Emission of Volatile Sulfur Compounds from Spruce Trees[1]

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ABSTRACT

Spruce (*Picea abies* L.) trees from the same clone were supplied with different, but low, amounts of plant available sulfate in the soil (9.7–18.1 milligrams per 100 grams of soil). Branches attached to the trees were enclosed in a dynamic gas exchange cuvette and analyzed for the emission of volatile sulfur compounds. Independent of the sulfate supply in the soil, H_2S was the predominant reduced sulfur compound continuously emitted from the branches with high rates during the day and low rates in the night. In the light, as well as in the dark, the rates of H_2S emission increased exponentially with increasing water vapor flux from the needles. Approximately 1 nanomole of H_2S was found to be emitted per mole of water. When stomata were closed completely, only minute emission of H_2S was observed. Apparently, H_2S emission from the needles is highly dependent on stromatal aperture, and permeation through the cuticle is negligible. In several experiments, small amounts of dimethylsulfide and carbonylsulfide were also detected in a portion of the samples. However, SO_2 was the only sulfur compound consistently emitted from branches of spruce trees in addition to H_2S. Emission of SO_2 mainly proceeded via an outburst starting before the beginning of the light period. The total amount of SO_2 emitted from the needles during this outburst was correlated with the plant available sulfate in the soil. The diurnal changes in sulfur metabolism that may result in an outburst of SO_2 are discussed.

Numerous laboratory experiments with cut branches, detached leaves, leaf discs, or tissue cultures have shown that green cells of higher plants can release volatile sulfur compounds into the atmosphere (6, 14, 16, 17). Volatile sulfur compounds were produced by plant cells exposed to an excess of sulfur (6, 16). In response to the uptake of sulfate, SO_2, L- and D-cysteine, H_2S was found to be emitted (6, 17); in response to S-methyl-L-cysteine, L- and D-methionine, emission of methyl mercaptan was observed (18). From these observations, it has been suggested that emission of volatile sulfur by plants may be a regulatory device to get rid of an excess of sulfur taken up by the roots or by the leaves (6, 16).

Whole plant studies in the laboratory and in the field have confirmed this assumption (6, 17). H_2S was emitted in significant amounts by attached leaves of *Geranium* plants and needles of pine trees exposed to a SO_2-containing atmosphere (11, 19); the same sulfur compound was found to be released by soybean plants and yellow poplar trees when additional sulfate was offered to the roots (8, 20). In leaf chamber studies with charcoal/Purafil-filtered air, volatile sulfur was also released in small amounts from several crops growing in the field without addition of sulfur-containing fertilizers (6). The amount of volatile sulfur emitted was considerably higher in the light than in the dark. But even in the light, emissions were too small to enable identification of the chemical nature of individual compounds by the GC system available to the authors. These observations indicate that volatile sulfur compounds may be emitted by plants also in the absence of excess atmospheric or pedospheric sulfur. The present experiments with spruce trees were performed to test this assumption, to identify the compounds emitted, and to obtain information on the factors modulating the emission of sulfur compounds under these conditions.

MATERIALS AND METHODS

Plant Material

The experiments were performed during October 1988 with 6 yr old Norway spruce trees from the same clone (*Picea abies* L. Karst., cv Vorallgäuer Fichte). Plants were obtained from the Staatliche Samenklenge und Pflanzgarten, Laufen (FRG) and grown in 20 L pots on compost/soil mixtures in the field close to the Fraunhofer-Institut in Garmisch-Partenkirchen, FRG. Once a week the trees were supplied with tap water.

Gas Exchange Studies

Plants were adapted to laboratory conditions for several hours and watered immediately before the experiments. A branch with three needle generations was enclosed in a dynamic gas exchange cuvette of 2.6-L volume. Sulfur-free compressed air (Messer-Griesheim, München, FRG) containing 350 ppm CO_2 was continuously passed through the cuvette at a flow rate of 1 L min^{-1}. Temperature and relative humidity in the cuvette were measured by a Vaisala humidity and temperature transmitter (type HMP 114Y, Helsinki, Finland). The temperature inside the cuvette was automatically adjusted to the outside temperature (day: 20.6–26.5°C; night: 20.5–23.0°C). Depending on the individual plant analyzed,

