Atmospheric H$_2$S exposure does not affect stomatal aperture in maize

Ties Ausma$^1$ · Jeffrey Mulder$^1$ · Thomas R. Polman$^1$ · Casper J. van der Kooi$^1$ · Luit J. De Kok$^1$

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Abstract

Main conclusion Stomatal aperture in maize is not affected by exposure to a subtoxic concentration of atmospheric H$_2$S. At least in maize, H$_2$S, thus, is not a gaseous signal molecule that controls stomatal aperture.

Abstract Sulfur is an indispensable element for the physiological functioning of plants with hydrogen sulfide (H$_2$S) potentially acting as gasotransmitter in the regulation of stomatal aperture. It is often assumed that H$_2$S is metabolized into cysteine to stimulate stomatal closure. To study the significance of H$_2$S for the regulation of stomatal closure, maize was exposed to a subtoxic atmospheric H$_2$S level in the presence or absence of a sulfate supply to the root. Similar to other plants, maize could use H$_2$S as a sulfur source for growth. Whereas sulfate-deprived plants had a lower biomass than sulfate-sufficient plants, exposure to H$_2$S alleviated this growth reduction. Shoot sulfate, glutathione, and cysteine levels were significantly higher in H$_2$S-fumigated plants compared to non-fumigated plants. Nevertheless, this was not associated with changes in the leaf area, stomatal density, stomatal resistance, and transpiration rate of plants, meaning that H$_2$S exposure did not affect the transpiration rate per stoma. Hence, it did not affect stomatal aperture, indicating that, at least in maize, H$_2$S is not a gaseous signal molecule controlling this aperture.

Keywords Stomata · Transpiration · Signal molecule · Gasotransmitter · Sulfur metabolism · Air pollution

Introduction

Sulfur is an essential macronutrient for plants, which plants usually acquire as sulfate via the root (Hawkesford and De Kok 2006). After its uptake, sulfate is reduced via several intermediates to sulfide, which is subsequently incorporated in cysteine via the reaction of sulfide with O-acetylserine (OAS), catalyzed by the enzyme O-acetylserine(thiol)lyase (OAS-TL; Hawkesford and De Kok 2006). Cysteine functions as the precursor and reduced sulfur donor for the synthesis of other organic compounds.

It is often assumed that sulfur-containing metabolites might modulate physiological processes in plants. Hydrogen sulfide (H$_2$S) might act as endogenous gasotransmitter that affects plant development and stress tolerance (Sirko and Gotor 2007; Calderwood and Kopriva 2014; Maniou et al. 2014; Hancock 2018). Moreover, H$_2$S might control the aperture of stomata (Lisjak et al. 2010, 2011; Scuffi et al. 2014; Honda et al. 2015; Li et al. 2016; Aroca et al. 2018; Zhang et al. 2019). It is assumed that H$_2$S is metabolized into cysteine to stimulate the synthesis of abscisic acid (ABA), which is the canonical trigger for stomatal closure (Batool et al. 2018; Rajab et al. 2019).

