*Symbiosis*, 31 (2001) 95–111
Balaban, Philadelphia/Rehovot



# Evidence for Ethanolic Fermentation in Lichens during Periods of High Thallus Water Content

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Received September 6, 2000; Accepted November 6, 2000

**Abstract**

Direct gas exchange between 'landplants' and the atmosphere represents a continuos process of vital importance and it involves more gas species than $CO_2$, $O_2$, and water vapour. Within these exchange processes emissions of volatile organic compounds (VOC) contribute significantly to the oxidation capacity and hence the $O_3$ level of the atmosphere. In order to investigate the VOC exchange of lichens, some abundant boreal macrolichens were enclosed in a dynamic cuvette system and measured under climate chamber conditions. Air samples were simultaneously collected from a cuvette with lichens enclosed and from an empty reference cuvette. The results obtained from lichens measured under prepurified air conditions pointed to a temporary emission of acetaldehyde occurring mainly within the range of higher thallus water contents (TWC) when diffusion resistances are high and the $CO_2$ and $O_2$ exchange is impeded. We supposed the acetaldehyde emission being caused by a temporary $O_2$ deprivation. Therefore subsequent VOC measurements were conducted under synthetic air conditions with and without $O_2$ and with increased $CO_2$ content. The switch from oxigenated to anoxic conditions resulted in an

*Presented at the Fourth International Association of Lichenology Symposium, September 3–8, 2000, Barcelona, Spain*

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0334-5114/2001/$05.50 

increase of the acetaldehyde emission of about 100%. A simultaneous emission of ethanol could be monitored by the operation of a proton-transfer-reaction mass spectrometer (PTR-MS). The acetaldehyde/ethanol co-emission confirmed the occurrence of the ethanolic fermentation within lichens. As ethanolic fermentation enables the lichens to bypass intrathalline oxygen deficiency at high TWC levels, it represents both a source of VOC emissions to the atmosphere and a so far unconsidered carbon loss for lichens.

Keywords: Boreal lichens, volatile organic compounds (VOC) emission, water content, gas exchange, acetaldehyde

## 1. Introduction

Ethanolic fermentation is a process to cope with anoxic conditions and delivers acetaldehyde and ethanol. Obviously these compounds can be emitted into the atmosphere (Kimmerer and Kozlowski, 1982) where they contribute to the budget of volatile organic compounds (VOCs) and are involved in two sets of processes which contribute to the so-called "Global Change", i.e. the "Photochemical Smog" formation and the "Greenhouse Warming" effect (Graedel and Crutzen, 1993). With reference to Isidorov et al. (1999) the global phytogenic VOC emission amounts to 1100 to 1500 Tg $a^{-1}$. Thus, the total VOC emission of the vegetation exceeds the emission of non-methan hydrocarbons (NMHCs) from all known anthropogenic sources, which is 103 Tg $a^{-1}$, by approximately one order of magnitude. Most of the numerous VOC studies have had an emphasis on shrubby or woody species and crop plants (review: Kesselmeier and Staudt, 1999). As the world's largest continuous vegetation zone is represented by the circumpolar boreal belt which covers ca. 12% of the land surface of the earth, numerous studies on the VOC exchange of boreal trees were conducted. In contrast, similar research involving lower vegetation such as lichens is very limited, even though particularly lichens represent one of the most characteristic elements of the boreal vegetation. There are at least three reasons that strongly support research on the VOC exchange of lichens: (1) The gas exchange of lichens occurs over the whole surface area of the thallus (Nash and Gries, 1995). (2) The lichens can be active to extremely low temperature conditions (Schulze and Lange, 1968; Kappen and Lange, 1970; Nash et al., 1987; Kappen et al., 1990; Kappen et al., 1998a; Kappen et al.; 1998b) so that lichens represent a potential source or sink of VOCs especially within those times when the growing season of higher plants is over and their VOC emissions are low. (3) Because the symbiotic association of a lichen can include two or three organisms from different kingdoms (Fungi, Protista, Monera), the lichen represents an accumulation of different metabolic processes (Huneck, 1999) with each representing a potential source or sink for VOCs. In addition, there is a

quasi-synergistic effect as the whole lichen produces secondary substances which are not produced by the single bionts (Fahselt, 1994). Numerous classes of lichen substances are known (overview Huneck and Yoshimura, 1996) involving lots of intermediates which may be volatilized. Accordingly, we considered lichens to be important contributors concerning the overall biogenic share of the VOC budget of remote boreal areas. First results concerning the VOC exchange of lichens indicated several VOCs to be deposited or emitted (Wilske and Kesselmeier, 1999; Kesselmeier et al., 1999).

