

Exchange of Oxygenated Volatile Organic Compounds Between Boreal Lichens and the Atmosphere

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Summary

To our knowledge these are the first measurements of the exchange of organic acids and aldehydes between lichens and the atmosphere under controlled climate chamber conditions. We found a formic acid deposition to lichens depending on thallus water content and significantly influenced by ambient atmospheric concentrations. Investigation of aldehyde exchange revealed a small and (time limited) emission of acetaldehyde which is enhanced by anoxic conditions. It is too early to transfer these findings to natural environmental conditions. For this purpose the studies must be continued in order to get more insight into the physiological regulation of the observed exchange. However, the studies point to a potential contribution of lichens and mosses to atmospheric hydrocarbons, being of special interest for regions dominated by this kind of vegetation.

Objectives

The objectives were

- 1) to reconstruct and adapt lichen (and tree twig enclosures) for field and laboratory investigations,
- 2) to sample and store lichen material for the next step of investigations,
- 3) to undertake some basic ecophysiological measurements during the BIPHOREP Joint field experiment at the Pallas site and
- 4) to investigate the exchange of C₁ and C₂ oxygenated volatiles (aldehydes and organic acids) as well as some other hydrocarbons between lichens and the atmosphere.

The investigations of VOC exchange were done under controlled environmental conditions with cuvettes implemented into a climate chamber. In addition we investigated an abundant moss species, which is known to be one of the important companions of boreal ground lichen flora.

Materials and Methods

Arrangement of enclosures within a climate chamber system

The laboratory experimental system for hydrocarbon measurements consists of five main components, (i) the climate chamber, (ii) the cuvette system including sample and reference cuvette, both connected to an IRGA, (iii) the cuvette air supply and processing, (iiii) the aldehyde (CC) sampling and (iiiii) the organic acid (OA) sampling units (Figure 1). Each Teflon bag cuvette (ID 14 cm; length 11 cm; volume of ca. 1.7 litres) was connected by four Teflon (PFA) tubings to the air inlet, IRGA, OA- and to CC-sampling units. The cuvettes were equipped with a fan ventilation at the bottom, a thermocouple to monitor inside temperature and a Teflon net to support the exposition of the lichen material. Pressurised air from a central supply was pre-purified by passing a molecular sieve and two humidifiers filled with pure Milli-Q water (HPLC-grade; $R > 18$ MS). The Humidifiers were installed in parallel or serial and total airflow through the cuvette ranged between 2-5 litres min^{-1} , depending on the kind experiments. Cuvette air was exchanged 1-3 times per minute. Climate chamber conditions for experiments ranged from 6-30 °C and PAR light intensity from 0-600 $\mu\text{E} / \text{m}^2 \text{ s}$ at upper cuvette level.

Lichen and moss sampling, storage and preconditioning

Lichen specimen were sampled in Finland and stored frozen at -23 °C according to Feige and Jensen (1987). To achieve full physiological activity the lichens were moistened and acclimatised over 3 days in a climate chamber. Climate chamber conditions for preconditioning were 14 h light ($> 400 \mu\text{mol} / \text{m}^2 \text{ s}$ PAR, 50-60 % RH, 10-15 °C) and 10 h dark (70 % RH, 8-10 °C). The lichen material was cleaned, humidified with ultra clean water ($R < 18$ MS) and pre-rinsed in an extra cuvette before being transferred to the sample-cuvette for measurement. Due to the generally higher sensitivity of mosses to transport and storage, the moss *Pleurozium schreberi* was not sampled in Finland, but in the Wisper-Valley, Taunus Mountains, Germany. The moss material was stored in the climate chamber and moistened each day. Further conditions and preparations were the same as for the lichens.