The physiological significance of H$_2$S for stomatal closure should, however, be questioned. Research with thale cress (Arabidopsis thaliana), maize (Zea mays), cabbage (Brassica oleracea), pumpkin (Cucurbita pepo), spruce (Picea abies), and spinach (Spinacea oleracea) showed that exposure to atmospheric H$_2$S did not affect transpiration rates, measured at the whole plant level, at various concentrations and under all exposure periods applied (which ranged from minutes to days; De Kok et al. 1989; Van der Kooij and De Kok 1998; Stuiver and De Kok 2001; Tausz et al. 1998).
Accordingly, there are at least two caveats pertaining studies that reported impacts of H$_2$S on stomatal dynamics. First, uncontrolled, potentially very high, levels of H$_2$S have been used (e.g., Scuffi et al. 2014; Zhang et al. 2019). Sodium hydrosulfide (NaHS) has been used as H$_2$S donor and it was added to nutrient or tissue incubation solutions at pH < 7.0. However, if NaHS is used at this pH range, HS$^-$ is rapidly converted to gaseous H$_2$S (HS$^-$$+$$H^+$$\rightleftharpoons$$H_2$$S;$$pK_a$$=7.0;$$Lee$$et$$al.$11). Since H$_2$S is rather poorly soluble in water (the Henry’s law solubility constant for H$_2$S is 0.086 M atm$^{-1}$ at 25 °C), it is quickly released into the atmosphere, where it may transiently reach phytotoxic (growth-inhibiting) levels (Lee et al. 2011; Riahi and Rowley 2014). H$_2$S may bind to metallo-groups in enzymes and other proteins (Beauchamp et al. 1984; Maas and De Kok 1988). Reported impacts of H$_2$S on stomatal aperture could possibly be the consequence of such toxicity, instead of being specifically related to H$_2$S functioning as gasotransmitter. One should further bear in mind that especially thale cress, which functioned as model plant, is rather susceptible to atmospheric H$_2$S (Van der Kooij and De Kok 1998; Birke et al. 2015).

Secondly, in some studies (e.g., Zhang et al. 2019), mutants with a modified H$_2$S homeostasis were used. Genetic manipulation of H$_2$S homeostasis may not only alter tissue H$_2$S content, but also the contents of other metabolites. These associated changes in metabolite contents may impact stomatal aperture. Hence, perceived impacts on stomatal aperture in mutants cannot directly be ascribed to the modification in H$_2$S homeostasis (viz., genotypic variation cannot directly be translated to phenotypic variation; Piersma and Van Gils 2011; Noble 2013; Noble et al. 2014).

The application of controlled, subtoxic (non-growth-inhibiting) levels of atmospheric H$_2$S to non-mutant plants can provide a physiologically realistic view of the role of H$_2$S in stomatal regulation. Plants absorb atmospheric H$_2$S via stomata, since the leaf’s cuticle is hardly permeable for gases (Ausma and De Kok 2019). At the pH of leaf cells (i.e., ~5–6.4) absorbed H$_2$S remains largely undissociated, causing it to easily pass cellular and subcellular membranes (Lee et al. 2011; Riahi and Rowley 2014). Foliar H$_2$S levels increase significantly upon H$_2$S fumigation (Ausma and De Kok 2019). For instance, exposure of thale cress to 0.5 and 1.0 µl l$^{-1}$ H$_2$S enhanced leaf H$_2$S levels by approximately twofold and threefold, respectively (Birke et al. 2015). Since H$_2$S is rapidly and with high affinity metabolized in cysteine, H$_2$S fumigation also strongly enhanced foliar cysteine content and that of the tripeptide glutathione (De Kok et al. 1997; Birke et al. 2015; Ausma et al. 2017; Ausma and De Kok 2019). Thus, fumigation with low H$_2$S levels may profoundly alter tissue sulfur status, without affecting plant growth (Ausma and De Kok 2019).

Plants may switch from using sulfate to using H$_2$S as sulfur source: H$_2$S absorbance by the foliage may partially downregulate the uptake and subsequent metabolism of sulfate (Buchner et al. 2004; De Kok et al. 1997). Plants may even grow with atmospheric H$_2$S as the only sulfur source (viz., in the absence of a root sulfate supply; De Kok et al. 1997; Koralewska et al. 2007, 2008). Whereas sulfate deprivation may reduce plant growth rate as well as endogenous cysteine and glutathione levels, fumigation with a sufficiently high H$_2$S level may fully alleviate these reductions.

Here, we study the importance of H$_2$S as gaseous signal molecule for the regulation of stomatal aperture in maize (Zea mays). Initially, we determined the H$_2$S level that is subtoxic for maize, though sufficiently high to fully cover the plant’s sulfur demand for growth (viz., the H$_2$S concentration at which H$_2$S-fumigated plants have a similar biomass as non-fumigated sulfate-sufficient plants). We then exposed plants for several days to this atmospheric H$_2$S level in the presence or absence of a root sulfate supply. We measured plant growth, sulfur status, stomatal density, stomatal resistance, and transpiration rates. We conclude that, at least in maize, H$_2$S is not a gaseous signal molecule that controls stomatal opening.