In contrast to isoprenoids, fewer studies have focussed on emissions of acetaldehyde and ethanol from plants. Acetaldehyde emissions from plants in connection with a simultaneous ethanol emission were detected by Kimmerer and Kozlowski (1982) and Kimmerer and MacDonald (1987). Within the study of Kimmerer et al. (1982) both the acetaldehyde and the ethanol emission was correlated to stress affecting the plant, e.g. high $SO_2$, high $O_3$, and freezing exposure. Kesselmeier et al. (1997) found the emissions of acetaldehyde to be in the same range for the two Mediterranean species *Pinus pinea* and *Quercus ilex*. Field measurements on a pasture site in Australia (Kirstine et al., 1998) revealed acetaldehyde to be one of the predominant VOCs which were emitted from grass, whereas the acetaldehyde emission from clover was very low. Acetaldehyde was also found to be emitted from Norway spruce (Janson et al., 1999) and, together with ethanol, from flooded poplar trees (Kreuzwieser et al., 1999; Kreuzwieser et al., 2000) and holm oak (Holzinger et al., 2000).

Acetaldehyde and ethanol are prominent products of the ethanolic fermentation and the basic fermentation processes in principle are well known pathways. However, the hitherto collected knowledge about the significance of e.g. biogenic ethanol emission for atmospheric chemistry is rather poor. The reason of disregarding potential ethanol input from vegetation may have been caused by the assumption that fermentation plays only a minor role in the life of land plants. Nevertheless, plants of either temperate and extreme environments certainly have to compete with periodical or accidental "flooding" events. The occurrence of the ethanolic fermentation in lichens may also be a feature of their poikilohydric lifecycle.

## 2. Materials and Methods

*Lichen samples, storage and preparation*

Lichens of the species *Cetraria islandica*, *Cladina rangiferina*, *Cladina stellaris*, and *Bryoria* spp. (mainly comprising *B. fuscescens*, *B. fremontii* and *B. capillaris*) were collected in Finland, spread on site to dry in ambient air and were afterwards bagged into unbleached paper for shipping to the laboratory.

The handling of lichen material in the laboratory was exclusively performed with one-way polyethylene gloves. The lichen material was stored at –23°C according to Feige and Jensen (1987). Samples to be measured were at first kept at 4°C for a few hours. To achieve full physiological activity the lichens were moistened and acclimatised over 3 days in a climate chamber (HPS1500/60/S, Heraeus-Vötsch, Germany) under a light to dark cycle of 14 to 10 hours. Light conditions during acclimatisation were around 300 µmol $m^{-2}s^{-1}$ photosynthetical active radiation (PAR), 50 to 60% relative air humidity (RH), and 10 to 15°C. Conditions in the dark were 70% RH and 8 to 10°C. Lichens were sprayed with ultraclean water (Milli-Q water; HPLC-grade; R>18 MΩ cm) several times per day. To avoid a persistent moisture the lichens were generally remoistened after a short period of desiccation. On the day of measurement the lichens were cleaned of all non-lichen matter and degrading thallus material. The prepared lichen sample was rinsed with MQ water and subsequently flushed with prepurified air for at least one hour in an extra enclosure. After the phase of cuvette adaption and decontamination under prepurified air conditions the lichen was submerged into MQ water, subsequently blotted to remove superficial water droplets, then weighed and installed in the sample cuvette. Lichens were generally enclosed for ca. 4 hours. After cessation the lichen was weighed again. The absolute dry weight (DW) of the lichen sample was determined after drying for 24 h at 105°C.

*Application of the phosphate carriers AMP and ATP*

The phosphate carriers adenosine monophosphate (AMP) and adenosine triphosphate (ATP) were applied by submerging the thalli immediately before measurements in a solution of either AMP or ATP (both Boehringer/Roche Diagnostics). A solution of 1 g AMP or 1 g ATP dissolved in MQ-water and filled up to a total volume of 200 ml was transferred to a vessel which was connected to a vacuum pump. The lichen samples were submerged for 10 min under low pressure (ca. 300 mbar) in order to (1) enhance the uptake of AMP or ATP into the thallus and (2) to standardize the relative uptake of water and both substances. The treatment of control samples was similar except that they were submerged in pure MQ-water.