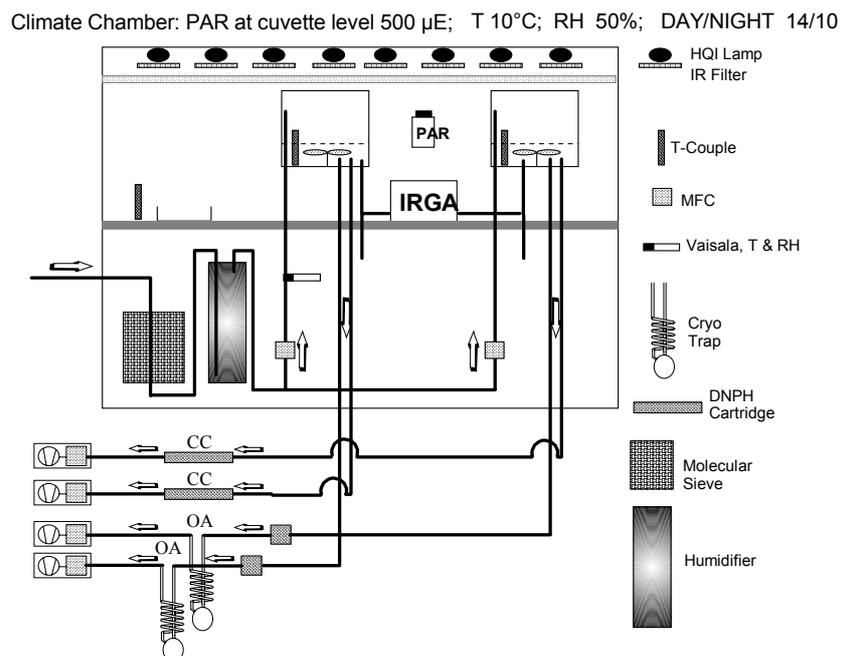


Figure 1. Arrangement of cuvettes and sampling units within the climate chamber.

Incubation and trace gas sampling

All lichen species and the moss were investigated by enclosing thallus material in Teflon bag cuvettes flushed with purified and humidified air. Sample and reference cuvette enclosures were simultaneously flushed with prepurified air or a gas-cylinder mixture. Inlet mixing ratios reflect the atmospheric environment in remote boreal areas for carbonyls (Janson R., pers. comm.) ranging between 0.05-0.5 C_1 and 0.05-0.2 C_2 [ppbv]. Organic acid concentrations were about 0.05-0.3 C_1 and 0.02-0.15 C_2 [ppbv]. Gas mixtures were obtained by mixing pressurised or synthetic air with pure gases such as oxygen, nitrogen and carbon dioxide using mass flow controllers. Total airflow of gas mixtures through the cuvette was in the order of 2-4 litres min^{-1} . Difference measurements were made by comparing a lichen cuvette with an empty reference cuvette. In most cases the lichen material was investigated over a 3h period. Minimum time resolution for trace gas sampling was 1h. The outlets of the cuvettes were connected to an infrared dual channel gas analyser (LICOR 6262) for difference measurements of CO_2 and water vapour to calculate CO_2 -assimilation and water loss. Monitoring of light, relative humidity, and temperature was performed with standard sensors for air temperature, relative humidity and photosynthetic active radiation. All data were recorded as 3 min averages on a

data logger (CSI Ltd. (UK), model 21X). In case of CO₂ depletion the absolute inlet CO₂ concentration was adjusted and measured by a portable EGM-1 gas monitor (PP Systems, UK).

Organic acids were cryogenically co-trapped with atmospheric water. The technique is described in detail elsewhere (Hofmann *et al.*, 1997). The samples were analysed on a SHIMADZU HIC-6A ion chromatograph equipped with a DIONEX Ion Pac AS 11, 4 mm column. The temperature of the system was 25°C, the flow rate of the eluent was 1 ml*min⁻¹. Aldehydes (Form- and Acetaldehyde) were trapped in accordance with Zhou and Mopper (1990) on specially prepared C₁₈ glass cartridges (C₁₈ Baker bond) (Kesselmeier *et al.*, 1997). The C18 phase was coated with an acidified solution of 2,4-dinitrophenylhydrazine (DNPH). Trapped aldehydes were eluted with acetonitrile and analysed by high performance liquid chromatography (HPLC) with a UV/VIS-detector at a wavelength of 365 nm.

Results and Discussion

Ecophysiological investigations of lichen species at the Pallas site

During a joint field experiment (July / August 1996) we investigated the actual daily physiological activities of the epiphytic lichen species at the Pallas site under natural conditions. Field data for some terrestrial lichens are available. However, investigations of physiology under field conditions with the epiphytic *Bryoria* species were not published so far. Such data are needed to consider the significance of laboratory trace gas exchange data for a natural environment. All investigations were accompanied by monitoring microclimate conditions. In the Pallas region pendulous epiphytic lichens of the genera *Bryoria* are the main contributors to the lichen biomass of the spruce/pine forests. Depending on a synergistic effect of a north-south / east- west changes of the species spectrum plus rising anthropogenic influence, other abundant genera (*Usnea*, *Hypogymnia*, *Parmelia*) become more dominant southwards. In Lapland the biomass of the naturally abundant to dominant terrestrial *Cladonia* species is heavily reduced because of reindeer husbandry. Normally, *Cladonia* is most common in the boreal zone, mainly restricted by fertile soils.

