, resulted in a strong decrease in GSH content, which was replaced by cysteine (Table 3). After BSO incubation the cysteine content accounted for 76% of the sulfhydryl compounds. The observed decrease of GSH content in presence of BSO again demonstrated a rapid in situ turnover of this sulfhydryl compound in the light. In addition, the observed high levels of cysteine in spinach leaf discs after incubation with BSO, demonstrated that its intracellular level was not controlled by feedback inhibition of cysteine synthetase. BSO only slightly reduced H_2S-induced accumulation of sulfhydryl compounds (Table 3). Also here cysteine was the major sulfhydryl compound present in the leaf discs (71%). In comparison with untreated spinach leaf discs, the GSH content was unaltered by a combined exposure to BSO and H_2S. This may indicate that GSH was not rapidly metabolized under conditions where cysteine was present in levels which exceed the metabolic needs for reduced sulfur. The observation that H_2S induced sulfhydryl

Table 3.

Effect of H_2S, Na_2SO_4, Na_2SeO_4 and buthionine sulfoximine (BSO) on the levels of water soluble non-protein sulfhydryl compounds of spinach leaf discs. Leaf discs were exposed in continuous light for 24 hours. Experimental conditions and determination of the sulfhydryl compounds were according to DE KOK & al. 1988.

	Treatment			
	Untreated[1])	Na_2SO_4[2]) (25 mM)	Na_2SeO_4[2]) (0.25 mM)	BSO[2]) (1 mM)
SH content (nmol leaf disc^{-1})				
0 µl l^{-1} H_2S	40 ± 4	99 ± 5	19 ± 1	33 ± 1
0.5 µl l^{-1} H_2S	140 ± 13	121 ± 5	80 ± 5	120 ± 4
Cysteine content (nmol leaf disc^{-1})				
0 µl l^{-1} H_2S	5 ± 1	29 ± 3	5 ± 1	25 ± 1
0.5 µl l^{-1} H_2S	61 ± 5	45 ± 3	39 ± 4	85 ± 4
Calculated GSH content (nmol leaf disc^{-1})				
0 µl l^{-1} H_2S	35 ± 3	70 ± 3	14 ± 1	8 ± 0
0.5 µl l^{-1} H_2S	79 ± 9	75 ± 2	41 ± 0	36 ± 0

[1]) Means of 9 measurements and
[2]) means of 3 measurements with 10 leaf discs each.

accumulation was hardly affected under conditions where synthesis of τ-glutamyl-cysteine and thus GSH was inhibited, indicated that metabolization of the absorbed atmospheric H_2S was not subject to feedback regulation by cysteine or GSH.

Physiological significance of H_2S-induced sulfhydryl accumulation

The sulfur assimilation in plants is subject to regulation. A feedback control of the sulfate reduction by reduced sulfur compounds, especially on the site of adenosine 5'-phosphosulfate sulfotransferase, was noticed (BRUNOLD & SCHMIDT 1976, 1978, WYSS & BRUNOLD 1980, VON ARB & BRUNOLD 1986, BRUNOLD & al. 1987). DE KOK & KUIPER (1986) proposed that the sulfate concentration at the ATP sulfurylase reaction site may also play an important role in the regulation of sulfur assimilation in the plant. Also the ability of plants to emit volatile sulfur compounds (H_2S) by the leaves may play a role in the regulation of sulfur assimilation (RENNENBERG 1982, FILNER & al. 1984).

The observed rapid accumulation of sulfhydryl compounds in the presence of H_2S indicated a direct entry of the reduced sulfur into cysteine and subsequently into GSH. RENNENBERG 1984 proposed that sulfhydryl compounds accumulate in plants, when the reduced sulfur supply exceeds the metabolic sulfur needs. Exposure of leaf tissue to high sulfate levels (GRILL & al. 1979, RENNENBERG & BERGMANN 1979, DE KOK & al. 1981, DE KOK & KUIPER 1986, BRUNOLD & al. 1987; Table 3) or SO_2 (GRILL & ESTERBAUER 1972, 1973, GRILL & al. 1979, 1980, 1982, CHIMENT & al. 1986, MAAS & al. 1987a, b, c, DE KOK & al. 1988) also resulted in enhanced sulfhydryl levels. In comparison to H_2S, the level of sulfhydryl accumulation upon SO_2 exposure was much lower at an equal concentration (MAAS & al. 1987 a, b, c). However, in contrast with H_2S, SO_2 exposure resulted in a more rapid accumulation of sulfate. Here, a substantially increased sulfate content was already noted after 24 h of exposure to 0.25 $\mu l\ l^{-1}$. At this concentration the accumulation of sulfate in spinach shoots was almost linear with the duration of fumigation for at least up to 6 days (MAAS & al. 1987b). Similar to H_2S, the increase in sulfhydryl content upon exposure of spinach leaf tissue to SO_2 or high levels of sulfate, could also be ascribed to enhanced levels of GSH and cysteine (BUWALDA & al. 1988, DE KOK & al. 1988; Table 2).