*Setup of the laboratory cuvettes experiments*

The VOC exchange of the lichens was investigated with an "all teflon" cuvette system which was installed in a climate chamber. For details of the chemically inert "all teflon" cuvette system designed for trace gas exchange measurements see Kuhn et al. (2000). A detailed description of important

characteristics of the cuvette type used for the experiments with lichens is provided by Kuhn (1997).

VOC exchange measurements were conducted in the differential mode by intercomparing air samples from a teflon bag cuvette containing lichens with simultaneous air samples from an empty reference cuvette. The cuvette volume was 1.7 L and total air flow through each cuvette was generally between 2 to 4 L $min^{-1}$. Sample and reference cuvette enclosures were simultaneously flushed with either compressed prepurified and humidified air from laboratory supply or from commercially available gas cylinder. Prepurification of the air was achieved by a molecular sieve (2.0 beads, 0.5 mesh, Merck, Germany ) and two humidifiers filled with ultraclean water (Milli-Q™ water; HPLC-grade; R>18 MΩ cm). Inlet mixing ratio of acetaldehyde under prepurified air conditions ranged mainly between 0.05 to 0.5 ppb, values reflecting the atmospheric environment in remote boreal areas. Recently Viskari et al. (2000) reported a bit higher background mixing ratio of acetaldehyde between 0.6 to 1.7 ppb from Eastern Finland. Although, this range of background values derived from the environment of a mature conifer forest, the sampling site was located in a distance of just 200 m from a national highway. Therefore it is probably not representative for remote boreal areas. Synthetic air gas composition was obtained by mixing pure gases of nitrogen ($N_2$ purity grade 5.0), oxygen ($O_2$ 4.5), and carbon dioxide ($CO_2$ 4.5) using mass flow controllers. The absolute $CO_2$ concentration of the air at the inlet port was measured by a portable EGM-1 gas monitor (PP Systems, UK) and adjusted to 350 or 1100 ppm. Net photosynthesis (NP) and evapotranspiration of the lichens were measured using an infra-red dual channel gasanalyzer (IRGA, LI-6262, LI-COR, USA). Additionally, a set of standard climatic sensors were used to monitor the conditions during the experiments, e.g. one quantum sensor (LI-190, LI-COR, USA) to measure photosynthetically active radiation (PAR), one thermohygrometer (HMP133Y, Vaisalla, Finland) to measure temperature and air humidity at cuvette inlet, and two thermocouples to measure temperature inside cuvettes. A second thermohygrometer (modified YA1000-C-00, Walz, Germany) was used to monitor temperature and humidity conditions during the lichen acclimatisation.

*Sampling and analysis of acetaldehyde and ethanol*

Acetaldehyde was trapped in accordance with Zhou and Mopper (1990) on specially prepared $C_{18}$ glass cartridges ($C_{18}$ Baker bond) (Kesselmeier et al., 1997). The $C_{18}$ 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 an UV/VIS-detector at a wavelength of 365 nm. The total error of emission rates obtained with the overall technique was calculated according to Kesselmeier et al. (1997). Using the DNPH-trapping technique the time resolution for acetaldehyde was one hour. The VOC sampling started 15 min after the lichen was enclosed in the cuvette. A limited set of experiments was conducted using a proton-transfer-reaction mass spectrometer (PTR-MS). The PTR-MS facilitated online measurements providing an excellent minute-ranging time resolution for acetaldehyde and ethanol. Furthermore, these experiments revealed that a 15 min flushing interval represents a safe time period to re-establish a cuvette atmosphere of prepurified or gas cylinder mixture air conditions, and hence to minimize contamination. For details on technique and operation of a PTR-MS see Lindinger et al. (1998). Particular details on VOC studies of plant emissions using the PTR-MS in combination with the "all teflon" type of cuvette technique were recently reported (Holzinger et al., 2000).

## 3. Results

VOC measurements of lichens under purified air conditions exhibited a small but significant emission of acetaldehyde. The main emission of acetaldehyde occurred during the first hour of an experiment when TWC was highest. The higher acetaldehyde emission and highest TWC of the recently moistened lichen samples pointed to a potential relationship. Within Fig. 1 the acetaldehyde exchange rates from *Cladina stellaris*, *Bryoria* spp., and *Cetraria islandica* were plotted versus the corresponding TWC range as allocated for the hour of acetaldehyde sampling. The highest emissions occurred mainly at higher TWC. It is known that high TWC affects the gas exchange of lichens (Green and Snelgar, 1982; Green et al., 1994; Lange et al., 1997; Lange et al., 1999) through increased $CO_2$ diffusion resistance and lower $CO_2$ uptake. We assume a similar relation between TWC and $O_2$. Accordingly, low and high acetaldehyde emission at similar TWC ranges are considered as being caused by different gradients of water saturation developing across the thalli during one hour of VOC sampling. To investigate whether acetaldehyde emissions were due to a temporary $O_2$ deficiency, further measurements were conducted with synthetic air under oxic or anoxic cuvette conditions avoiding water condensation effects which may interfere with VOC measurements.