As the summer season in boreal region of Finland is subjected to the influence of continental air masses from Russia, water supply by rain is very limited. In some cases the epiphytes are even shielded against short precipitations due to dense growth of spruces. Hence, diel activities vary between 0 and 24 hours. Within 523 hours of precipitation monitoring inside a

phorophyte spruce, wetness by rain occurred only for 70 hours. Furthermore, more than 30% of these precipitations occurred at night or under light conditions, which hardly matched the needs for a positive net photosynthesis of epiphytic lichens. Using a porometer (LCA-4, ADC) coupled to a small Teflon bag type cuvette, the net photosynthesis (NP) of *Bryoria spec.* has been measured with respect to relative thallus water content (%TWC), temperature conditions and fast changing light intensity (PAR) in a pine/spruce stand. We measured daily cycles of net photosynthesis in relation to the light regime (PAR), cuvette air temperature and lichen thallus water content. The available data set is not sufficient to explain lichen physiology under field conditions in detail but it will be of fundamental value to discuss our laboratory data. The data show evidence that photosynthesis is strongly suppressed if %TWC is too high (for *Bryoria* for example > 270%). Daytime activities of *Bryoria* were mostly found at a TWC below 100%. A low light compensation point for NP between 25-35 μE was detected with a TWC higher than 60% and temperature around 12 °C. In most cases the ambient temperature or cuvette temperature (T_{cuv}), respectively, has been the limiting factor of positive NP. The measurements give evidence that temperatures higher than 16 °C lead to strong respiration, especially if TWC or light intensity is low, resulting in a negative net photosynthesis. In the course of our cuvette measurements *Bryoria* showed lowest photosynthesis rates as compared to other pendulous lichen species that were less abundant. Generally, we assume that *Bryoria spec.* is better adapted to lower temperatures. Hence, *Bryoria* should show highest activities during spring and autumn.

Physiological studies under climatically controlled conditions

The Photosynthesis or, in general, physiology of lichens is strongly influenced by the hydration status of the thallus. Figure 2 gives an impression of the overall conventional characteristics of the CO_2 exchange in relation to the thallus water content of *Cetraria islandica* (L.) ACH and under temperature and light regimes. At very low water contents no physiological activity occurs, but with increasing hydration the activity, here the uptake of CO_2 , increases markedly. This is a typical behaviour for a poikilohydric organism (Nash, 1996). At water saturation of the lichen thallus, the diffusion resistance of CO_2 leads to a lower net photosynthesis (Green et al., 1994). This overall behaviour reflects the potential difficulties in measuring the gas exchange of lichens, because of water loss and drying during the experimental phase.

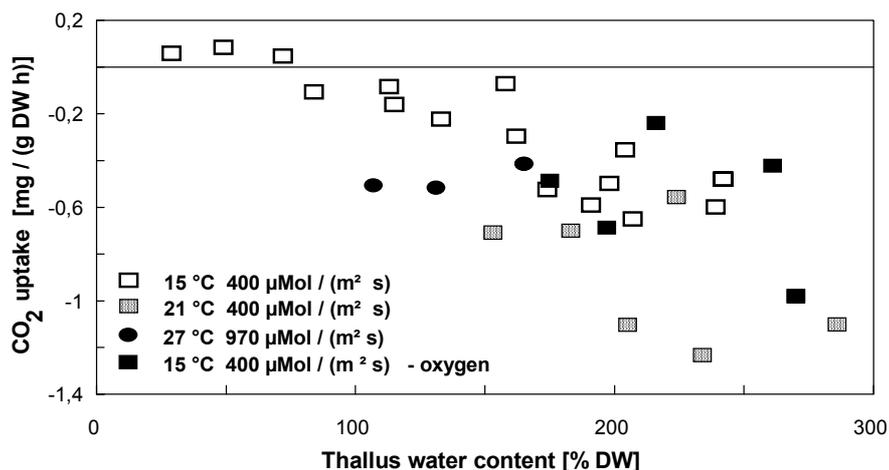


Figure 2. Exchange of carbon dioxide for *Cetraria islandica* (L.) ACH in relation to the thallus water content. CO₂ uptake (negative values) is depending on thallus water content.