Materials and methods

Plant material and growth conditions

Seeds of maize (Zea mays; cultivar number 669; Van Der Wal; Hoogeveen; The Netherlands) were germinated between moistened filter paper in the dark at 23 °C. After 3 days, the seedlings were put on 15 l boxes containing aerated tap water, which were placed in a climate-controlled room. Air temperature was 23 °C (± 1 °C), relative humidity was 60–70%, and the photoperiod was 16 h at a photon fluence rate of 300 ± 20 µmol m$^{-2}$ s$^{-1}$ (within the 400–700 nm range) at plant height, supplied by Philips GreenPower LED (deep red/white 120) production modules.

After 7 days, the seedlings were transferred to 13 l stainless-steel boxes (10 sets of plants per box, 6 plants per set in the first experiment, and 4 plants per set in the second experiment) holding aerated 50% Hoagland nutrient solutions, which were placed in 50 l cylindrical stainless-steel cabinets (0.6 m diameter) with a polymethyl-methacrylate top (Supplementary Fig. S1). Day and night air temperatures were 21 and 18 °C (± 1 °C), respectively, relative humidity was 30–40%, and the photoperiod was 16 h at a photon fluence rate of 300 ± 20 µmol m$^{-2}$ s$^{-1}$ (within the 400–700 nm range) at plant height, supplied by Philips GreenPower LED (deep red/white 120) production modules. Air exchange inside the cabinets was 40 l min$^{-1}$ and the air inside the cabinets was stirred continuously by a ventilator. Nutrient solutions either contained 1 mM sulfate (+ S; sulfate-sufficient; solution’s composition being 2.5 mM CaCl$_2$, 2.5 mM KCl, 0.5 mM KH$_2$PO$_4$, 1 mM MgSO$_4$, 3.75 mM NH$_4$NO$_3$, 0.11 mM FeSO$_4$·7H$_2$O, 0.115 mM ZnSO$_4$·7H$_2$O, 0.0475 mM MnSO$_4$·H$_2$O, 0.097 mM CuSO$_4$·5H$_2$O, 0.0215 mM Ca$_3$(PO$_4$)$_2$, 0.0215 mM Na$_2$MoO$_4$·2H$_2$O, and 0.0715 mM Mg$_3$(PO$_4$)$_2$).
23.4 µM H3BO3, 4.8 µM MnCl2, 0.48 µM ZnSO4, 0.16 µM CuSO4, 0.26 µM Na2MoO4 and 45 µM Fe3+EDTA), or 0 mM sulfate (-S; sulfate-deprived; all sulfate salts replaced by chloride salts).

Plants were fumigated either with 0, 0.5, 1.0, or 1.5 µl l−1 H2S. Pressurized H2S diluted with N2 (1.0 ml l−1) was injected into the incoming air stream and the concentration in the cabinet was adjusted to the desired level using electronic mass flow controllers (ASM; Bilthoven; The Netherlands). H2S levels in the cabinets were monitored by an SO2 analyzer (model 9850) equipped with a H2S converter (model 8770; Monitor Labs; Measurements Controls Corporation; Englewood; CO; USA). Sealing of the lid of the boxes and plant sets prevented absorption of H2S by the nutrient solutions.

In the first experiment, plants were harvested after 10 days of exposure. In the second experiment after 7 days of exposure per treatment, sets of 4 plants were weighted (viz., total biomass was determined). Subsequently, each plant set was transferred to a separate vessel containing 1.1 l of a similar 50% Hoagland nutrient solution as the set was grown on before (Supplementary Fig. S1). Vessels with plant sets were placed in the stainless-steel cabinets described above (with similar H2S levels) and plants were grown for an additional 3 days before harvest.