Acetaldehyde emission from *Cetraria islandica* was measured under three different regimes for gas compositions during cuvette flushing: (1) Synthetic air conditions (i.e. $N_2/CO_2/O_2$) reflecting the composition of ambient air for nitrogen, carbondioxide (350–400 ppm), and oxygen. (2) Alternatively, the cuvettes were flushed with oxygen free air (i.e. $N_2/CO_2$) or (3) with oxygen free

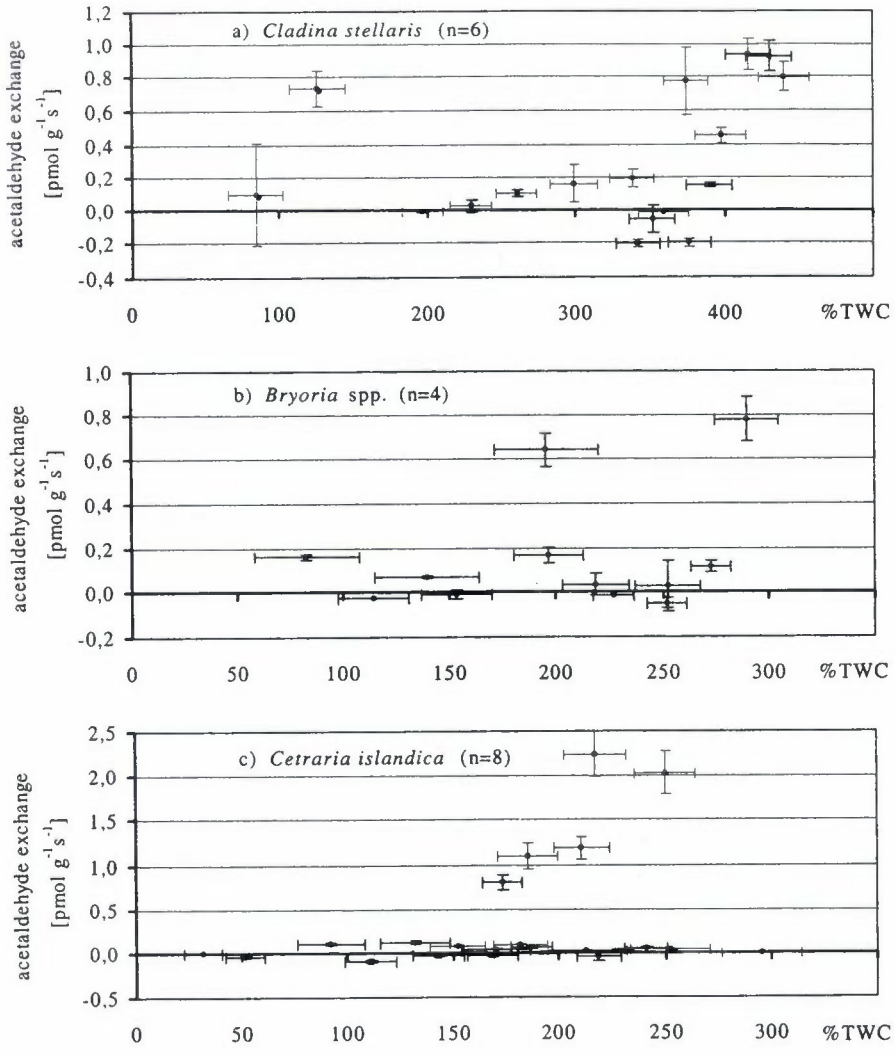


Figure 1. Acetaldehyde exchange of a) *Cladina stellaris*, b) *Bryoria* spp, i.e. mainly *B. fuscescens* and *B. capillaris*, c) *Cetraria islandica*, in relation to the thallus water content (%TWC). Abscissa bars represent the TWC range allocated for the hour of measurement. Positive and negative ordinate sections represent emission or deposition of acetaldehyde, respectively. Error bars represent the total emission rate error of the acetaldehyde measurement (Kesselmeier et al., 1997). Acetaldehyde emission occurred mainly under the regime of high TWC.