Exchange of volatile organic compounds: aldehydes

We did not detect any significant exchange of formaldehyde. However, we found a time-limited acetaldehyde emission in the case of *Cetraria islandica* (L.) ACH as well as for lichens from the genera *Cladina*, *Ramalina* and *Bryoria*. For *Cetraria islandica* (L.) ACH the acetaldehyde emission ranged between <0.01 and 0.13 nmol / g DW min. Highest emissions go along with low net photosynthesis (CO₂-uptake) or even respiration (Figure 3). The acetaldehyde emissions could be triggered by anoxic conditions (without oxygen) and were further increased by elevated CO₂-concentrations (Figure 4).

The influence of sub-optimal O₂ and/or elevated CO₂ conditions on the acetaldehyde emission varied within different lichen samples but was found to be a general result. These dependencies of total acetaldehyde emissions from distinct lichen samples indicate a physiological regulation process, which depends on the disposability of either enzyme, substrate, or co-substrate (Fahselt, 1988). The most important anoxic pathway with acetaldehyde as an intermediate is the well known ethanolic fermentation pathway.

Many corresponding factors may influence Acetaldehyde emission. We would like to stress just one aspect. Within the group of preliminary investigated lichens, *Cetraria islandica* (L.) ACH and *Cladina stellaris* (OPIZ) POUZ. et VEZDA showed the highest Acetaldehyde emissions

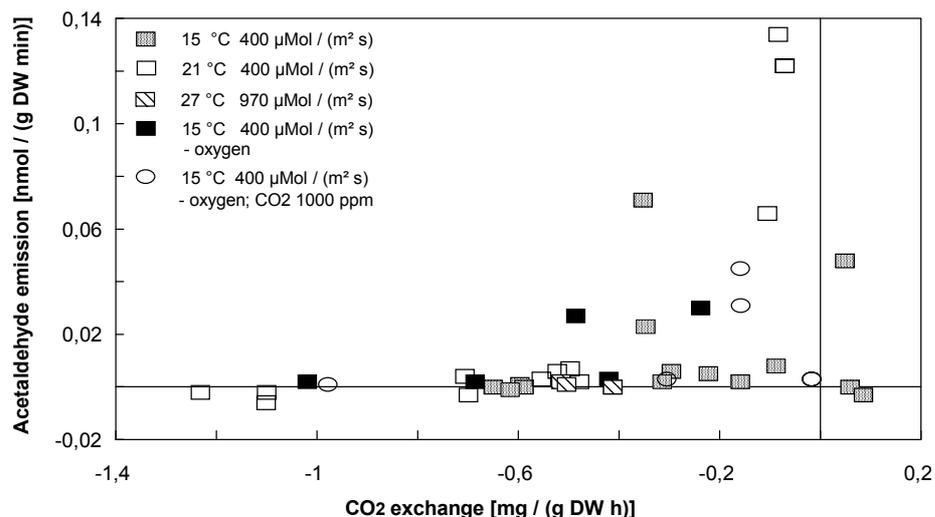


Figure 3. Acetaldehyde emissions observed with *Cetraria islandica* (L.) ACH in relation to CO₂ uptake under varying temperature and light regimes as well as under anoxic conditions.

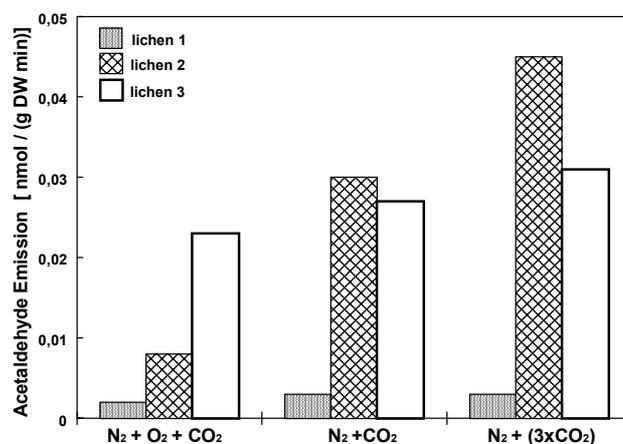


Figure 4. The influence of the availability of oxygen on the emission of acetaldehyde from *Cetraria islandica* (L.) ACH thalli.

