University of Groningen

Effects of atmospheric hydrogen sulfide on plant metabolism
de Kok, Luit J.

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1989

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Both natural and industrial processes may locally cause high levels of H₂S in the atmosphere. H₂S is a phytotoxic gas, which may reduce plant growth already at relatively low levels of 0.1 μl l⁻¹ and higher. The physiological and biochemical basis for the effects of H₂S on plants is largely unknown.

In order to obtain more insight into the background of the effects of H₂S on plants, the relation between foliar deposition and metabolism of H₂S was studied in the present thesis. The effects of H₂S on sulfur metabolism were compared with those observed in leaf tissue exposed to high levels of sulfate. Because of the strong coupling between sulfur and nitrogen metabolism in plants, the effect of H₂S on nitrate reduction was also studied.

Plant shoots form a sink for atmospheric H₂S. Short-term (1 h) exposure of maize, pumpkin, spinach and spruce to various atmospheric H₂S concentrations (0.03 to 0.9 μl l⁻¹) resulted in high H₂S fluxes to the shoots (Chapter 3). In all species there was a nearly linear relation between the H₂S flux to shoots and an atmospheric H₂S concentration, up to around 0.3 μl l⁻¹. At higher H₂S concentrations the H₂S flux reached a maximum, which varied between species and ranged from 0.03 to 0.1 μmol g fresh weight⁻¹ h⁻¹. The transpiration rate was not affected during H₂S exposure. Up to a level of 0.3 μl l⁻¹ H₂S, the deposition velocity (H₂S flux to shoots / H₂S concentration) for the above species varied between 61 and 92 % of H₂S deposition velocity predicted from the conductance for aqueous vapor efflux (stomatal conductance). This demonstrated that below an atmospheric H₂S concentrations of 0.3 μl l⁻¹, the internal resistance of the shoots for H₂S was close to zero.

H₂S fluxes to shoots remained rather constant during a 3 to 4 days exposure to about 0.2 and 0.8 μl l⁻¹ H₂S (Chapter 3). Fluxes of H₂S to shoots were high during the light and low during the dark period. The H₂S flux to transpiration ratio was constant during light and dark periods, indicating that the H₂S flux to shoots predominantly proceeded via the stomata. There was no direct relation between the H₂S fluxes to the shoots and the sensitivity of the species towards H₂S.

The deposited H₂S was rapidly metabolized in the plant. Short-term exposure of plants to H₂S resulted in a rapid accumulation of water-soluble non-protein sulfhydryl compounds in the shoots, which occurred both in light and in darkness (Chapter 4). Already after 1 h of exposure a sub-
stantial increase in sulphydryl content of the shoots was detectable and maximum accumulation, three- to four-fold of that in untreated plants, was observed after 1 to 2 days of exposure to 0.25 μl 1⁻¹ H₂S. The sulphydryl content of the water-soluble proteins and the amino acid content were not affected by short-term H₂S exposure. Sulfate accumulation in spinach shoots was only observed after an H₂S exposure (0.25 μl 1⁻¹), longer than 2 days.

In general the tripeptide glutathione (γ-glutamyl-cysteinyl-glycine) functions as the storage form of reduced sulfur in plants. Even though in spinach leaves glutathione was the major sulphydryl compound present, also after H₂S exposure, the accumulation of water-soluble non-protein sulphydryl compounds in leaves upon H₂S exposure could be ascribed to strongly enhanced levels of both cysteine and glutathione. In darkness also the level of γ-glutamyl-cysteine was strongly enhanced upon H₂S exposure. The cysteine content of untreated leaves accounted for 12% or less of the water-soluble non-protein sulphydryl compounds. However, after a 24 h exposure to 0.25 μl 1⁻¹ H₂S, the cysteine content increased up to more than seven-fold (versus maximal three- to four-fold of that of the total sulphydryl content) and its content could account for up to 50% of the sulphydryl compounds. The present results demonstrated a rather poor regulation of the cysteine level in the leaves. Even after prolonged (2 weeks) exposure still a high cysteine level was observed, demonstrating that regulation of sulfur assimilation was not altered in such a way that low levels of cysteine were obtained. Exposure of spinach to 0.33 μl 1⁻¹ SO₂ for 24 h also resulted in a two-fold increase in the sulphydryl content of the leaves and a four-fold increase in the cysteine content.

Glutathione was predominantly present in the reduced form (> 85%), also after H₂S exposure, and the glutathione reductase activity was not substantially affected by short-term H₂S exposure.

After a 3 days exposure of spinach to 0.24 and 0.74 μl 1⁻¹ H₂S, at both concentrations 25% of the deposited H₂S during the entire exposure period was revealed as accumulated water-soluble non-protein sulphydryl compounds in the shoots. After 4 days exposure of pumpkin to 0.22 and 0.82 μl 1⁻¹ H₂S this was 13 and 35%, respectively (Chapter 4). From the observation that sulphydryl accumulation already was maximal after 1 to 2 days of exposure, it was concluded, that a direct metabolism of the deposited H₂S played a significant role in the high foliar deposition of H₂S; it was responsible for the low internal (mesophyll) resistance of H₂S. It is proposed, that cysteine synthase is directly involved in the fixation and metabolism of atmospheric H₂S by the plant.
Termination of the H$_2$S exposure resulted in a rapid disappearance of the accumulated sulfhydryl compounds in spinach shoots (Chapter 4). The decrease in sulfhydryl content was accompanied by a similar decrease in the level of oxidized glutathione, and the emission of H$_2$S by the shoots was below the detection limit. The last observation indicated, that desulfhydration of cysteine did not significantly contribute to the decrease in the sulfhydryl compounds. It was suggested, that the excessive sulfhydryl compounds were rapidly metabolized (or partly translocated to the roots) and used as a sulfur source by the plant.