air plus a threefold increase of carbondioxide concentration (i.e. $N_2/(3x\ CO_2)$). The higher $CO_2$ was provided to obtain better assimilation rates by the

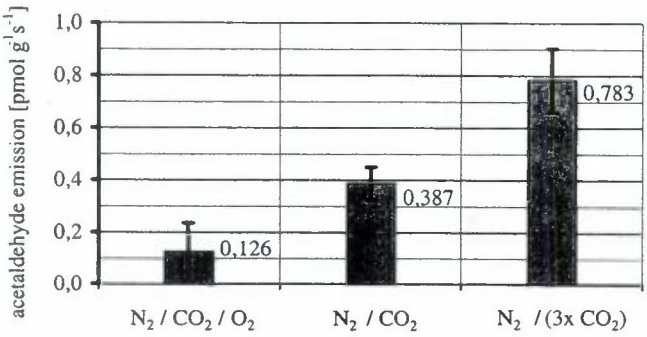


Figure 2. Acetaldehyde emission from *Cetraria islandica* (average ± sd, n=7) measured under constant irradiance but different atmospheric compositions: synthetic air ($N_2/CO_2/O_2$), anoxic atmosphere ($N_2/CO_2$), and anoxic atmosphere plus threefold carbon dioxide ($N_2/(3xCO_2)$). Acetaldehyde emission was significantly enhanced by anoxic conditions indicating oxygen deficiency to be the emission. Further increase of the emission under conditions of a threefold increase of carbondioxide (ca. 1100 ppm) pointed to a dependence on the $CO_2$ assimilation by the RUBISCO enzyme; thus confirming the involvement of lichen physiology.

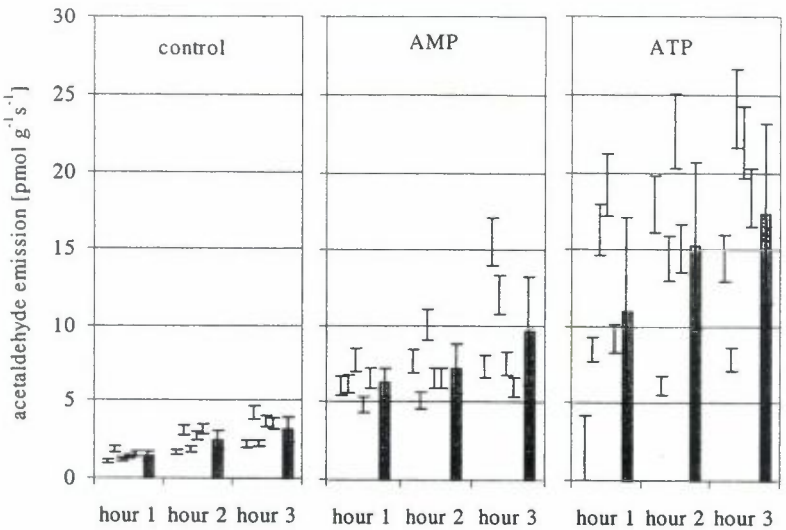


Figure 3. Acetaldehyde emission from *Cetraria islandica* triggered by the treatment with energy balancing adenosine phosphates. For each treatment five samples were measured over three hours under anoxic conditions. Error bars represent total errors of single measurements. Columns with error bars represent the average ± sd (n=5) of the respective hour of measurement within each treatment. The average emission of the samples triggered by the phosphate carriers is significantly higher than the emission of the control samples.

Ribulosebisphospate carboxylase (RUBISCO). *Cetraria islandica* clearly showed a significantly higher acetaldehyde emission under anoxic conditions and further enhanced emission under anoxic conditions with threefold $CO_2$ (Fig. 2). Thus, the results pointed to (1) oxygen deficiency being responsible for the acetaldehyde emission, and (2) to there being a substrate-dependent trigger as expected.

The correlation between $O_2$ availability and acetaldehyde emission by 10 samples of *Cetraria islandica* were measured under increasing oxygen content ranging from 1 to almost 21% $O_2$. Emission rates did not decrease with increasing oxygen but were variable and reflected different levels of initial TWCs. The average acetaldehyde emission rate of three measurements with each sample ranged between 0.1 to 0.3 pmol $g^{-1}s^{-1}$. With respect to the previous experiment (see Fig. 2) this result demonstrated that anoxic conditions led to a 100% increase of the acetaldehyde emission rate, whereas, under hypoxic air conditions containing just 1% $O_2$, the TWC of the samples was the dominant influence on acetaldehyde emission.