the relative humidity in the chamber varied from 33 to 94% during the day and from 10 to 75% during the night. Photosynthetic active radiation was measured by a Li185B Radiometer equipped with a Li190SB Quantum sensor (Lambda Instr., Lincoln, NE); CO_2 concentrations were determined with an infrared gas analyzer (BINOS 4b, Leybold-Heraeus Hanau, FRG). All variables were recorded every minute during the experiments. Rates of transpiration and CO_2 fixation were calculated from differences between the inlet and the outlet port of the cuvette as previously described (4). Branches enclosed in the cuvette were exposed to light intensities shown to be saturating with the spruce trees analyzed (370 μmol m^{-2} s^{-1}) for 10.5 h and to a 30 min period of diminished light (15 μmol m^{-2} s^{-1}) at the beginning and the end of the dark period. Illumination was provided by an Osram Powerstar HQI-T 400 W/DH lamp. During the dark periods, the lamp was switched off and the cuvette was covered with a black cotton blanket; this resulted in light intensities below 1.0 μmol m^{-2} s^{-1} inside the cuvette.

Analysis of Volatile Sulfur Compounds

At the outlet port of the cuvette, aliquots of 167 mL min^{-1} were led via Teflon tubing (⅛ inch o.d.) to a sampling loop (27 cm length, 8 mm i.d.) made out of glass (Duran, Schott, Mainz, FRG). For cryo-enrichment of sulfur compounds, the loop was inserted into liquid argon (−186°C, Linde, Munich, FRG). After 30 min, sampling was terminated by closing valves (Rheodyne Teflon Rotary valve, type 50, Cotati, CA) at both ends of the loop. Sampling loops were stored in liquid argon until GC analysis. For desorption of trapped sulfur compounds, the loops were inserted into a Dewar flask containing a mixture of EtOH and Dry Ice at −80°C; for cryo-focusing, desorbed gases were carried with a stream of 20 mL N$_2$ min^{-1} via Teflon tubing (¹⁄₁₆ inch o.d.) into a second glass loop (25 cm length, 1.5 mm i.d.) kept in liquid argon and connected to the GC column (Carbopack BHT-100, Supelco, Belafonte, PA). After 30 s the second loop was transferred into a water bath at 25°C, so that the entire sample was swept by the carrier gas stream (20 mL N$_2$ min^{-1}) to the GC column. Separation of volatile sulfur compounds was achieved by maintaining the column temperature at −5°C for 5 min and subsequent heating to +55°C at a rate of 30°C min^{-1}. Sulfur compounds were measured with an FPD[4] sulfur analyzer (Tracor, Bilthoven, The Netherlands) and quantified with a Shimadzu CR3A integrator (Kyoto, Japan). Identification and calibration was performed by means of standard permeation tubes (Dynacal, UPK, Bad Nauheim, FRG).

Other Methods

Soil pH, plant available sulfate, and total nitrogen content of the soils were analyzed by standard methods at the Bayerische Hauptversuchsanstalt für Landwirtschaft (3). Total sulfur in spruce needles was determined after conversion to sulfate; for this purpose needles were wet-ashed in a Wickbold apparatus and subjected to ion chromatography (Dionex, Sunnyvale, CA). The projected leaf area of spruce needles was

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4 Abbreviation: FPD, flame photometric detector.

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determined by optical measurements with an area meter (Delta-T Devices Ltd., Cambridge, UK) connected to a video camera (TC2000, RCA, Lancaster, PA). From these measurements, the surface area of the needles was calculated by multiplication with the factor 2.65 (15).

RESULTS

Branches of spruce trees were enclosed in a dynamic gas exchange cuvette. Transpiration and CO_2 fixation rates determined under these conditions (Table I) were consistent with those reported for spruce by other authors (2, 13). The branches enclosed continuously emitted hydrogen sulfide into the atmosphere during the day and in the night (Table I). In several experiments, trace amounts of dimethylsulfide and carbonylsulfide were also detected in some of the samples; emission of carbon disulfide, methyl mercaptan, or dimethyldisulfide was not observed (data not shown). For an individual plant, the rates of emission of H$_2$S in the light were considerably higher than those in dark, suggesting that light-dependent and light-independent processes may contribute to the emission of H$_2$S from spruce needles (Table I). However, the rapid light/dark or dark/light transitions performed did not result in rapid changes in the rate of H$_2$S emission; several hours were needed before a constantly enhanced/reduced rate of emission was determined (Fig. 1).