(~0.07 nmol / g DW min), followed by *Bryoria* spec. (~0.04 nmol / g DW min), while the desert lichen *Ramalina maciformis* (DEL) BORY showed the smallest Acetaldehyde emission (0.035 nmol / g DW min). These data are preliminary and need further measurements. But within the context of a recent publication (Green et al. 1994) they seem to confirm the principal influence of sub-optimal O₂ and/or elevated CO₂ conditions. Green et al. (1994)

discussed the different, species specific CO₂ diffusion resistances and defined the CO₂ compensation points for several lichens species among them three of the lichens we investigated. Based on these data *Cetraria* showed the highest resistance followed by *Cladina* whereas *Ramalina* had the lowest resistance. Assuming that the pure physical resistance is the same for O₂ as for CO₂, the increasing Acetaldehyde emission would match a species dependant increasing diffusion resistance leading to a TWC dependant and therefore time limited O₂ deficiency in lichen thallus, which needs the production of acetaldehyde as a bypass to recover NAD. At least *Cetraria* and *Ramalina* fit into this scheme. Also a stress-induced release of acetaldehyde from a wide range of higher plants is known (Kimmerer and Kozlowski, 1982; Kimmerer and MacDonald, 1987). Furthermore, the production of acetaldehyde is well known in the case of *Saccharomyces cerevisiae* (an Ascomycete like most lichen fungi). However, further investigations are needed to show whether the central enzyme pyruvic decarboxylase is the key enzyme for the observed acetaldehyde production also in lichens.

Exchange of volatile organic compounds: aldehyde exchange of a moss

The moss *Pleurozium schreberi* was taken from a pine stand on September 9, 1997, October 23, 1997, and February 2, 1998. With respect to the weather conditions of late summer 1997 both collections from '97 must still have been adapted to summer climate. In contrast to the moss material from 1997, the samples from early 1998 experienced low temperatures and continuous moisture during several weeks. In consequence the '97 samples are titled as „summer“ and the '98 sample as „winter“ material.

First measurements of the summer samples revealed acetaldehyde emissions, which were a magnitude higher than those from the investigated lichens. The mean acetaldehyde emissions were around 0.2 to 0.3 nmol / g DW min. with a maximum over 3 nmol / g DW min. The NP of these samples were around 1 mg / g DW min (CO₂ uptake). Acetaldehyde emissions from the samples of the second moss collection (Oct. 97) were in the same range. Comparable data from both series of measurements are shown in Figure 5.

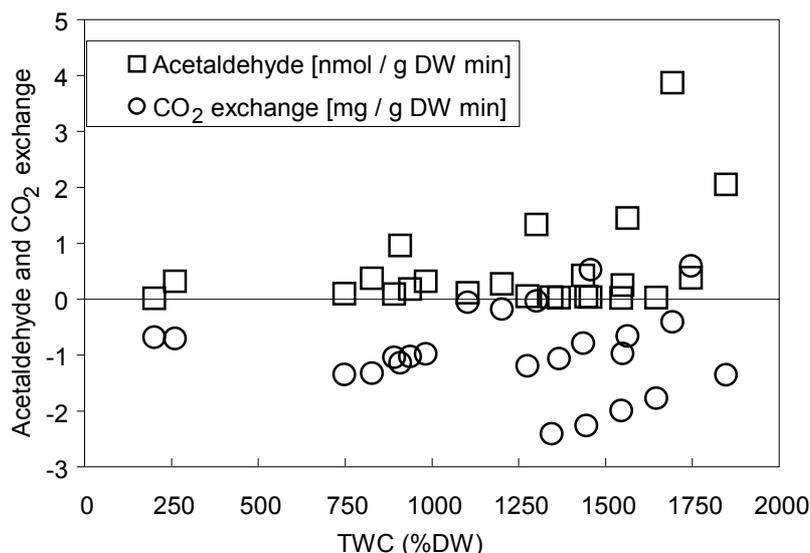


Figure 5. Relation between CO₂ exchange and acetaldehyde emission and thallus water content (TWC) of the moss *Pleurozium schreberi*.