Growth analyses

Plant harvesting took place 3 h after the onset of the light period. To remove ions and other particles attached to the root, plants were placed in their roots in ice-cold de-mineralized water (3 × 20 s). Thereafter, the root and shoot were separated and weighted. In the second experiment, the shoot was additionally separated in leaf blades and the whorl of leaf sheaths (viz., the seedlings did not yet possess a true stem, since all leaves emerged from the shoot base). Moreover, the total leaf blade area (abaxial plus adaxial) of the plants was determined by drawing the outlines of all leaf blades on graph paper.

Stomatal resistance

On the harvest day, stomatal resistance was analyzed at the abaxial and adaxial side of nascent leaf blades using a portable leaf porometer (AP4 Leaf Porometer; Delta-T-Devices Ltd.; Cambridge; UK). Measurements were performed 2–3 h after the onset of the light period.

Stomatal density

For the determination of stomatal density, silicone impression paste was prepared by 1:1 mixing of catalyst and base material (Provil Novo Light; Kulzer GmbH; Hanau; Germany). Subsequently, freshly harvested nascent leaf blades were gently pressed in the paste with either their abaxial or adaxial side. Once the paste had solidified, the leaf blades were removed and the mould was filled with transparent nail polish, as described by Kraaij and van der Kooi (2020). The positive (nail polish) replica was next examined under an Olympus CX-41 microscope and photographed using a Euromex CMEX 5000 camera with ImageFocus v3.0 software. From the obtained photographs, stomatal density (number of stomata per leaf area) was determined. Importantly, during trial experiments, also leaf sheaths were examined, but these did not hold stomata.

Transpiration rate

The transpiration rate of plants, expressed on a whole plant fresh weight basis, was calculated over the 3-day period that plants were grown on the vessels as follows:

\[ I_t = I_u - I_g \] (1)

\[ I_u = \left( \frac{\ln P_2 - \ln P_1}{3} \right) \times \left( \frac{(I_{m2} - I_{m1} - 8.95)}{(P_2 - P_1)} \right) \] (2)

\[ I_g = \left( \frac{\ln S_2 - \ln S_1}{3} \right) \times 0.9 + \left( \frac{\ln R_2 - \ln R_1}{3} \right) \times 0.95 \] (3)

where \( I_t \) represents the transpiration rate, \( I_u \) the water uptake rate, and \( I_g \) the amount of water required for plant growth (all expressed as g H2O g−1 FW plant day−1). Furthermore, \( P \) represents the whole plant’s fresh weight, \( S \) the shoot’s fresh weight, \( R \) the root’s fresh weight, and \( I_u \) the total solution weight in the vessels, with the subscripts 1 and 2 denoting the parameters’ value at the start and at the end of the 3-day exposure period, respectively. Moreover, whereas the factor 3 in the formulas refers to the 3-day duration of the experiment, the factor 8.95 refers to the average difference in solution weight of 4 vessels, which did not hold a plant set, between the start and end of the 3-day exposure period, respectively (standard deviation of this measurement was 0.61). Finally, the factors 0.9 and 0.95 represent the fraction...
of a maize shoot and root consisting of water, respectively (Ausma et al. 2017). It deserves mentioning that during the 3-day exposure period, the proportion of biomass allocated to the different plant organs was not affected.

**Statistics**

Statistical analyses were performed in GraphPad Prism (version 8.4.1; GraphPad Software; San Diego; CA; USA). Treatment means were compared using a two-way analysis of variance (ANOVA) with a Tukey’s HSD test as post hoc test at the $P \leq 0.05$ level.

**Results and discussion**

To test the relevance of H$_2$S for the regulation of stomatal aperture, maize seedlings were grown with atmospheric H$_2$S in the presence or absence of sulfate in the root environment.

We first assessed what H$_2$S level is subtoxic for maize, albeit sufficiently high to fully cover the plant’s sulfur demand for growth. Sulfur-deficiency symptoms manifested after 10 days of sulfur deprivation (Table 1). The biomass of sulfate-deprived seedlings was on average 36% lower than that of sulfate-sufficient seedlings, which could be ascribed to both a lower root (33%) and shoot (37%) biomass (Table 1).