When light and dark emissions of H$_2$S from different plants were compared, dark emissions from some plants were found to be higher than light emissions from other plants. This observation can be explained by differences in transpiration between trees, as the rate of emission of H$_2$S increased exponentially with transpiration (Fig. 2) independent of whether the branches were kept in the light or in the dark. Approximately 1 nmol of H$_2$S was emitted per 1 mol water transpired. Apparently, H$_2$S emission from the needles is entirely dependent on stomatal aperture. Slow changes in the rate of H$_2$S emission during light/dark or dark/light transitions may therefore be the consequence of slow changes in stomatal conductance. The finding that H$_2$S is also emitted at zero

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Table I. *Gas-Exchange Characteristics of Spruce Branches Enclosed in a Dynamic Chamber*

Branches of spruce trees were enclosed in a dynamic cuvette flushed with sulfur-free compressed air at a rate of 1 L min^{-1}. The air in the chamber was stirred by a fan so that concentration gradients inside the chamber could be avoided. The rates of transpiration and CO_2 exchange were calculated from measurements at the chamber inlet and outlet. Sulfur compounds emitted were collected from the gas stream leaving the chamber in a liquid argon trap and were separated and quantified by GC/FPD analysis.

Parameter	Light	Dark
CO_2 exchange (mmol CO_2 m^{-2} la h^{-1})[b]	−8.99 ± 0.22[a]	+1.27 ± 0.16
Transpiration (mol H$_2$O m^{-2} la h^{-1})	1.50 ± 0.13	1.98 ± 0.13
Stomatal conductance (cm s^{-1})	0.19 ± 0.03	0.09 ± 0.02
H$_2$S emission (nmol H$_2$S m^{-2} la h^{-1})	2.10 ± 0.15	1.04 ± 0.19

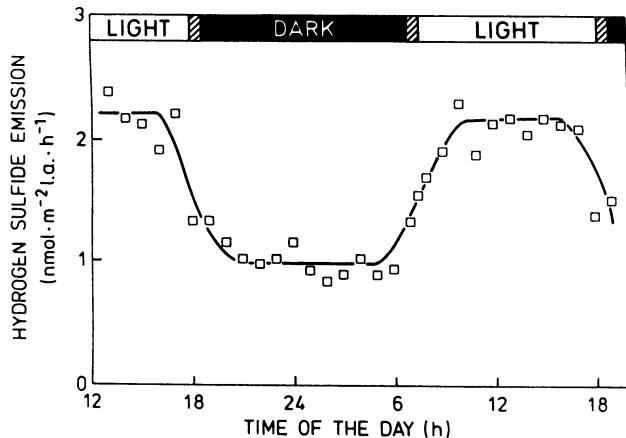[a] ± SD.   [b] la: projected leaf surface area × 2.65 (see ref. 15).

Figure 1. H_2S emission from branches of spruce trees. Branches of a spruce tree were enclosed in a dynamic chamber flushed with sulfur free compressed air at a rate of 1 L min^{-1}. The air in the chamber was stirred by a fan so that concentration gradients inside the chamber could be avoided. Branches were exposed to saturating light intensities for 10.5 h and to a 30-min period of diminished light at the beginning and the end of the dark period. At the times indicated, sulfur compounds emitted were collected from the gas stream leaving the chamber in a liquid argon trap. Sulfur compounds trapped were separated and quantified by GC/FPD analysis. The data shown represent one individual cycle out of 20 measured with similar results. l.a., leaf surface area.

transpiration suggests that small amounts of this sulfur compound may also be emitted via the cuticle when the stomata are closed (Fig. 2).

In experiments performed with different spruce trees from the same clone, the sulfate available in the soils differed by a factor of two; soil pH and total nitrogen contents of the soils were similar. Still, H_2S emission was not dependent on the sulfate available in the soil, and the total sulfur content of the needles was similar in all the trees studied (Table II). These findings may be explained by the relatively low sulfate content of all the soils studied (7).

