Biogenic Sulfur in the Environment

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Sulfur Emissions from Roots of the Rain Forest Tree
*Stryphnodendron excelsum*

Ecosystem, Community, and Physiological Implications

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Roots of *Stryphnodendron excelsum* trees in a lowland rain forest in eastern Costa Rica emit sulfur gases. Extrapolated annual estimates of emissions, based on *S. excelsum* tree density, are on the order of 0.29 kg S.ha⁻¹.yr⁻¹. At the ecosystem level, this flux is too small to account for the 11 kg S.ha⁻¹.yr⁻¹ SO₄-S input-output discrepancy and acid rain reported earlier. At the physiological level, emission of CS₂ is stimulated by disturbance to the roots of *S. excelsum*. Considering the known toxicity of CS₂ to nematodes, root rot fungi, insects, and nitrifying bacteria we suggest that CS₂ emission may, at the community level, be a defensive mechanism against root predators, and pathogens and a nitrogen conserving mechanism.

Sulfur gas flux from living vascular plants to the atmosphere is a little studied part of the global sulfur cycle. Sulfur fluxes from other parts of the biosphere to the atmosphere are more studied. For example, sulfur gas emissions are known from marine systems (1-6), from coastal estuarine and marsh systems (7-16), from fresh water floodplain lakes (17), from temperate soils (18-20), and from tropical forests (20-22). Laboratory studies have demonstrated sulfur gas emissions from soils (23-26), from intact higher plants (27-30), and plant parts (see review by Rennenberg 31). An inventory of reduced sulfur gas emissions from soil, crops, and trees in the United States has been started (32,33,34). General reviews of the sulfur cycle are provided by Smil (35) and by Ivanov and Freney (36). From these studies, the geographic distribution of biogenic sulfur gas source strengths on a global basis and the phylogenetic distribution of sulfur gas emissions within the plant kingdom are still relatively unknown.

During a survey of sulfur gas emissions from a central American rainforest (37), *Stryphnodendron excelsum* Harms (Mimosaceae) was found to be a sufficiently strong sulfur emitter that its location in the forest could be detected by odor. The present study attempted to quantify sulfur emissions from *S. excelsum* to the atmosphere.
Study Site and Methods

Sampling was performed at the La Selva Biological Station of the Organization for Tropical Studies, Puerto Viejo de Sarapiquí, Provincia Heredia, Costa Rica, 10° 24 '26 "N lat, 84° 00 '02 "W long. Three *S. excelsum* trees ranging from 0.87 to 0.96 m in diameter and from 22 to 32 m in height were selected within 100 m of the laboratory to facilitate rapid processing of gas samples.

Preliminary sampling suggested that roots of one of the trees extended more than 16 m from the trunk. Later sampling was performed at 4 m intervals on transects on each of the compass quadrants centered on two trees. For one tree near a forest edge, one transect line was run toward the forest interior for 16 m. At each sampling point on each transect a 25 x 25 cm template was used to cut leaf litter and roots. Litter was removed to a 250 ml Nalgene polypropylene wide mouth centrifuge jar (Nalge Co. Rochester, NY). Soil and associated roots were excavated to a depth of 10 cm and removed to the lab where they were separated by 4 mm sieves without addition of water. Dry sieving avoided the possibility of flooding and anaerobism changing the quality and amount of reduced sulfur gas emission. The total root mass was sorted by odor into sulfur emitting roots and non-emitting roots. Sulfur emitting roots were placed in 250 ml polypropylene centrifuge jars and incubated at ambient rainforest temperature. The time course of sulfur gas accumulation in the centrifuge jars was quantified by use of a Perkin-Elmer Sigma 4B gas chromatograph (Perkin-Elmer, Norwalk, CT) fitted with a sulfur specific flame photometric detector, a 2 mm internal dia. 9 m teflon column packed with 5% polyphenyl ether + 0.5% H3PO4 on 40/60 teflon, at 90°C, with 99 ml N2 carrier min-1. Reference hydrogen sulfide, carbon disulfide, dimethyl sulfide, and ethyl mercaptan were supplied from permeation tubes (VICI Metronics, Santa Clara, CA) inserted into a Tracor Model 432 Tri-Perm Permeation Calibration system (Tracor, Inc., Austin, TX). Standards and unknown samples were pulled either from sample incubation bottles or from the permeation system to the gas chromatograph through multiposition zero dead volume sampling valves and a 2 ml teflon sample loop using a 10 ml syringe. Sample loop, valves, and connecting teflon lines were heated to 65°C to minimize surface adsorption of sulfur gases.