The occurrence of high levels of both cysteine and GSH (and τ-glutamyl-cysteine in darkness) upon H_2S, SO_2 or sulfate exposure (BUWALDA & al. 1988, DE KOK & al. 1988; Table 2, 3), and the observation that the specific increase of the cysteine pool by BSO, an inhibitor of GSH synthesis, did not significantly affected the H_2S-induced sulfhydryl accumulation (Table 3), indicated a poorly controlled intracellular level of

cysteine in plants in the presence of excess sulfur (DE KOK & al. 1988). This is in contrast with that, which had been suggested before (RENNENBERG 1982, 1984, FILNER & al. 1984). Even after prolonged exposure of spinach to H_2S, high levels of both cysteine and GSH were present. E. g. a two weeks exposure of spinach plants to 0.4 µl l^{-1} H_2S resulted in almost thirty-fold and three-fold increase in cysteine and GSH content of the second pair of leaves, respectively (L. J. DE KOK, unpublished results). This demonstrated that upon prolonged exposure to H_2S, regulation of the intracelluar sulfhydryl compounds was not altered in such a way that low cysteine levels were obtained.

From H_2S flux measurements it was estimated that 25% of the total H_2S taken up by spinach shoots could be revealed in the water-soluble non-protein sulfhydryl fraction after an exposure to 0.2 or 0.8 µl l^{-1} H_2S for 3 days (DE KOK & al. 1989). These data indicated that during the first 24 h of exposure the greater part of the H_2S taken up by the shoots was incorporated into sulfhydryl compounds, since maximum accumulation of these compounds was reached after this period. In addition, the observation that H_2S flux to shoots was rather constant up to 4 days of exposure, indicated that the rate of accumulation of sulfhydryl compounds in the shoots was not a reflection of the actual H_2S uptake by plants (DE KOK & al. 1989). The fate of the remaining fraction of H_2S taken up by the plant was unclear. Part of the reduced sulfur could be utilized for plant growth (MAAS & al. 1987 c, DE KOK & al. 1989) or may be oxidized and responsible for the observed increased sulfate content upon prolonged H_2S exposure (MAAS & al. 1985, 1987 a, b, c; Table 1). Whether photosynthetic electron transport is involved in the oxidation of sulfide needs further study (DE KOK & al. 1983 a). At least part of the accumulated reduced sulfur compounds were transported to the roots, since exposure of spinach to H_2S resulted also in a two-fold increase in GSH content in the roots (from 2.6 to 5.1 µmol g dry weight^{-1}) after an exposure to 0.25 µl l^{-1} for 2 days (F. BUWALDA & W. VISSER, personal communication). They observed no substantial increase in the cysteine content of the roots upon H_2S exposure, which supported the suggestion of RENNENBERG & al. (1982, 1984), that GSH is the major transport form of reduced sulfur in the plant.

From the observed high H_2S fluxes to shoots and the rapid incorporation of H_2S into cysteine, γ-glutamyl-cysteine and GSH, a direct involvement the sulfur assimilatory pathway in the rapid H_2S absorption was suggested (DE KOK & al. 1989). They proposed that cysteine synthase played an direct role in fixation of H_2S.