SO_2 was the only volatile sulfur compound consistently found to be emitted from branches of spruce trees in addition to H_2S (Fig. 3). High emissions of SO_2 were observed only early in the morning; SO_2 emission usually started shortly before the beginning of the light period and continued for 2 to 3 h. During other times of the day or the night, SO_2 was only sporadically emitted in minor amounts. The outburst of SO_2 in the morning was compared between trees supplied with different sulfate contents in the soil (Table II). Although the sulfur contents of the needles were similar, the total amount of SO_2 emitted early in the morning increased with increasing amounts of sulfate available in the soil.

DISCUSSION

H_2S emission by higher plants has previously been observed in response to an excess sulfur supply to the roots or to the leaves (16, 17). The present experiments with spruce trees show that H_2S is also emitted in the absence of significant atmospheric sulfur at low sulfate contents in the soil. Therefore, emission of volatile sulfur compounds from vegetation may be of higher significance in the global biogeochemical

cycle of sulfur than previously assumed (1). Dimethylsulfide, found to be emitted from several crop species in the absence of excess sulfur (5, 10, 12), was not released in significant amounts from needles of spruce trees. H_2S emission from needles of spruce trees was observed in the light and in the dark, indicating a participation of light-dependent and light-independent processes, *e.g.* sulfate reduction and cysteine desulfhydration (17). This conclusion is consistent with the finding that for the individual tree the rates of emission were much higher in the light than in the dark. However, when emissions from different trees were compared, the rates of H_2S emission were entirely dependent on transpiration and, hence, on stomatal aperture. Therefore, it cannot be excluded that only light-independent processes contribute to the emission of H_2S in the absence of excess sulfur. The present finding that emissions in the dark from one tree can be higher than emissions in the light from another tree, provided transpiration is sufficiently high, supports this idea. The observation that only minute amounts of H_2S may be released from the needles via the cuticle is consistent with recent experiments on the permeability of isolated cuticles to sulfur gases (K Lendzian, H Rennenberg, unpublished results).
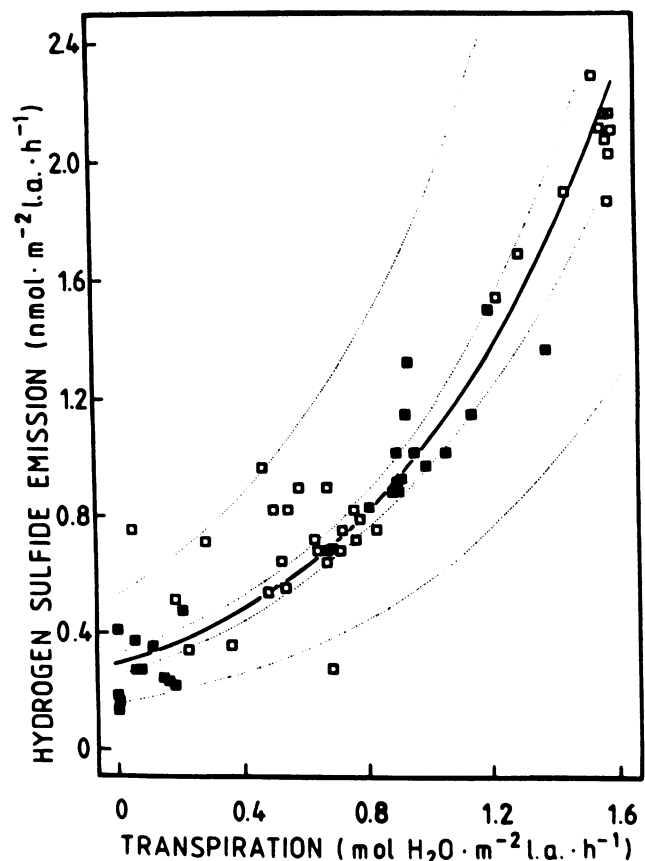
SO_2 was the only sulfur compound consistently found to

Figure 2. H_2S emission and water vapor flux from branches of spruce trees. H_2S emission and water vapor flux were determined during the day (open squares) and during the night (closed squares) with branches of three spruce trees, differing in the availability of sulfate in the soil. The data shown represent the entire set of single measurements performed with the trees l.a., leaf surface area.

Table II. *Soil Composition, Volatile Sulfur Emissions, and Sulfur Content of Spruce Needles*

Total nitrogen, plant available sulfate, and pH in the soil as well as total sulfur in the needles were measured as described in "Materials and Methods." H_2S and SO_2 emissions from spruce branches enclosed in a dynamic cuvette were determined after trapping in liquid argon by GC/FPD analysis. Transpiration was calculated from water vapor measurements at the inlet and outlet port of the cuvette.