Verification of CS2 as the principal sulfur gas from the roots of each of the three trees sampled in this study was performed with a Finnigan OWA 3B gas chromatograph-mass spectrograph (Finnigan Corp., Sunnyvale, CA).

Three incubation regimes were used for sulfur emitting roots.

1) Field moist roots. All root samples were physically pulled from field collected soil and incubated at existing moisture conditions.

2) Vigorously washed roots. Following incubations of field moist roots, they were removed from the bottles and squeezed repeatedly under running water to dislodge adhering soil particles in preparation for dry weight determinations. When the odor of increased sulfur emissions was noted during washing, one subset of samples was re-incubated and reanalyzed by gas chromatography to determine the magnitude of change in emission rate.

3) Gently rinsed roots. For another set of 12 root samples, following incubation at field moist conditions, the roots were kept in the incubation jars and the gas was displaced by 3 changes of water in immediate succession. This was done to determine if wetting alone or both wetting and squeezing were required to stimulate sulfur gas emissions.

Following the various incubation regimes, all roots were washed free of soil and dried to constant weight at 65°C. Dry roots were sorted into diameter
classes of 0-1.9, 2.0-4.9, 5.0-9.9 and greater than 10 mm. Means, standard deviations, and correlations were computed with the Statistical Analysis System (38).

Results

Field Moist Samples. Emissions of the sulfur gases hydrogen sulfide, carbon disulfide, dimethyl sulfide + ethyl mercaptan from forest floor litter, roots, and soil calculated from the initial slopes of time course incubations are given in Table I. Rates are given for distances of 4, 8, 12, and 16 m from trunks of S. excelsum trees as g S. m⁻².min⁻¹. Summing outward from the tree trunk, the annual extrapolated emission rate multiplied by the areas of each successive circle (Figure 1), estimated an overall emission of 100 g S. tree⁻¹.year⁻¹ from a 200 m² area. The fractional contribution of S. excelsum trees to the stem cross sectional area (basal area) of the forest is 0.06 (39). From the extrapolated annual emission rate and the proportional contribution of S. excelsum to the cross sectional area, an annual emission of 0.29 kg S. ha⁻¹.yr⁻¹ was calculated. This assumes constant emission rates instead of possible diurnal and seasonal cycling of rates.

Vigorously Washed Roots. The time courses of CS₂ at field moist conditions and after two vigorous washings are given in Figure 2a. Results from each of the two washings are replotted in Figures 2b and 2c synchronizing the beginnings of the re-incubations in order to facilitate comparisons of initial slopes. For this particular set of roots, CS₂ was detectable in 5 incubations of field moist roots, but detectable in 7 incubations after the first wash and in 8 after the second wash. Following rapid initial accumulation of CS₂, concentrations either increased more slowly, remained the same or decreased.

Gently Rinsed Roots. The field moist incubations showed rapid CS₂ accumulation followed by declining concentration. CS₂ production was not continuous. The initial slopes for the CS₂ time courses were greater for the field moist incubations (Figure 3a) than for post-rinse incubations (Figure 3b). CS₂ was detectable in 7 of the field moist incubations but continued in only 5 of them following rinsing.

Root Weight and S Emission per Unit Root Weight. Dry weights of non-sulfur emitting and of sulfur emitting roots (Table II) show about the same total amount of roots but decreasing amounts of sulfur emitting roots with increasing distance away from S. excelsum trunks. The dry weights of 0-1.9 mm dia. non-sulfur emitting roots were negatively correlated (r = -0.3, P < 0.049, n = 36) with the dry weights of 2-4.9 mm diameter sulfur emitting roots. We do not interpret this as evidence for allelopathy. Emission rates first calculated as g.m⁻² forest in Table I are recalculated using individual sample root weights to give sulfur emission rates per gram root dry weight in Table III to facilitate comparison of data with data from future studies.

Discussion

Results of this study have implications for ecosystem ecology, community ecology, and for physiological-evolutionary ecology.

Ecosystem Level. This study was designed to estimate the potential proportional contribution of sulfur gas emissions from S. excelsum to the 11 kg.ha⁻¹.yr⁻¹ SO₄-S input-output discrepancy of the rainforest (40). A gas
Table I. Potential Sulfur Emission, Mean (Standard Deviation) g S x 10^-9 m^-2.min^-1 from Roots, Litter and Soil at 4 Distances from S. excelsum Trees. Sample Size = 9 at Each Distance