H$_2$S fumigation can alleviate sulfur-deficiency symptoms. If maize was H$_2$S fumigated in the absence of a sulfate supply, the plants did not develop any sulfur-deficiency symptoms (Table 1). The biomass of sulfate-deprived plants that were fumigated with 0.5 or 1.0 µl l$^{-1}$ H$_2$S was comparable to that of sulfate-sufficient, non-fumigated plants (Table 1), meaning that, analogous to the many plant species tested previously (Ausma et al. 2017; Ausma and De Kok 2019), maize can use H$_2$S as a sulfur source. The results further demonstrate that maize is rather insusceptible for the potential phytotoxicity of H$_2$S. Only exposure to 1.5 µl l$^{-1}$ H$_2$S negatively affected plant growth (Table 1). Generally, monocots are highly H$_2$S tolerant (Stulen et al. 1990, 2000). In monocots, the shoot’s meristem is sheltered by the whorl of leaves. Therefore, H$_2$S can hardly penetrate the meristem, which may explain why grasses are relatively H$_2$S insusceptible (Stulen et al. 1990, 2000).

Tissue H$_2$S, cysteine, and glutathione levels may be more profoundly affected at higher H$_2$S levels (Birke et al. 2012; Ausma and De Kok 2019). Thus, in a second experiment, plants were fumigated with 1.0 µl l$^{-1}$ H$_2$S instead of 0.5 µl l$^{-1}$ H$_2$S. Similar to our previous observations (Table 1), sulfate-deprived plants had a lower biomass than sulfate-sufficient plants, owing to a lower root (34%) and leaf sheath biomass (22%; Table 2). Leaf blade biomass was comparable between sulfate-sufficient and sulfate-deprived plants (Table 2).

Sulfate deprivation lowered tissue sulfate and (water-soluble non-protein) thiol levels. Whereas a 10-day sulfate deprivation of maize reduced shoot and root sulfate levels by 92% and 75%, respectively, it reduced shoot and root thiol levels by 73% and 60%, respectively (Fig. 1). In plants, the thiol pool is mainly comprised of glutathione, though cysteine is a minor thiol (Buwalda et al. 1993). In maize, cysteine accounted for only 12% and 16% of the shoot and root thiol pool, respectively (Fig. 1). Sulfate deprivation decreased tissue cysteine contents: it lowered root and shoot

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### Table 1

| Treatment | Biomass of maize as affected by various levels of atmospheric H$_2$S and sulfate deprivation. 10-day old maize was grown on a 50% Hoagland nutrient solution, containing 0 (−S) or 1.0 mM sulfate (+S) and simultaneously fumigated with 0, 0.5, 1.0, and 1.5 µl l$^{-1}$ H$_2$S for 10 days. Data (g FW) represent the mean (± SD) of 5 measurements with 6 plants in each and different letters indicate significant differences between treatments ($P\leq0.05$; two-way ANOVA; Tukey’s HSD test as a post hoc test) |
|-----------|-------------------------------------------------------------------------------------------------|
| 0 µl l$^{-1}$ H$_2$S | 1.0 µl l$^{-1}$ H$_2$S |
| +S | − S | +S | − S | +S | − S |
| Plant | 3.60 ± 0.12a | 2.31 ± 0.17b | 3.78 ± 0.15a | 3.66 ± 0.06a | 3.71 ± 0.07a | 3.63 ± 0.11a | 1.74 ± 0.15c | 1.77 ± 0.08c |
| Roots | 1.30 ± 0.12a | 0.87 ± 0.06b | 1.39 ± 0.08a | 1.33 ± 0.05a | 1.36 ± 0.06a | 1.33 ± 0.05a | 0.96 ± 0.09b | 0.96 ± 0.05b |
| Shoots | 2.30 ± 0.08a | 1.45 ± 0.17b | 2.39 ± 0.11a | 2.33 ± 0.04a | 2.35 ± 0.11a | 2.30 ± 0.08a | 0.78 ± 0.08c | 0.81 ± 0.04c |