The results described above pointed to ethanolic fermentation as the source for the acetaldehyde emission from lichens. Fermentation regulation is known to depend on the ratio between the phosphate carriers adenosine monophosphate (AMP) and adenosine triphosphate (ATP) within cells. Within this basic regulation system a low ATP level or a higher AMP level, respectively, enhances the substrate turnover in order to gain sufficient ATP from fermentation. Three groups of lichen samples of *C. islandica* were measured for their acetaldehyde emission rate: (1) an untreated control group, (2) an AMP treated group, and (3) an ATP treated group. The samples of each group were measured over a three hour period under anoxic conditions. The results are presented in Fig. 3: acetaldehyde emission of the AMP treated samples was significantly increased compared to the control samples. However, as the phosphate carriers were not injected into cells but were applied via water uptake, both AMP and ATP had to pass membranes. Membrane passage of the phosphate carriers is known to be controlled and to occur for low energy phosphates, which results in the case of ATP in a hydrolysis of the high energy phosphates. Therefore, supplying extracellular ATP simulates the application of a higher dose of low energy signaling AMP. Hence, a further increase of the acetaldehyde emission resulted from the ATP application. The results clearly show that the acetaldehyde emission was enhanced by triggering the turnover of the fermentation and the overall results from the experiments corroborated the ethanolic fermentation pathway of being the source for the acetaldehyde emission from lichens.

Because of potential long term carbon loss and relatively low energy gain with ethanolic fermentation it was suspected that a down regulation may occur. Accordingly, samples of *Cladina rangiferina* were subjected to (oxic)

preincubation periods ranging from 2 to 16 hours. During preincubation in the flushing cuvette the samples were kept under continuously well hydrated thallus conditions and good light conditions (ca. 400 µmol $m^{-2}s^{-1}$ PAR). Subsequently the samples were measured over three hours under anoxic conditions. Acetaldehyde emissions ranged between 0.2 to 1.4 pmol $g^{-1}s^{-1}$ (n=12, average 0.7 pmol $g^{-1}s^{-1}$) for samples preincubated for 2 to 8 hours. In contrast, emission were mainly below 0.3 pmol $g^{-1}s^{-1}$ (n=18, average 0.2 pmol $g^{-1}s^{-1}$) for samples preincubated more than 8 hours pointing to a down regulation of the fermentation with the increase of the hydration duration.

A set of experiments was conducted by means of a proton-transfer-reaction mass spectrometer (PTR-MS) in order to improve the time resolution for the measurement of acetaldehyde emission and to detect the supposed higher ethanol emission from lichens. Results obtained from the PTR-MS