<table>
<thead>
<tr>
<th>Source</th>
<th>Distance, m</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur Compound</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS_2-S</td>
<td>2959(2010)</td>
<td>1663(1417)</td>
<td>1049(1242)</td>
<td>281(416)</td>
<td></td>
</tr>
<tr>
<td>H_2S-S</td>
<td>41.6(83.2)</td>
<td>3.5(10.7)</td>
<td>0(0)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Litter (CH_3)_2S-S + C_2H_5SH-S</td>
<td>0.006(0.22)</td>
<td>0.23(0.62)</td>
<td>0.18(0.49)</td>
<td>0.17(0.38)</td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3000(1981)</td>
<td>1666(1420)</td>
<td>1049(1242)</td>
<td>281(410)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Estimation scheme for sulfur emission from an average Stryphnodendron tree where emissions from roots, leaf litter, and soil were sampled at 4 m intervals on transects radiating out from trunk. Emissions at each distance (Table I) were multiplied by the area of circular sampling band between that distance and the previous distance, and the emissions summed through the circular bands for the whole tree.
Figure 2. Time course of CS$_2$-S concentrations in incubations of *S. excelsum* roots. A) time course for individual incubations (identified by numbers) starting as field moist roots and later subjected to two episodes of vigorous washing (W). B) time courses following the first washing replotted synchronizing the starting times of re-incubation. C) replot for the second wash as in B.

Figure 3. Time course of CS$_2$-S gas concentrations incubations of *S. excelsum* roots. A) time courses for individual incubations starting as field moist roots then gases displaced by 3 gentle rinsings with water (W). B) time courses after washing re-drawn with starting times synchronized.
Table II. Root Dry Weights, Means (Standard Deviation), in Grams by Diameter Classes, and Total for Non-sulfur Emitting Roots (R) and Sulfur Emitting Roots (RS) from 25 X 25 X 10 cm Deep Soil Volumes Sampled on Transects Radiating out From 3 S. Excelsum Trees. Sample Size = 9 at Each Distance

<table>
<thead>
<tr>
<th>Distance from tree, m</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter classes, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>RS</td>
<td>R</td>
<td>RS</td>
</tr>
<tr>
<td>0-1.9</td>
<td>4.2(2.9)</td>
<td>2.3(1.3)</td>
<td>5.2(2.3)</td>
<td>1.2(0.76)</td>
</tr>
<tr>
<td>2.0-4.9</td>
<td>2.9(2.5)</td>
<td>1.6(2.4)</td>
<td>4.2(3.8)</td>
<td>0.57(0.65)</td>
</tr>
<tr>
<td>5.0-9.9</td>
<td>2.0(2.1)</td>
<td>1.1(2.4)</td>
<td>3.3(3.4)</td>
<td>0(0)</td>
</tr>
<tr>
<td>&gt; 10.0</td>
<td>4.2(9.9)</td>
<td>0(0)</td>
<td>4.6(4.9)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Total</td>
<td>13.4(13.4)</td>
<td>5.0(5.5)</td>
<td>17.3(7.8)</td>
<td>1.8(1.1)</td>
</tr>
</tbody>
</table>

Table III. Potential Sulfur Emissions, Mean (Standard Deviation) g S x 10^-9, g Root Dry Weight^-1 min^-1, Sample Size = 9 at Each Distance

<table>
<thead>
<tr>
<th>Distance from S. excelsum tree, m</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS2-S</td>
<td>63.2(53.5)</td>
<td>78.7(120)</td>
<td>32.3(51.2)</td>
<td>30.6(40)</td>
</tr>
<tr>
<td>H2S-S</td>
<td>0.67(1.7)</td>
<td>0.49(1.5)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>
emission of 11 kg S ha\(^{-1}\) yr\(^{-1}\) to the atmosphere its subsequent oxidation to SO\(_4^{2-}\) and dilution in rain, might explain the acid rain (41) reported for this forest (40). A literal interpretation of emissions estimates calculated from roots incubated at field moist conditions and extrapolated to annual estimates, then adjusted for the proportion of *S. excelsum* trunk cross sectional area of the forest indicates annual emission rates of 0.29 kg S ha\(^{-1}\) yr\(^{-1}\). Emissions from *S. excelsum* may be incorrectly estimated for three reasons.

First, maximum emission rates may have been missed. Emission rates were rapid at first, then declined. For individual samples, between 15 and 25 min were needed to carry the soil from the forest to the laboratory, to extract sulfur emitting roots from the soil, and to place the roots in the incubation bottles. Incubations for determining rates were usually between 5 and 10 min duration.

Second, the decrease in concentration of CS\(_2\) in the headspace of the incubation jars, Figure 2, suggests that two reactions namely CS\(_2\), production and CS\(_2\) consumption were simultaneously in progress. As long as production was greater than consumption, the concentration of CS\(_2\) appeared to increase. When consumption exceeds production, the concentrations appears to decrease. With both reactions in progress, the gross gas production would be the sum of the two rates. Whether CS\(_2\) was simply adsorbed to the surface of the incubation vessel or was oxidized to SO\(_2\) and SO\(_4^{2-}\) is unknown, thus the magnitude of the consumption rate is unknown. Potential CS\(_2\) emission rates may thus have been underestimated in these incubations.