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### Table 2

| Treatment | Biomass of maize as affected by H$_2$S fumigation and sulfate deprivation. 10-day old maize was grown on a 50% Hoagland nutrient solution, containing 0 (−S) or 1.0 mM sulfate (+S) and simultaneously fumigated with 0 or 1.0 µl l$^{-1}$ H$_2$S for 10 days. Data (g FW) represent the mean (± SD) of 10 measurements with 4 plants in each and different letters indicate significant differences between treatments ($P\leq0.05$; two-way ANOVA; Tukey’s HSD test as a post hoc test) |
|-----------|-------------------------------------------------------------------------------------------------|
| 0 µl l$^{-1}$ H$_2$S | 1.0 µl l$^{-1}$ H$_2$S |
| +S | − S | +S | − S |
| Plant | 3.46 ± 0.11a | 2.57 ± 0.10b | 3.55 ± 0.23a | 3.42 ± 0.19a |
| Roots | 1.32 ± 0.11a | 0.87 ± 0.08b | 1.41 ± 0.14a | 1.29 ± 0.14a |
| Leaf sheaths | 1.90 ± 0.08a | 1.49 ± 0.06b | 1.92 ± 0.12a | 1.90 ± 0.09a |
| Leaf blades | 0.23 ± 0.01a | 0.21 ± 0.02b | 0.22 ± 0.01a | 0.23 ± 0.02a |
cysteine content by 79% and 100%, respectively (Fig. 1). Clearly, the lower biomass production upon sulfate deprivation was accompanied by lower sulfate, glutathione, and cysteine contents (Fig. 1).

The biomass of plants that were fumigated with 1.0 µl l⁻¹ H₂S was comparable to that of sulfate-sufficient non-fumigated plants (Table 2). Thiol levels were higher in H₂S-fumigated plants compared to non-fumigated plants (Fig. 1). Under sulfate-sufficient conditions, shoot total water-soluble non-protein thiol and cysteine levels were 1.4- and 2.0-fold higher in fumigated plants compared to non-fumigated plants, respectively (Fig. 1). Moreover, under sulfate-deprived conditions, fumigated plants had a 5.0-fold higher shoot total water-soluble non-protein thiol level, a 1.9-fold higher root water-soluble non-protein thiol level, and a 3.0-fold higher root cysteine level compared to non-fumigated plants (Fig. 1). Shoot cysteine levels in sulfate-deprived fumigated plants were even 1.5-fold higher compared to sulfate-sufficient non-fumigated plants (Fig. 1).

Fig. 1 The content of sulfate, total water-soluble non-protein thiols, and cysteine in maize as affected by H₂S fumigation and sulfate deprivation. For experimental details, see the legend of Table 2. Data, representing 3 measurements with 4 plants in each, are presented as boxes with a 5–95 percentile and whiskers. Different letters indicate significant differences between treatments ($P \leq 0.05$; two-way ANOVA; Tukey’s HSD test as a post hoc test).
higher shoot sulfate content compared to sulfate-sufficient non-fumigated plants, sulfate-deprived fumigated plants had a 5.0-fold higher shoot sulfate content compared to sulfate-deprived non-fumigated plants (Fig. 1). The higher sulfate content in fumigated plants might be related to the oxidation of absorbed H₂S and/or the degradation of excessively accumulated organic compounds (Ausma and De Kok 2019). However, it may also be due to H₂S absorbance only partially downregulating root sulfate uptake (Ausma and De Kok 2019). Further research should elucidate the source of the accumulated sulfate.

Exposure of maize to 1.0 µl l⁻¹ H₂S did not affect the total leaf blade area and stomatal density at the abaxial and adaxial side of nascent leaves (Figs. 2 and 3). There were approximately 75 stomata mm⁻² at the adaxial leaf side and 50 at the abaxial leaf side (Fig. 3). Similar densities were reported previously (e.g., Zheng et al. 2013). Based on these observations, it is concluded that H₂S fumigation does not affect the total number of stomata per plant.

