Chapter 3

Uptake and metabolism of atmospheric H$_2$S in *Allium*

**Abstract.** The impact of atmospheric H$_2$S deposition on growth and sulfur and nitrogen metabolism has been studied in onion (*Allium cepa* L.). The H$_2$S uptake followed saturation kinetics with respect to the H$_2$S concentration. The maximum H$_2$S uptake rate (J$_{H_2S}^\text{max}$) was approx. 1 µmol g$^{-1}$ fw h$^{-1}$ and the KH$_2$S (H$_2$S concentration at which ½J$_{H_2S}^\text{max}$ was reached) was approx. 1.5 µl l$^{-1}$, which demonstrated that onion had a rather high H$_2$S uptake rate when compared with other species. Upon exposure of onion to 0, 0.075, 0.15, 0.225 and 0.3 µl l$^{-1}$ H$_2$S for two weeks, growth was only slightly reduced at 0.3 µl l$^{-1}$ H$_2$S. H$_2$S exposure resulted in an increased content of sulfate, total thiols and total sulfur in the shoot, while that in the roots was unaltered. There was a slight increase in total nitrogen content in the shoot, whereas the content of nitrate and amino acids was hardly affected. Prolonged exposure to 0.15 µl l$^{-1}$ H$_2$S resulted in an accumulation of sulfate and organic sulfur and in a decrease in the organic N/S ratio in shoots of different cultivars of *Allium cepa*, *A. fistulosum* and *A. porrum*. The substantial increase in the organic sulfur fraction in the shoot was probably due to an increase in the content of secondary sulfur compounds, e.g. γ-glutamyl peptides and/or alliins.

**Introduction**

Atmospheric H$_2$S has a paradoxical impact on plants. It may negatively affect growth at atmospheric levels of 0.03 µl l$^{-1}$ and higher and may even cause visible injury and defoliation at ≥ 0.3 µl l$^{-1}$, however, there is a wide variation in susceptibility between species to H$_2$S (De Kok *et al*., 1998, 2000, 2002b). On the other hand, foliarly absorbed H$_2$S may be utilized as sulfur source for growth and may be beneficial if the pedospheric sulfur supply is limited (De Kok *et al*., 2000, 2002b; Westerman *et al*., 2000a). H$_2$S is taken up via the stomates, metabolized with high affinity into cysteine and subsequently into other sulfur compounds (De Kok *et al*., 1998; Stuiver and De Kok, 2001). In *Brassica oleracea* L. there was a direct interaction between foliar H$_2$S deposition and the uptake and metabolism of pedospheric sulfate (De Kok *et al*., 2000; Westerman *et al*., 2000a, 2001a,b).

*Allium cepa* L. (onion) was possibly one of the first domesticated vegetables by man and it was already cultivated by the ancient Egyptians. *Allium cepa* appears to be a species with a high sink capacity for reduced sulfur. This species belongs to the genus *Allium* (about 400 species), which derives its name from an allyl group in its secondary sulfur compounds. These compounds, specific for the family Alliaceae, are known as alliins and consist of different stereoisomers of S-alk(en)ylcysteine sulfoxides. Cysteine is the major precursor for the synthesis of alliins and sulfur-containing γ-glutamyl peptides are thought to act as intermediates (Block, 1992). The content of alliins in the leaves can di-
rectly be related to the sulfur status and can be affected by the sulfate supply to the roots (Randle et al., 1993, 1999; Haneklaus et al., 1997). Alliins may account for up to 80% of the total organic sulfur fraction in onion and they are thought to be metabolically inert end-products, which can not be re-metabolized as sulfur source (Schnug, 1993).

The aim of the present study is to get insight into the interaction between atmospheric and pedospheric sulfur nutrition in onion and the significance of secondary sulfur compounds, e.g. γ-glutamyl peptides and alliins, as sink for foliarly absorbed sulfur gases. The present study is focused on the kinetics of H₂S uptake by onion and on the impact of H₂S exposure on growth and sulfur and nitrogen content in different species and cultivars of Allium.

Results and discussion

Uptake of atmospheric H₂S and its impact on growth and sulfur metabolism

The uptake of gases by plant shoots is predominantly determined by both the diffusive conductance of the stomates and the mesophyll (internal) resistance (De Kok and Tausz, 2001). The uptake of H₂S by plant shoots appears to be largely dependent on the mesophyll resistance, viz. the rate of H₂S metabolism into cysteine and subsequently into other sulfur compounds (De Kok et al., 1998, 2000, 2002b; De Kok and Tausz, 2001). The maximum uptake rates of H₂S differ considerably among species (De Kok et al., 2002b).