Third, the stimulation of gas emission from roots by washing raises the question about the effect of the passage of wetting fronts through the rainforest soil with each of the numerous rain showers.

Future quantification of sulfur gas emissions from *S. excelsum* roots should avoid possible mechanical damage to roots caused by their excavation from soils as in the present study. Instead, plants need to be grown in soil in containers to which simulated rainfall additions are made while sampling for sulfur gases above the soil surface. Once the relations of sulfur gas emission rates to the frequency and amounts of soil wetting are determined, these data can be coupled with a rainfall frequency and quantity model to simulate potential sulfur gas emission for *S. excelsum* on an annual basis.

Perhaps more important than the exact quantities of these emissions with the three sources of uncertainty, are the qualities of the sulfur emissions. Qualitatively, CS\(_2\) emissions have community and physiological implications.

**Community Level.** Sulfur gas emissions from *S. excelsum* roots may influence the species composition of biological communities through actions as anti-bacterial, anti-fungal, anti-nematode, anti-herbivore, anti-plant (allelopathy) agents. Anti-bacterial properties of CS\(_2\) can inhibit nitrification, the oxidation of ammonia to nitrate (42-44). The anti-fungal properties of CS\(_2\) have been used to control root rot fungi in agriculture and in forestry (45-47). It is a registered fungicide and nematicide (48). Its anti-nematode properties have been used in soil fumigation in agriculture (49). Its anti-herbivore properties have been used to kill insects in stored grains (50). The anti-plant or allelopathic effects have not been investigated as far we know.

**Physiological and Evolutionary Level.** In field and pot studies *Acacia pulchella* roots suppressed the soil fungus *Phytophthora cinnamomi* and promoted the survival of *Eucalyptus marginata* trees. This fungus is an important pathogen for *Eucalyptus*. Investigating the *Acacia-Phytophthora-Eucalyptus* interaction, Whitfield et al. (51) found CS\(_2\) to be a major constituent of the *Acacia* root volatile compounds.
Carbon disulfide emissions are known from only five plant species: minced leaves of Brassica oleracea (52), intact leaves of Medicago sativum L., Zea mays, L., Quercus lobata Nee (27) and steam distilled roots of Acacia pulchella (51). Continuation of surveys of plant species for sulfur gas emission will expand our understanding of the phylogenetic and biogeographic distribution of the sulfur emission phenomenon.

The physiological controls of \( \text{CS}_2 \) emission from plants apparently are unknown. We offer two interrelated suggestions about controls to \( \text{CS}_2 \) emission. Removal of \( \text{CS}_2 \) from around the roots either by extracting roots from soil (Figures 2 & 3) or by vigorous washing (Figure 2) or by gentle rinsing (Figure 3) leads to rapid \( \text{CS}_2 \) emission followed by relatively steady concentration (Figures 2 & 3). This suggests a feedback mechanism in which lowered \( \text{CS}_2 \) concentration at the root surface leads to rapid \( \text{CS}_2 \) release from the root.

A related control of \( \text{CS}_2 \) release from \( S. \) excelsum roots is disturbance. In the present study the gentle disturbance of rinsing with water stimulated \( \text{CS}_2 \) release (Figure 3). More vigorous washing stimulated higher rates of release (Figure 2). In the field, the odor of \( \text{CS}_2 \) became stronger during the beginning of a rain storm. Given the toxicity of \( \text{CS}_2 \), to nematodes and insects and the stimulation of \( \text{CS}_2 \) release by disturbance, we suggest that disturbance to \( S. \) excelsum roots by root-eating nematodes and insects stimulates \( \text{CS}_2 \) production as a defense mechanism.

The emission of \( \text{CS}_2 \) from these legumes may also be a mechanism removing excess \( \text{SO}_4^{2-} \) from the rhizosphere where \( \text{SO}_4^{2-} \) might otherwise inhibit the uptake of the Mo required for nitrogen fixation. Rennenberg (31) argues that \( \text{H}_2\text{S} \) emission from plants is a mechanism against excess sulfur accumulation in plants. While Rennenberg's study (31) dealt with \( \text{H}_2\text{S} \), emission of \( \text{CS}_2 \) and other sulfur gases could have similar roles. Cole et al (53) showed that \( \text{SO}_4^{2-} \) can inhibit Mo uptake by algae and bacteria. Sulfur emissions from these trees may have multiple selective advantages.

Acknowledgements

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