Responding to lower chamber temperature as well as regular water availability, the moss from the second collection adapted within 2-3 weeks to the new conditions and the NP increased over two weeks. Coincidentally the acetaldehyde emissions decreased. The moss samples of the third collection (winter) started with higher NP (mean 1.7 mg / g DW min) than the summer samples, but they emitted no or little amounts of acetaldehyde, with highest emissions around 0.03 nmol / g DW min. Significant decrease of photosynthetic capacity became obvious after 6 weeks in the climate chamber. In Figure 6 the data from the first experiments with winter samples are plotted in comparison with data obtained with summer samples.

Four main hypothesises have to be discussed in order to explain the different results of acetaldehyde emissions from summer and winter samples of *Pleurozium schreberi* : (i) A general damage of the moss, which suffered from hot and dry season, (ii) a strong contamination load on by immissions, (iii) a fungal or bacterial infection and a (iiii) seasonal-dependant metabolic adaptation of the moss. Points 1 and 2 are unlikely, firstly because of the overall photosynthetic activity of the moss and secondly, because the emissions were lower but constant over a longer measurement period. The third hypothesis would be in accordance with a generally rapid development of fungal mycelia under late summer or fall conditions, changing direct emissions into indirect ones. Both, a possible fungal infection as well as a

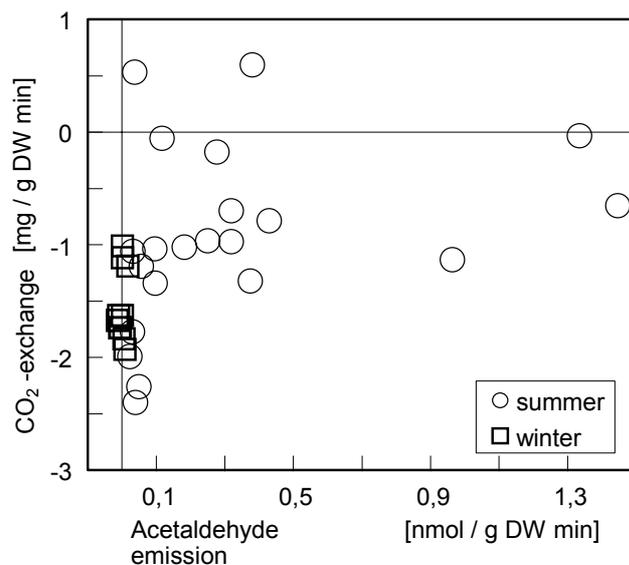


Figure 6. Relation between CO₂ exchange and acetaldehyde emission of the moss *Pleurozium schreberi* in summer and winter samples.

seasonal metabolic adaptation point to the coincidence of acetaldehyde emissions with the emission of other VOCs, which have been found for the summer samples (see table 2). These questions are under investigation now.

Exchange of volatile organic compounds with lichens: organic acids

A production and a release of both, formic as well as acetic acid from lichens, was never observed. On the contrary, a deposition was always found. In contrast to tree species but very similar to crop plants (Kesselmeier et al., 1998) formic and acetic acid were deposited in all experiments with all lichen species investigated. Therefore, we regard lichens always to represent a sink for both organic acids, at least as long as they are damp. Figure 7 shows representative data sets obtained in the case of the reindeer lichen *Cladina stellaris* (OPIZ) POUZ. et VEZDA).

Both acid species show a correlation with the inlet mixing ratios; no significant difference was found between formic and acetic acid. This correlation might even become better if it would be possible to keep the enclosed species at constant water content. Nevertheless, though lichens dry during the measurement periods, the deposition shows a quite linear correlation with the ambient concentrations. Emission or an release of deposited acids can only be expected if the