![Fig. 1. Kinetics of H₂S uptake in shoots of onion (Allium cepa L. cv. Nerato). Two-week-old seedlings were grown at 24 °C on a 25% Hoagland nutrient solution for 10 days after which the H₂S uptake (JH₂S) and the ratio of H₂S deposition velocity to aqueous vapor efflux (gH₂S/gH₂O) were measured at various atmospheric H₂S levels. The mean transpiration rate (JH₂O) was 18.6 ± 0.8 mmol g⁻¹ fw h⁻¹. KH₂S and JH₂Smax are expressed as µl l⁻¹ and µmol g⁻¹ fw h⁻¹, respectively. Data represent the mean of four measurements with 12 plants in each (± SD). Repeating the experiment gave approx. the same results (1.38 and 1.04 for KH₂S and JH₂Smax, respectively).](image-url)
Table 1. Impact of H$_2$S exposure on growth of onion (Allium cepa L. cv. Nerato). Two-week-old seedlings were grown at 24 °C on a 25 % Hoagland nutrient solution for two weeks and exposed to 0, 0.075, 0.15, 0.225 and 0.3 µl l$^{-1}$ H$_2$S. Data on fresh weight (g), shoot to root (S/R) ratio and dry matter content (DMC, %) represent the mean of six and three measurements, respectively, with six plants in each (± SD). Data on relative growth rate (RGR, % day$^{-1}$) represent the mean of three experiments (± SD). There were no significant differences between treatments (P < 0.05, Student’s t-test).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight</th>
<th>Shoot</th>
<th>Roots</th>
<th>S/R ratio</th>
<th>RGR Plant</th>
<th>DMC Shoot</th>
<th>DMC Roots</th>
</tr>
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<tbody>
<tr>
<td>0 µl l$^{-1}$ H$_2$S</td>
<td>0.31 ± 0.07</td>
<td>0.32 ± 0.07</td>
<td>0.12 ± 0.02</td>
<td>2.55 ± 0.30</td>
<td>9.5 ± 1.0</td>
<td>6.59 ± 0.65</td>
<td>4.46 ± 0.49</td>
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<tr>
<td>0.075 µl l$^{-1}$ H$_2$S</td>
<td>0.34 ± 0.05</td>
<td>0.34 ± 0.05</td>
<td>0.13 ± 0.03</td>
<td>2.55 ± 0.32</td>
<td>9.9 ± 1.4</td>
<td>6.39 ± 0.54</td>
<td>4.21 ± 0.35</td>
</tr>
<tr>
<td>0.15 µl l$^{-1}$ H$_2$S</td>
<td>0.31 ± 0.07</td>
<td>0.34 ± 0.05</td>
<td>0.13 ± 0.02</td>
<td>2.55 ± 0.31</td>
<td>10.5 ± 1.5</td>
<td>6.65 ± 0.80</td>
<td>4.19 ± 0.55</td>
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<tr>
<td>0.225 µl l$^{-1}$ H$_2$S</td>
<td>0.31 ± 0.07</td>
<td>0.34 ± 0.05</td>
<td>0.12 ± 0.02</td>
<td>2.48 ± 0.21</td>
<td>9.7 ± 1.2</td>
<td>6.51 ± 0.48</td>
<td>4.22 ± 0.50</td>
</tr>
<tr>
<td>0.3 µl l$^{-1}$ H$_2$S</td>
<td>0.26 ± 0.05</td>
<td>0.31 ± 0.07</td>
<td>0.11 ± 0.02</td>
<td>2.36 ± 0.24</td>
<td>8.7 ± 1.0</td>
<td>6.65 ± 0.58</td>
<td>3.99 ± 0.36</td>
</tr>
</tbody>
</table>

Similar to observations with other species, the H$_2$S uptake by shoots of onion followed saturation kinetics with respect to the H$_2$S concentration and the saturation kinetics fitted well with the Michaelis-Menten equation. The maximum H$_2$S uptake rate ($J_{H_2S_{max}}$) was 1.1 µmol g$^{-1}$ h$^{-1}$ and the H$_2$S concentration at which $\frac{1}{2}J_{H_2S_{max}}$ was reached ($K_{H_2S}$) was 1.5 µl l$^{-1}$ H$_2$S (Fig. 1), which showed that onion had a rather high capacity for H$_2$S uptake and a higher $K_{H_2S}$ value when compared with other species (De Kok et al., 2002b). The ratio of H$_2$S deposition velocity to aqueous vapor efflux ($g_{H_2S}/g_{H_2O}$) was calculated to obtain insight into the factors determining H$_2$S uptake in the shoots of onion. The $g_{H_2S}/g_{H_2O}$ ratio remained constant and close to 1 at levels up to 0.3 µl l$^{-1}$ H$_2$S, which indicated that uptake of H$_2$S was limited by its diffusion through the stomates only and that the mesophyll (internal) resistance was close to zero (Fig. 1).