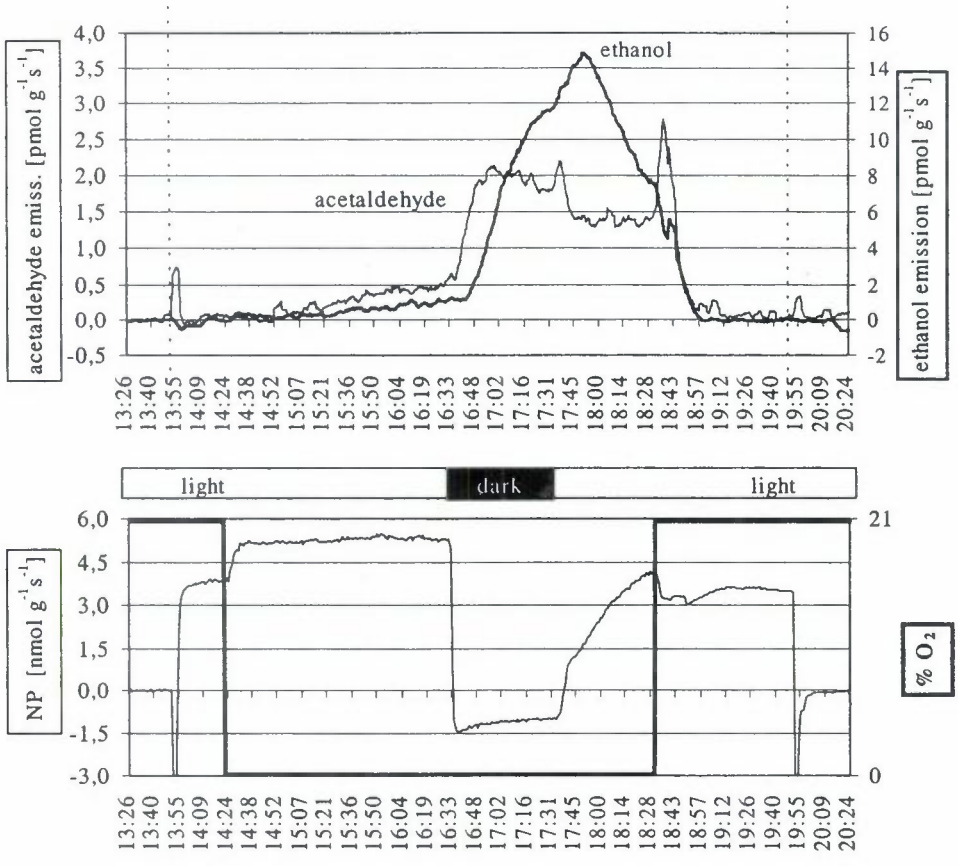


Figure 4. See legend on opposite page.

measurements demonstrate the co-emission of acetaldehyde and ethanol by *Cladina stellaris* (Fig. 4). Similar results were obtained with *Cetraria islandica*, *Cladina rangiferina*, *Bryoria* spp., and (one measured sample) *Alectoria sarmentosa* (data not shown).

The following five items summarise the results: (1) The PTR-MS measurements clearly documented the acetaldehyde emission from lichens due to anoxic conditions, and hence, confirmed the previous measurements. (2) The detection of the co-emission of ethanol finally confirmed the ethanolic fermentation within lichens to be the source. (3) The measurement of the co-emission confirmed the assumption that an ethanol release should be significantly larger than an acetaldehyde emission. This was expected because only formation of the final product of the pathway meets the implicated needs of the organism. (4) There was an increased co-emission during dark conditions potentially caused by the missing of photosynthetically produced oxygen within the lichen. (5) The constant levels of the acetaldehyde emission observed during the dark phase and the subsequent light phase can be explained by a limited acetaldehyde leakage from the fermentation. Thus, limitation of acetaldehyde emission additionally underlines the meaning of this pathway for the organism, which, apart from gaining ATP, is the recovery of NAD.

Figure 4. Co-emission of acetaldehyde and ethanol from *Cladina stellaris* (ethanol: upper plot and presented with bold line) under synthetic air and anoxic conditions ($O_2$ content: lower plot and presented with bold line). The lichen was measured from 13:55 to 19:55 (assigned by vertical broken line in the upper plot). Co-emission started after turning $O_2$ off at around 14:20. The delay of starting co-emission was probably due to the decreasing $O_2$ content. Within the first period under light the emission rate of ethanol was around twice the respective emission rate of acetaldehyde. Co-emission was further enhanced by turning off the light at ca. 16:33 (see light and dark signature in the insertion). Acetaldehyde emission started earlier than ethanol emission then, for a short time, both emission rates increased similarly. The acetaldehyde emission reached a constant level whereas ethanol emission still increased up to values of almost six times that of acetaldehyde. The increase of the fermentation rate under dark conditions is assumed to result from the missing $O_2$ production by the algae. The constant level of acetaldehyde emission might indicate the maximal "leakage" rate of acetaldehyde escaping from the pathway. When light conditions were re established the constant acetaldehyde emission shifted back to a lower level after a short increase of release. This release may correspond to the overshooting ethanol emission pointing to a delay of effectiveness of different regulating systems which are involved. When oxygen was turned on the immediately enhanced decrease of ethanol was contrasted by a short burst of acetaldehyde emission. Finally, both emissions decreased consistently at least within 10 min after oxygen was turned on.