Physiological background of the phytotoxicity of H_2S

The physiological consequences of enhanced levels of sulfhydryl compounds in plants in the presence of H_2S are unknown. Evidently, some

species may tolerate high levels of sulfhydryl compounds in the shoots in the presence of H_2S, without phytotoxic effects (MAAS & al. 1987c). However, the composition of the sulfhydryl pool and the subcellular localization of the accumulated sulfhydryl compounds in the different species needs to be studied. It was proposed that GSH may function as a temporary storage compound of excess sulfur (RENNENBERG 1982, 1984; DE KOK & al. 1986a). However, GRILL & al. 1979, 1980, 1982 suggested that permanent high levels of GSH may alter regulation of cellular metabolism and may result in phytotoxic effects. RENNENBERG 1981 has reported that high intracellular levels of cysteine are toxic to plant cells. DE KOK & al. 1988 observed that in vitro cysteine may react with aldehydes. Whether reactions of cysteine with metabolically active aldehydes are involved in its toxicity, and the relation between its accumulation and the phytotoxicity of H_2S (SO_2) needs further investigation.

DE KOK & al. 1989 observed that there was no direct relation between the H_2S absorption by shoots and the sensitivity of the species to H_2S. This indicated, that the toxic reaction of H_2S with cellular constituents, resulting in a reduced growth, was not directly related to the quantity of H_2S incorporated into the shoots with time. They proposed, that the in situ sulfide concentration at the reaction site, or the cellular localization of produced toxic components, or the degree of penetration of sulfide to e. g. the meristems were more important factors in the occurrence of H_2S induced growth reduction.

Even though photosynthetic processes may be reduced upon H_2S exposure (TAYLOR & SELVIDGE 1984, MAAS & al. 1988), it has been demonstrated that a direct effect on photosynthesis was not the initial cause of the phytotoxicity of H_2S for spinach. MAAS & al. 1987a observed equal yield reduction after a 14 days exposure of spinach to 0.25 $\mu l\ l^{-1}$ H_2S, either only during the 12 h photoperiod or only during the 12 h dark period.

H_2S may also alter amino acid metabolism and nitrogen assimilation in plants (VAN DIJK & al. 1986, DE KOK & al. 1986b). However, it is unclear whether the effects on nitrogen metabolism are involved in the phytotoxicity of H_2S.

The activity of NADH oxidizing enzymes of spinach shoots was reduced by H_2S exposure. This reduction was maximum after a two day exposure and then could be related to reduction in relative growth rate by H_2S (MAAS & DE KOK 1988). The in vitro NADH oxidation capacity by shoot extracts showed promise as an early indicator for growth reduction by H_2S.

Acknowledgements

The author wishes to thank Dr. I. STULEN, Mrs. C. E. E. STUIVER and Mr. F. BUWALDA for critical reading of the manuscript.

References

BRUNOLD C. & ERISMANN K. H. 1974. H_2S als Schwefelquelle bei *Lemna minor* L.: Einfluß auf das Wachstum, den Schwefelgehalt und die Sulfataufnahme. – Experientia 30: 465–467.

BRUNOLD C. & ERISMANN K. H. 1975. H_2S as sulfur source in *Lemna minor* L.: II. Direct incorporation into cysteine and inhibition of sulfate assimilation. – Experientia 31: 508–510.

BRUNOLD C. & SCHMIDT A. 1976. Regulation of adenosine 5'-phosphosulfate sulfotransferase activity by H_2S in *Lemna minor* L. – Planta 133: 85–88.

BRUNOLD C. & SCHMIDT A. 1978. Regulation of sulfate assimilation in plants. 7. Cysteine inactivation of adenosine 5'-phosphosulfate sulfotransferase in *Lemna minor* L. – Plant Physiol. 61: 342–347.

BRUNOLD C., SUTER M. & LAVANCHY P. 1987. Effect of high and low sulfate concentrations on adenosine 5'-phosphosulfate sulfotransferase activity from *Lemna minor* – Physiol. Plant. 70: 168–174.

BUWALDA F., DE KOK L. J., STULEN I. & KUIPER P. J. C. 1988. Cysteine, γ-glutamylcysteine and glutathione contents of spinach leaves as affected by darkness and application of excess sulfur. – Physiol. Plant. 74: 663–668.

CHIMENT J. J., ALSCHER R. & HUGHES P. R. 1986. Glutathione as an indicator of SO_2-induced stress in soybean. – Environ. Exp. Bot. 26: 147–152.

COPE D. M. & Spedding, D. J. 1982. Hydrogen sulphide uptake by vegetation. – Atmos. Environ. 16: 349–353.

DE KOK L. J. & KUIPER P. J. C. 1986. Effect of short-term dark incubation with sulfate, chloride and selenate on the glutathione content of spinach leaf discs. – Physiol. Plant. 68: 477–482.