Onion is a rather slow growing species with a RGR of approx. 9.5 % day$^{-1}$ (on a plant fresh weight basis at a day/night temperature of 24/18 °C) and appeared to be resistant to relatively high levels of H$_2$S (Table 1). The plant biomass production was hardly affected upon a two-week exposure to atmospheric H$_2$S levels up to 0.3 µl l$^{-1}$. Shoot to root ratio and dry matter content in shoot and roots were not affected upon H$_2$S exposure (Table 1).

Exposure of onion seedlings to atmospheric H$_2$S resulted in an increased content of sulfate, thiols and total sulfur in the shoots. There was a slight increase in total nitrogen content of the shoot, whereas that of nitrate and amino acids was hardly affected (Fig. 2). Metabolite content of the roots was not affected upon H$_2$S exposure. At 0.3 µl l$^{-1}$ H$_2$S sulfate and total sulfur contents in the shoot were increased with 6.9 and 17.7 µmol g$^{-1}$ fw, respectively, whereas the total nitrogen content in the shoot increased with 29 µmol g$^{-1}$ fresh weight and the nitrate content was not significantly affected (Fig. 2). This demon-
strated that in particular for sulfur the greater proportion of the increase in its content was due to an increase in the organic sulfur fraction. In unexposed plants the organic N/S ratio was around 41, predominantly representing the molar ratio of N/S in the proteins. However, upon H$_2$S exposure the organic N/S ratio decreased with the H$_2$S concentration, which likely had to be attributed to an increase in non-protein organic sulfur compounds (Fig. 3). It has been observed that onion has a sink capacity for sulfur which is metabolized into secondary sulfur compounds viz. $\gamma$-glutamyl peptides and alliins (Randle et al., 1993, 1999; Haneklaus et al., 1997). The molar ratio of N/S in $\gamma$-glutamyl peptides and alliins is $\leq 2$. The present data provide circumstantial evidence that a great part of the increase in total sulfur content upon H$_2$S exposure may be attributed to an accumulation of $\gamma$-glutamyl peptides and/or alliins. The impact of H$_2$S exposure on the synthesis of $\gamma$-glutamyl peptides and alliins will further be investigated. The experimental techniques will have to be optimized, since these compounds are rather unstable during extraction and subject to degradation.

H$_2$S had a strong impact on thiol levels of the shoot. At 0.3 µl l$^{-1}$ H$_2$S the thiol content in the shoot was 2.8 times higher than that in the shoot of the control plants but at 0.075 µl l$^{-1}$ H$_2$S it was only 1.1 times higher, indicating that at low levels of H$_2$S the incorporation of cysteine (and glutathione) into other metabolic compounds, possibly $\gamma$-glutamyl peptides and/or alliins, was high and prevented a substantial accumulation of thiols (Fig. 2). Future studies will include measurements on the composition of the thiol pool, since H$_2$S exposure may not only affect the content but also the composition of the thiol pool (Buwalda et al., 1993; Poortinga and De Kok, 1997; Tausz et al., 1998; Westerman et al., 2000a).

**Impact of H$_2$S in different species and cultivars of Allium**

The accumulation of sulfur-containing compounds upon H$_2$S exposure was studied in different species and cultivars of *Allium*. *Allium cepa* had a higher biomass production and a lower dry matter content than *A. fistulosum* and *A. porrum* (Fig. 4). Prolonged H$_2$S exposure (0.15 µl l$^{-1}$) resulted in a reduced shoot growth of *A. cepa* cv. Wolf F1 and Bravo F1 and *A. porrum* cv. NiZ 33-2015 F1, whereas the dry matter content (DMC) was hardly affected in all species and cultivars.

Atmospheric H$_2$S exposure resulted in an accumulation of inorganic, organic and total sulfur compounds in all *Allium* species and cultivars studied (between 2 and 5 fold, Fig. 5). The accumulation in organic sulfur compounds was most likely due to an increase in the content of $\gamma$-glutamyl peptides and/or alliins and not in the content of proteins, as described above. Since, H$_2$S exposure resulted in a more than two-fold decrease in the organic N/S ratio (Fig. 5). The inorganic, organic and total nitrogen content was hardly affected by H$_2$S exposure and differences between cultivars could largely be explained by variations in dry matter content (Fig. 4, 5).
Fig. 2. Impact of long-term H$_2$S exposure on the sulfate, nitrate, thiol, amino acid, total sulfur and total nitrogen content in shoot and roots of onion (Allium cepa L. cv. Nerato). Two-week-old seedlings were grown at 24 °C on a 25 % Hoagland nutrient solution for two weeks and exposed to 0, 0.075, 0.15, 0.225 and 0.3 µl l$^{-1}$ H$_2$S. Metabolite contents in shoot (closed squares) and roots (open squares) are expressed in µmol g$^{-1}$ fw. Data on sulfate, nitrate, free amino acid, total sulfur and total nitrogen represent the mean of three measurements with 36 plants in each (± SD). Data on the total thiol content represent the mean of four measurements with six plants in each (± SD). Total nitrogen and total sulfur contents increased linearly with the H$_2$S concentration (P < 0.05, F-test).
Fig. 3. The impact of long-term H$_2$S exposure on the organic and inorganic N/S ratio in the shoot of onion. The organic (closed squares) and inorganic (open squares) N/S ratio (± SD) were derived from data presented in Fig. 2.