Similar to H$_2$S and SO$_2$, a 1 day incubation of spinach leaf discs with high levels of sulfate (10 to 200 mM Na$_2$SO$_4$) also resulted in enhanced levels of water-soluble non-protein sulfhydryl compounds (Chapter 5). The water-soluble protein-sulfhydryl and amino acid contents of the spinach leaf discs were not affected by 50 mM sulfate. Sulfate-induced accumulation occurred in both light and darkness and it was inhibited by selenate, an inhibitor of sulfate reduction. This demonstrated that sulfate-induced accumulation of water-soluble sulfhydryl compounds was due to an enhanced de novo reduction of sulfate and its subsequent incorporation into cysteine and its metabolites. The observation, that sulfate-induced accumulation of sulfhydryl compounds also occurred in darkness, demonstrated that no direct photosynthetic reducing capacity was needed for sulfate reduction. It was concluded, that the concentration of sulfate at the reduction site plays an important role in the regulation of sulfate assimilation in the plant.

Similar to the observations with H$_2$S, incubation of spinach leaf discs with sulfate resulted in a relatively stronger accumulation of cysteine (in darkness also γ-glutamyl-cysteine) than that of glutathione (Chapter 5). E.g., a 24 h incubation of spinach leaf discs with 50 mM Na$_2$SO$_4$ in the light resulted in a five-fold increase in the sulfhydryl content and in a thirteen-fold increase in the cysteine content. This finding demonstrated that even when sulfate reduction was limiting synthesis of sulfhydryl compounds, the cysteine pool was not regulated by feedback control of sulfate reduction.

A 1 day incubation of spinach leaf discs with selenate (0.25 to 10 mM) resulted in a substantial decrease in sulfhydryl compounds in the light, which indicated a rapid turnover of these compounds (Chapter 5). Apparently under conditions where the sulfate reduction is inhibited, the sulfhydryl compounds, especially glutathione, were utilized as a sulfur source. Selenate also strongly reduced H$_2$S-induced sulfhydryl accumulation, which may indicate that, due to inhibition of sulfate reduction by selenate, part of the deposited atmospheric H$_2$S was directly utilized for
synthesis of other sulfur-containing compounds, e.g. proteins.

A rapid turnover of glutathione in the light was observed, when spinach leaf discs were incubated with 1 mM buthionine sulfoximine (BSO), a selective inhibitor of γ-glutamyl-cysteine synthesis, for 24 h (Chapter 5). Light incubation of leaf discs with BSO only slightly reduced the sulfhydryl content; however, the glutathione content was strongly decreased, but it was replaced by cysteine. Also, BSO only slightly reduced H_{2}S-induced accumulation of sulfhydryl compounds in spinach leaf discs; cysteine again was the major sulfhydryl compound present upon exposure of the leaf discs to H_{2}S. It was concluded that metabolism of the deposited H_{2}S was not subject to feedback regulation by cysteine.

A combined 24 h exposure of spinach leaf discs to Na_{2}SO_{4} and H_{2}S demonstrated that the H_{2}S- and the sulfate-induced sulfhydryl accumulations were not additive (Chapter 5). This indicated that the sulfide, produced by sulfate reduction, and the atmospheric sulfide, absorbed by the leaf discs competed for the same substrate (O-acetyl-serine) utilized, for synthesis of cysteine and its metabolites.

In conclusion, the present results demonstrated that under conditions where sulfur, in both oxidized or reduced form, is directly supplied to the leaves, sulfur reduction and assimilation is beyond direct regulatory control (Chapter 4 and 5). Whether a disturbed regulation of sulfur assimilation and the subsequent enhanced levels of sulfhydryl compounds are significantly involved in the phytotoxicity of H_{2}S is still questionable (Chapter 7).

Short-term H_{2}S (0.25 μl l^{-1}) exposure did not affect in vitro nitrate reductase activity (NRA) in spinach leaves (Chapter 6). Likewise, H_{2}S exposure did not significantly affect in vivo NRA (the activity was about ten-times lower than the in vitro activity), measured under anaerobic conditions at a relative low photon fluence rate of 35 μmol m^{-2} s^{-1}. In vivo NRA of untreated plants was apparently inhibited in the presence of oxygen. However, short-term H_{2}S exposure increased in vivo "aerobic" NRA at already low levels (> 0.04 μl l^{-1}). The H_{2}S-induced increase in in vivo "aerobic" NRA depended on the H_{2}S concentration and after 1 day of exposure a maximal increase in activity (up to five-fold) was observed at 0.22 μl l^{-1}. However, after H_{2}S exposure the in vivo "aerobic" NRA was never higher than the in vivo "anaerobic" NRA. After cessation of the exposure the H_{2}S-induced increase in the in vivo "aerobic" NRA decreased again. It is proposed that the observed H_{2}S-induced inhibition of NADH oxidizing enzymes resulted in an altered competition and in an increased NADH supply to nitrate reductase (NR) in the presence of oxygen. It was unlikely
that the increase in in vivo "aerobic" NRA in H₂S-exposed plants was due to an altered competition between mitochondrial respiration and NR, since leaf respiration was not affected by an exposure to 0.25 μl l⁻¹ H₂S. A relation between the phytotoxicity of H₂S and reactions of part of the deposited H₂S with e.g. NADH oxidizing enzymes, appears to be quite possible (Chapter 7).