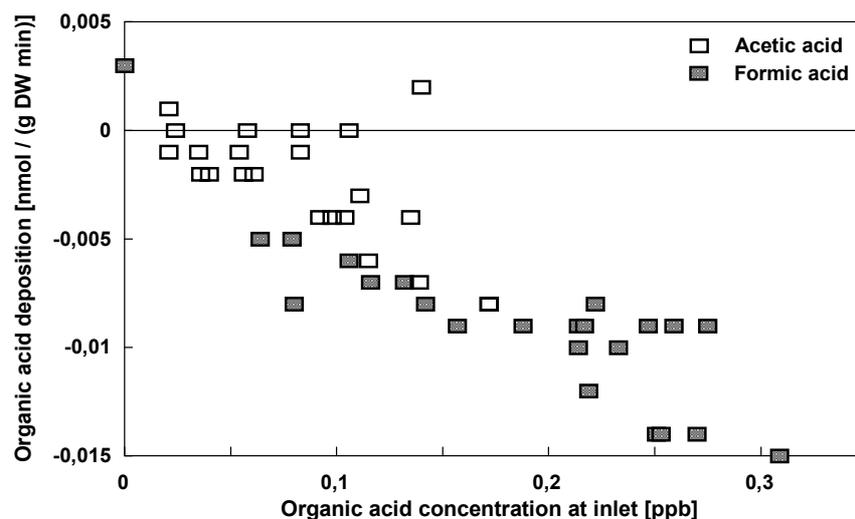


Figure 7. Exchange of formic and acetic acid with the reindeer lichen *Cladina stellaris* (OPIZ) POUZ. et VEZDA) in relation to the mixing ratio of both acids at the cuvette inlet.

atmospheric concentration is zero. As lichens represent a surface enlargement for the deposition of trace gases they can be regarded as an important sink for organic acids under humidified conditions.

Exchange of volatile organic compounds: Other VOCs

In close co-operation with the FMI (H. Hakola, Helsinki) and the CNR (P. Ciccioli, Rome) we investigated the emission of VOCs others than short chained aldehydes and organic acids from several lichens and the moss. Additional classes of hydrocarbons were sampled on either TENAX (FMI; sampling volume 3 litres) or CARBOTRAP (CNR; sampling volume 1 Litre) filled cartridges. For further technical information see Hakola et al. (1997) and Kesselmeier et al. (1996, 1997). Sampling of hydrocarbons on Carbotrap cartridges was unsuccessful, presumably due to a too small volume sampled. Those samples on TENAX (FMI) showed concentrations above the detection limits and the results are reported below (Tables 1 and 2).

Generally, the VOC emissions were very low as compared with higher plants. However there are some VOC species, which should be considered for a source discussion in the case of boreal regions. Figure 8 shows the release of Hexanal and 1-Pentanol, increasing with NP. In contrast, 1-Octen-3-ol emissions decrease with increasing NP. Such trends point to different

Table 1. Emissions [pg/s g DW] of VOCs from lichen species (Co-operation FMI / MPIC).

	<i>Cetraria islandica</i> mean (n=8)	<i>Cladina stellaris</i> mean (n=4)	<i>Bryoria spec.</i> mean (n=2)
1-penten-3-ol	0,41 ± 0,58	-1,68 ± 1,59	2,55 ± 2,16
1-pentanol	0,54 ± 0,35	1,46 ± 0,42	0,13 ± 0,13
hexanal	2,38 ± 1,98	3,04 ± 3,44	1,27 ± 0,78
2-hexenal	0,04 ± 0,1	---	---
3-hexen-1-ol	0,13 ± 0,33	---	---
1-hexanol	0,35 ± 0,39	0,82 ± 1,04	0,06 ± 0,06
a-pinene	0,6 ± 0,82	0,02 ± 0,17	-0,64 ± 0,41
camphene	0,04 ± 0,07	---	---
1-octen-3-ol	0,45 ± 0,49	0,69 ± 0,99	2,81 ± 0,02
sabinene	0,02 ± 0,03	0,03 ± 0,05	---
b-pinene	0,1 ± 0,09	0,3 ± 0,45	-0,05 ± 0,05
3-hexenylacetate	0,28 ± 0,45	0,02 ± 0,03	-0,01 ± 0,01
carene	0,4 ± 0,55	0,02 ± 0,07	-0,08 ± 0,08
limonene	0,07 ± 0,93	-0,03 ± 2,89	0,1 ± 0,25
linalool	1,33 ± 1,73	0,98 ± 1,07	0,55 ± 0,55

Table 2. Emissions of VOCs from the moss *Pleurozium schreberi* (Co-operation FMI / MPIC) [pg/s gDW] harvested in summer and winter (see text).