Based on these observations, it is also concluded that it is unlikely that H₂S regulates the formation of aerenchyma in maize leaves. Aerenchyma can be formed via programmed cell death (PCD) events and H₂S is hypothesized to be a signal molecule stimulating PCD (Maniou et al. 2014). However, H₂S fumigation did neither alter leaf biomass nor leaf area (Figs. 2 and 3). It did thus not affect the specific leaf weight, which implies H₂S did not induce aerenchyma formation in the foliage. In accordance with this result, previously, it was shown that exposure of maize to atmospheric H₂S did not trigger the aerenchyma formation in roots (Ausma et al. 2017).

![Fig. 2](image2.png)

**Fig. 2** Total leaf blade area of maize as affected by H₂S fumigation and sulfate deprivation. For experimental details, see the legend of Table 2. Data, representing 4 measurements with 4 plants in each, are presented as boxes with a 5–95 percentile and whiskers. Different letters indicate significant differences between treatments ($P ≤ 0.05$; two-way ANOVA; Tukey’s HSD test as a post hoc test)

![Fig. 4](image4.png)

**Fig. 4** Transpiration rate of maize as affected by H₂S fumigation and sulfate deprivation. For experimental details, see the legend of Table 2. Data, representing 4 measurements with 4 plants in each, are presented as boxes with a 5–95 percentile and whiskers. Different letters indicate significant differences between treatments ($P ≤ 0.05$; two-way ANOVA; Tukey’s HSD test as a post hoc test)

![Fig. 3](image3.png)

**Fig. 3** Stomatal density at the abaxial and adaxial side of leaf blades of maize as affected by H₂S fumigation and sulfate deprivation. For experimental details, see the legend of Table 2. Data, representing 4 measurements with 2 plants in each, are presented as boxes with a 5–95 percentile and whiskers. Different letters indicate significant differences between treatments ($P ≤ 0.05$; two-way ANOVA; Tukey’s HSD test as a post hoc test)
Apart from having no effect on the total number of stomata per plant, exposure to 1.0 µl l⁻¹ H₂S did not affect the plants’ transpiration rate (Fig. 4). Transpiration rates were approximately 3.6 g H₂O g⁻¹ FW plant day⁻¹ (Fig. 4). Accordingly, H₂S exposure did not affect stomatal resistance at the abaxial and adaxial side of nascent leaves (Fig. 5). Since H₂S fumigation did neither affect the total number of stomata per plant nor the plant’s transpiration rate and stomatal resistance, we conclude that fumigation did not affect the transpiration rate per stoma.

In maize and other plants, stomatal transpiration and conductance are strongly positively correlated with stomatal aperture (Shimshi 1963; Shimshi and Ephrat 1975; Lawson et al. 1998; Kaiser 2009). For instance, Shimshi (1963) reported for maize that stomatal conductance (y) depends on aperture (x) according to the formula y = 0.073 + 0.147x (R² = 0.88). It thus is safe to say that fumigation with 1.0 µl l⁻¹ H₂S of maize did not modify stomatal aperture. The absence of an effect is not caused by H₂S levels that are too low, because shoot cysteine levels were two-to-three fold higher in H₂S-fumigated plants compared to non-fumigated plants (Fig. 1), which is highly similar to the twofold increase of foliar cysteine levels that Batool et al. (2018) reported to strongly impact stomatal aperture. Clearly, at least in maize, H₂S does not interfere with the signal transduction cascade that regulates stomatal aperture.

**Conclusion**

Maize plants could use atmospheric H₂S as a sulfur source for growth. Foliar H₂S absorbance markedly affected the plant’s sulfur status; however, it did not affect the total leaf area, stomatal density, stomatal resistance, and transpiration rate of plants. We thus conclude that, at least in maize, H₂S does not function as signal molecule in the regulation of stomatal aperture.

**Author contributions statement** TA conceived and designed the study. TA, JM, and TRP collected the data. TA analyzed the data. TA, CJvdK, and LJDK wrote the manuscript.

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