Parameter	Tree		
	1	2	3
Soil pH	7.1	7.0	7.1
Sulfate (mg 100 g^{-1} soil)	18.1	12.9	9.7
Total N (% in the soil)	1.3	1.3	1.1
Total S (mg g^{-1} dry wt needles)	1.2	0.9	1.0
H_2S emission (nmol mol^{-1} H_2O)	1.0	1.1	0.9
SO_2 emission (nmol m^{-2} la 2 h^{-1})[a]	10.3	8.8	4.1

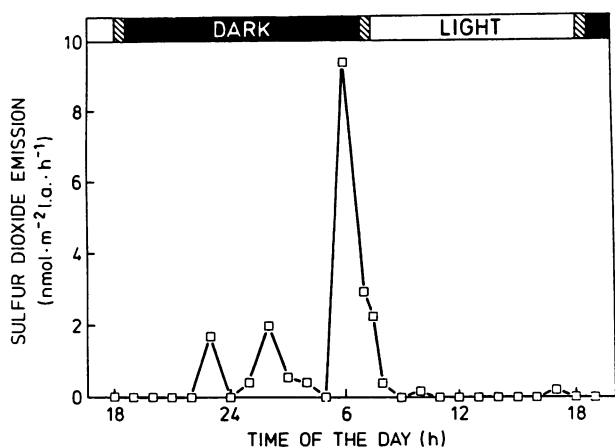[a] la: projected leaf surface area $\times$ 2.65 (see ref. 15).

Figure 3. SO_2 emission from branches of spruce trees. Branches of a spruce tree were enclosed in a dynamic chamber flushed with sulfur-free compressed air at a rate of 1 L min^{-1}. The air in the chamber was stirred by a fan so that concentration gradients inside the chamber could be avoided. At the times indicated, sulfur compounds emitted were collected from the gas stream leaving the chamber in a liquid argon trap. Sulfur compounds trapped were separated and quantified by GC/FPD analysis. The data shown represent one individual cycle out of six measured with similar results. l.a., leaf surface area.

be emitted by spruce needles in addition to H_2S. To our knowledge this is the first report demonstrating biogenic emission of SO_2. Because of its biophysical and biochemical properties (solubility, pH-dependent dissociation, oxidation), this atmospheric trace constituent is generally thought to be deposited in plants, but not to be emitted by them (*e.g.* 19). Materna (14) observed that SO_2 was released from spruce trees fumigated with SO_2 after the fumigation was terminated. However, it cannot be excluded that this release was due to desorption processes. In the present experiments with spruce trees exposed to sulfur-free air, sulfur dioxide was primarily emitted in an outburst starting shortly before the beginning of the light period. In a previous study, SO_2 was also observed

in the atmosphere above a remote conifer forest early in the morning (9). It may therefore be concluded that this finding is due to sulfur emissions by the trees; obviously, *in situ* measurements are required to test this assumption. The actual amount of SO_2 released in the outburst observed in the present study was found to be dependent on the sulfate available in the soil. This result is surprising because such a correlation was observed even though the differences in the sulfate content of the soil did not result in considerable changes in the sulfur content of the needles.

The metabolic events leading to the production and emission of SO_2 from spruce trees are obscure. It may be assumed that sulfate is converted to sulfite accumulating inside the cells during the night. The sulfite inside the cells spontaneously equilibrates with bisulfite and SO_2, depending on compartmental pH values. If not metabolized, SO_2 may reach equilibrium between intracellular and extracellular compartments (cell wall and intracellular air space). However, from the present results, it appears unlikely that opening of the stomata results in a sudden release of the SO_2 from the intracellular space; an outburst of SO_2 was also observed when the stomata were only partially closed during the night. On the other hand, stomatal conductance gradually increased during the time SO_2 was emitted in all experiments performed (data not shown). Acidification of a cellular compartment in which sulfite may have accumulated during the night can be an alternative explanation for the observed emission of SO_2. Obviously, further experiments are needed to obtain a better understanding of the processes leading to an emission of SO_2 from needles of spruce trees into the atmosphere.

ACKNOWLEDGMENTS

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