## 4. Discussion

*Plant and lichen emissions of acetaldehyde and ethanol to the atmosphere*

Only a few plant species have been previously investigated for emissions of acetaldehyde and ethanol (review: Kesselmeier and Staudt, 1999; and see present introduction). These species belong to very different groups concerning the phylogeny, the growth form, the vegetation zones and the habitat preferences. First results for acetaldehyde emission from lichens were previously reported (Wilske and Kesselmeier, 1999). With the new results presented here the investigated lichen species principally complement the pattern of phylogenetic diverse "landplants" emitting acetaldehyde (and ethanol) to the atmosphere. In contrast to higher plants the emission is only temporary and the variability of water supply leading to an acetaldehyde/ethanol co-emission from lichens cannot be readily estimated. However, the presented data will facilitate upscaling of the acetaldehyde/ethanol co-emission from lichens in future work. Two implications of the co-emission are: (1) The importance of lichens for the global trace gas budget was first found by studies on the emission ($H_2S$, DMS) and consumption (COS) of climatically relevant sulfur compounds (Gries et al., 1994; Kuhn and Kesselmeier, 1996; Kuhn, 1997; Kuhn et al., 2000; Kuhn and Kesselmeier, 2000). We must now add the acetaldehyde/ethanol co-emission, the deposition of short chained organic acids (Wilske and Kesselmeier, 1999), and the indicated emission of additional VOCs (Kesselmeier et al., 1999).

(2) With addition of the lichens we can now ask how many "landplants" emit acetaldehyde? The emissions by lichens suggest that fungi should not be disregarded. Plant production and emission of acetaldehyde and or ethanol must be given even more importance as both occur not just due to anoxic conditions or hypoxia, but also due to other stress effect like $SO_2$ or $O_3$ exposure, water deficit, freezing, and pathogen infection (Kimmerer and Kozlowski, 1982; Tadege et al., 1998).

*Occurrence of ethanolic fermentation in lichens and potential implications for the symbiosis*

The occurrence of ethanolic fermentation in lichens was demonstrated by measurements under ambient air conditions and further confirmed by means of measurements under artificial anoxic conditions. The investigated species, all boreal macrolichens, showed no significant differences in the overall range of the acetaldehyde and ethanol emission rates. We are aware that there are morphological, anatomical, and chemical characters which may promote or reduce the time periods of high TWC. Hence, the occurrence of ethanolic

fermentation may depend on the species specific characters which are involved in water storage or which represent repellents, seals and water barriers (review Poelt, 1986; Ott and Schieleit, 1994; Lange et al., 2000; Scherrer et al., 2000). However, the intensity of the ethanolic fermentation might split lichens into groups of lower and higher "flooding tolerance" as it is known from higher plants. Based on the present results we can not discern whether one or both symbionts emit acetaldehyde and ethanol nor identify the potential of stress effects other than hypoxia. Irradiance and preceeding hydration periods represent additional parameters affecting the rates of the co-emission due to hypoxia. Vice versa, the impact of heavy flooding after a long period of desiccation (e.g. under continental summer conditions within the boreal biome) would be of high interest. Therefore, the natural incidences of prevailing TWC sufficiently high to initiate and maintain ethanolic fermentation within lichens cannot be estimated at the moment. Field measurements would be necessary to check the abundance and time scale of acetaldehyde and or ethanol emission by certain lichen species under natural conditions.

The actual importance of the ethanolic fermentation as well as its meaning for the carbon budget of lichens remains to be determined. Even if we add other detected VOC emission rates (to be published elsewhere) the total carbon loss due to these emissions seems to be negligible compared with the carbon loss of higher plants which is within a range between one to a few percents of the simultaneously measured NP rates (Kesselmeier and Staudt, 1999). However, VOC emissions certainly depend on a lot of environmental factors which may change both emission rates and emitted VOC species. Thus, it cannot be totally ruled out that the actual carbon loss by VOC emission might be significant for the carbon budget of lichens.

With respect to both the functions of acetaldehyde and ethanol within the regulation network of plants (Tadege et al., 1999; Podd and van Staden, 1999) and the growing interest on phytohormone regulations within the lichen symbiosis (e.g. ethylene: Epstein et al., 1986; Lurie and Garty, 1991; Ott and Zwoch, 1992; Ott, 1993; Ott and Schieleit, 1994; Garty et al., 1995; Schieleit and Ott, 1996; Schieleit and Ott, 1997; Garty et al., 1997; and abscisic acid: Dietz and Hartung, 1998, 1999) temporary high concentrations of intrathalline acetaldehyde and ethanol may be considered as being potentially involved in the regulation of the lichen association. This makes sense regarding the close coupling of ethanolic fermentation to the conditions of TWC and the different meaning of lasting hydration for both symbionts.

**Acknowledgements**

This study was supported by the EU Commission within the frame of the project BIPHOREP (ENV4-CT95-0022) and by the Max Planck Society. We

thank the colleagues from the Finnish Meterological Institute (Air Quality Department) and from the University of Joensuu (Faculty of Forestry) and especially Dr. Harri Hypen for sampling the lichens.

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