DE KOK L. J., DE KAN P. J. L., TANCZOS O. G. & KUIPER P. J. C. 1981. Sulphate-induced accumulation of glutathione and frost-tolerance of spinach leaf tissue. – Physiol. Plant 53: 435–438.

DE KOK L. J., THOMPSON C. R. & KUIPER P. J. C. 1983 a. Sulfide-induced oxygen uptake by isolated spinach chloroplasts catalyzed by photosynthetic electron transport. – Physiol. Plant. 59: 19–22.

DE KOK L. J., THOMPSON C. R., MUDD J. B. & KATS G. 1983 b. Effect of H_2S fumigation on water-soluble sulfhydryl compounds in shotts of crop plants. – Z. Pflanzenphysiol. 111: 85–89.

DE KOK L. J., BOSMA W., MAAS F. M. & KUIPER P. J. C. 1985. The effect of short-term H_2S fumigation on water-soluble sulphhydryl and glutathione levels in spinach. – Plant. Cell & Environment 8: 189–194.

DE KOK L. J., MAAS F. M., GODEKE J., HAAKSMA A. B. & KUIPER P. J. C. 1986 a. Glutathione, a tripeptide which may function as a temporary storage of excessive reduced sulphur in H_2S fumigated spinach plants. – Plant & Soil 91: 349–352.

DE KOK L. J., STULEN I., BOSMA W. & HIBMA J. 1986 b. The effect of short-therm H_2S fumigation on nitrate reductase activity in spinach leaves. – Plant & Cell Physiol. 27: 1249–1254.

DE KOK L. J., BUWALDA F. & BOSMA W. 1988. Determination of cysteine and its accumulation in spinach leaf tissue upon exposure to excess sulfur. – J. Plant Physiol. 133: 502–505.

De Kok L. J., Stahl K. & Rennenberg H. 1989. Fluxes of atmospheric hydrogen sulfide to plant shoots. – New Phytol. 112 (4) (in press).

Faller N. 1972. Schwefeldioxid, Schwefelwasserstoff, nitrose Gase und Amoniak als ausschließliche S- bzw. N-Quellen der höheren Pflanzen. – Z. Pflanzenernähr. Düngg. Bodenkde. 131: 120–130.

Filner P., Rennenberg H., Sekya J., Bressan R. A., Wilson L. G., Le Cureux L. & Shimei T. 1984. Biosynthesis and emission of hydrogen sulfide by higher plants. pp. 291–312. In: Koziol, M. J. & Whatley, F. R. (Eds.). Gaseous Air Polutants and Plant Metabolism. – Butterworths, Borough Green, England.

Grill D. & Esterbauer H. 1973a. Quantitative Bestimmung wasserlöslicher Sulfhydrylverbindungen in gesunden und SO_2-geschädigten Nadeln von *Picea abies*. – Phyton (Austria) 15: 87–101.

Grill D., Esterbauer H. 1973b. Cystein und Glutathion in gesunden und SO_2-geschädigten Fichtennadeln. – Eur. J. For. Path. 3: 65–71.

Grill D. & Esterbauer H. & Klösch U. 1979. Effect of sulfur-dioxide on glutathione in leaves of plants. – Environ. Pollut. 17: 187–194.

Grill D., Esterbauer H., Scharner M. & Felgitsch Ch. 1980. Effect of sulfur-dioxide on protein-SH in needles of *Picea abies*. – Eur. J. For. Path. 10: 263–267.

Grill D., Esterbauer H. & Hellig K. 1982. Further studies on the effect of SO_2-pollution on the sulfhydryl-system of plants. – Phytopath. Z. 104: 264–271.

Krause G. H. M. 1979. Relative Phytotoxizität von Schwefelwasserstoff. – Staub-Reinhalt. Luft 39: 165–167.

Maas F. M. & De Kok L. J. 1988. *In vitro* NADH oxidation as an early indicator for growth reduction in spinach exposed to H_2S in the ambient air. – Plant & Cell Physiol. 29: 523–526.

Maas F. M., De Kok L. J. & Kuiper P. J. C. 1985. The effect of H_2S fumigation on various spinach *(Spinacia oleracea* L.) cultivars. Relation between growth inhibition and accumulation of sulphur compounds in the plant. – J. Plant Physiol. 119: 219–226.