Fig. 4. Impact of long-term H$_2$S exposure on biomass production and dry matter content of shoots of *Allium*. Two-week-old seedlings of *A. cepa* L. cv. Wolf F1, Bravo F1 and Nerato F1, *A. fistulosum* L. cv. Savel and *A. porrum* L. cv. Bluetan F1 (P1) and NiZ 33-2015 F1 (P2) were exposed to 0 and 0.15 µl l$^{-1}$ H$_2$S (light and dark bars, respectively) for 18 days. Data on biomass production (over an 18-day period, g) and dry matter content (DMC, %) represent the mean of 3 measurements with 80 plants in each (± SD). Asterisks indicate significant differences between 0 and 0.15 µl l$^{-1}$ H$_2$S (P < 0.05, Student’s t-test).
Fig. 5. Impact of long-term H$_2$S exposure on sulfur and nitrogen contents and N/S ratio’s of shoots of *Allium*. Two-week-old seedlings of *A. cepa* L. cv. Wolf F1, Bravo F1 and Nerato F1, *A. fistulosum* L. cv. Savel and *A. porrum* L. cv. Bluetan F1 (P1) and NiZ 33-2015 F1 (P2) were exposed to 0 and 0.15 µl l$^{-1}$ H$_2$S (light and dark bars, respectively) for 18 days. Sulfate, total sulfur, nitrate and total nitrogen contents were determined (µmol g$^{-1}$ fresh weight) and organic nitrogen and sulfur contents (µmol g$^{-1}$ fresh weight) and total, inorganic and organic N/S ratios were calculated. Data represent the mean of 3 measurements with 80 plants in each (± SD). Asterisks indicate significant differences between 0 and 0.15 µl l$^{-1}$ H$_2$S (P<0.05, Student’s $t$-test).

**Sulfur requirement of onion**

The plant’s sulfur requirement for growth can be expressed as the rate of sulfur uptake and its assimilation needed per gram plant biomass produced with time and can strongly vary between species (De Kok et al., 2000, 2002a). Although *Allium cepa* (onion) had a much lower sulfur requirement than *Brassica oleracea* (Table 2), the H$_2$S uptake rate was in the same range (Fig. 1), which might explain the observed increase in the total sulfur content upon H$_2$S exposure (Fig. 2). From calculations on the plant’s sulfur requirement and foliar uptake rate of sulfur gases, and subsequent laboratory experiments it is evident that atmospheric levels of ≥ 0.1 µl l$^{-1}$ of these sulfur gases should be sufficient to cover
the organic sulfur requirement for growth of most plant species, including onion (De Kok et al., 2000, 2002b; Table 2).

Table 2. Sulfur requirement of seedlings of Brassica oleracea (curly kale) and Allium cepa (onion). Data are expressed on a fresh weight basis. Plants were grown on 25 % Hoagland nutrient solution for 2 weeks in a climate-controlled room (for experiment details see Stuiver and De Kok, 2001; this chapter).

<table>
<thead>
<tr>
<th></th>
<th>RGR (g g⁻¹ plant day⁻¹)</th>
<th>Sulfur content (µmol g⁻¹ plant)</th>
<th>Sulfur requirement (µmol g⁻¹ plant day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allium cepa</td>
<td>0.095</td>
<td>9.4</td>
<td>0.89</td>
</tr>
<tr>
<td>Brassica oleracea</td>
<td>0.235</td>
<td>43.1</td>
<td>10.13</td>
</tr>
</tbody>
</table>

In Brassica oleracea there was a strong interaction between the uptake and metabolism of atmospheric H₂S and pedospheric sulfate. Upon H₂S exposure the sulfate uptake was down-regulated, whereas the total sulfur content was hardly affected (De Kok et al., 2000; Westerman et al., 2001a). It appeared from our experiments that the sulfur metabolism in Allium was differently regulated since the total sulfur content increased linearly with the H₂S concentration. The interaction between atmospheric and pedospheric sulfur nutrition and its consequences for the synthesis of γ-glutamyl peptides and alliins will further be evaluated.