	summer samples mean (n=3)	winter samples mean (n=7)
1-penten-3-ol	2221,72 ± 419,5	3,78 9,58
1-pentanol	316,21 ± 26,68	0,70 1,13
hexanal	565,69 ± 153,08	18,83 37,1
2-Hexenal	74,16 ± 26,04	0,38 0,6
3-Hexen-1-ol	347,67 ± 74,08	0,66 1,62
1-Hexanol	370,93 ± 56	1,46 2,7
a-Pinene	2,43 ± 1,07	0,25 0,66
Camphene	1,29 ± 0,91	---
1-octen-3-ol	169,11 ± 33,63	6,15 14,39
Sabinene	---	---
b-Pinene	4,01 ± 2,07	0,28 0,19
3-Hexenylacetate	1,88 ± 1,74	-0,01 0,18
Carene	0,48 ± 0,45	0,21 0,55
Limonene	4,87 ± 5,08	1,13 4,95
Linalool	---	0,90 1,1

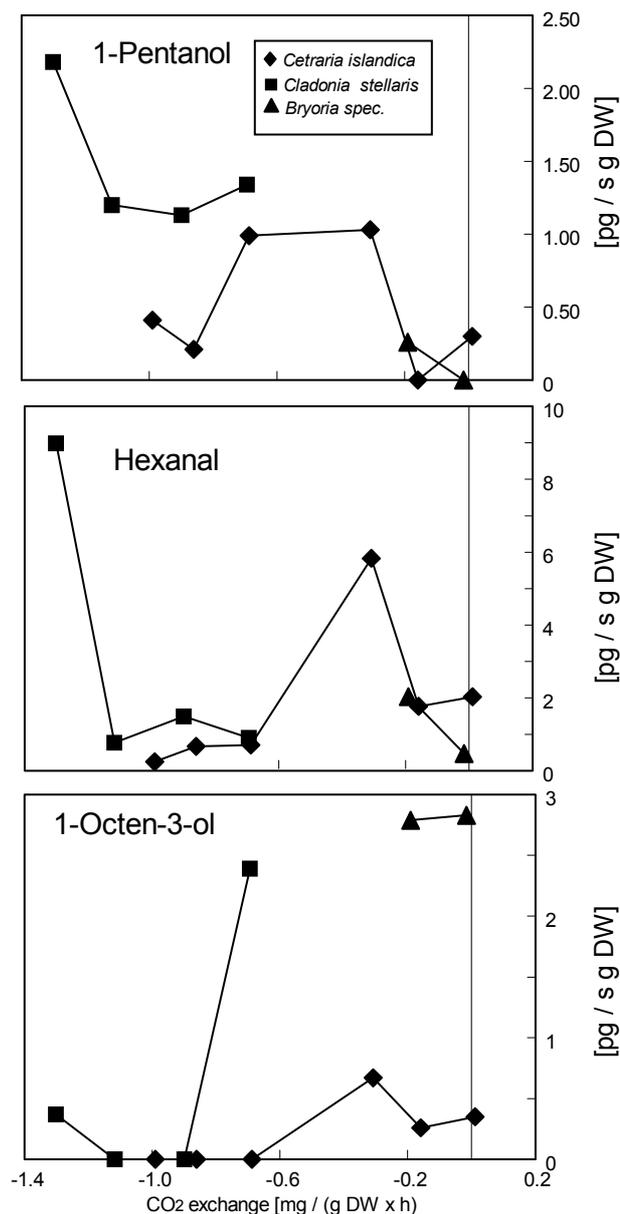


Figure 8. Emission of hexanal and 2 higher alcohols from three lichen species. Note the general increase of hexanal and 1-pentanol emission and the general decrease of 1-octen-3-ol emission with increasing CO₂ assimilation, pointing to different physiological conditions under which production and emission takes place.

metabolic pathways and in the case of lichens additionally to the two symbiotic partners, i.e the alga and the fungus. Secondary products, which do not exhibit a correlation with photosynthesis, are generally suspicious to be produced and released by the fungal partner. These results obtained within the joint (FMI / MPIC) experiments show the necessity to focus

on these VOC emissions from lichens in the future, especially including investigations on the VOC production by lichens triggered by environmental conditions and developmental stages.

Acknowledgements

We thank the colleagues from the Finnish Meteorological Institute (Air Quality Department) and from the University of Joensuu (Faculty of Forestry) as well as CNR (Rome) for collaboration, and especially Dr. Harri Hypen for sampling the lichen species. We acknowledge the financial support by EU Commission within the frame of BIPHOREP project (ENV4-CT95-0022).

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