Maas F. M., De Kok L. J., Hoffmann I. & Kuiper P. J. C. 1987a. Plant responses to H_2S and SO_2 fumigation. I. Effects on growth, transpiration and sulfur content of spinach. – Physiol. Plant. 70: 713–721.

Maas F. M., De Kok L. J., Strik-Timmer W. & Kuiper P. J. C. 1987b. Plant responses to H_2S and SO_2 fumigation. II. Differences in metabolism of H_2S and SO_2 in spinach. – Physiol. Plant. 70: 722–728.

Maas F. M., De Kok L. J., Peters J. L. & Kuiper P. J. C. 1987c. A comparative study on the effects of H_2S and SO_2 fumigation on the growth and accumulation of sulfate and sulfhydryl compounds in *Trifolium pratense* L., *Glycine max* Merr. and *Phaseolus vulgaris* L. – J. Exp. Bot. 38: 1459–1469.

Maas F. M., Van Loo E. N. & Van Hasselt P. R. 1988. Effect of long-term H_2S fumigation on photosynthesis in spinach. Correlation between CO_2 fixation and chlorophyll a fluorescence. – Physiol. Plant. 72: 78–83.

Meister A. 1983. Selective modification of glutathione metabolism. – Science 220: 472–477.

Oliva M. & Steubing L. 1976. Untersuchungen über die Beeinflußung von Photosynthese, Respiration und Wassergehalt durch H_2S bei *Spinacia oleracea*. – Angew. Bot. 50: 1–17.

RENNENBERG H. 1981. Differences in the use of cysteine and glutathione as sulfur source in photoheterotrophic tobacco suspension cultures. – Z. Pflanzenphysiol. 105: 31–40.

RENNENBERG H. 1982. Glutathione metabolism and possible biological roles in higher plants. – Phytochemistry 21: 2771–2781.

RENNENBERG H. 1984. The fate of excess sulfur in higher plants. – Ann. Rev. Plant Physiol. 35: 121–153.

RENNENBERG H. & BERGMANN L. 1979. Influences of ammonium and sulfate on the production of glutathione in suspension cultures of *Nicotiana tabacum*. – Z. Pflanzenphysiol. 92: 133–142.

RENNENBERG H., SEKIJA J., WILSON L. G. & FILNER P. 1982. Evidence for an intracellular sulfur cycle in cucumber leaves. – Planta 154: 516–524.

STEUBING L. & JÄGER H–J. 1978. Oekophysiologisch-biochemische Wirkung von H_2S auf *Pisum sativum* L. – Angew. Bot. 52: 137–147.

STUIVER C. E. E., DE KOK L. J. & KUIPER P. J. C. 1988. Freezing injury in spinach leaf tissue: Effects on water-soluble proteins, protein-sulfhydryl and watersoluble non-protein-sulfhydryl groups. – Physiol. Plant. 74: 72–76.

TAYLOR G. E., MCLAUGHLIN S. B., SHRINER D. S. & SELVIDGE W. J. 1983. The flux of sulfur containing gases to vegetation. – Atmos. Environ. 17: 789–796.

TAYLOR G. E. & SELVIDGE W. J. 1984. Phytotoxicity in bush bean of five sulfur-containing gases released from advances fossil energy technologies. – J. Environ. Qual. 13: 224–230.

THOMPSON C. R. & KATS G. 1978. Effect of continuous H_2S fumigation on crop and forest plants. – Environ. Sci. Technol. 12: 550–553.

VON ARB C. & BRUNOLD C. 1986. Ezymes of the assimilatory sulfate reduction in leaves of *Pisum sativum:* Activity changes during ontogeny and *in vivo* regulation by H_2S and cyst(e)ine. – Physiol. Plant. 67: 81–86.

VAN DIJK P. J., STULEN I. & DE KOK L. J. 1986. The effect of sulfide in the ambient air on amino acid metabolism of spinach leaves. pp. 207–209. In: LAMBERS H., NEETESON J. J. & STULEN I. (eds.). Physiological, Ecological and Agricultural Aspects of Nitrogen Metabolism in Higher Plants. – Martinus Nijhoff Publishers, Dordrecht.

WYSS, H-R. & BRUNOLD C. 1980. Regulation of adenosine 5'-phosphosulfate sulfotransferase by sulfur dioxide in primary leaves of beans *(Phaseolus vulgaris)*. – Physiol. Plant. 50: 161–165.